Table 1 Patient characteristics at the starting time of follow up†

	NAFLD group	HCV group	*P*-value
n	1600	1600	
Age (years)	62.5 ± 9.5	62.6 ± 8.7	0.936
Gender (male/female)	1200/400	1200/400	1.000
Body mass index	25.1 ± 2.6	21.8 ± 4.0	<0.001
Blood pressure			
(systolic, mmHg)	132 ± 17	133 ± 18	0.972
(diastolic, mmHg)	76 ± 11	77 ± 12	0.937
Hypertension (+/−)	279/1321	306/1294	0.252
Smoking (+/−)	421/1179	396/1141	0.807
AST (IU/L)	29 ± 15	77 ± 64	<0.001
ALT (IU/L)	37 ± 25	104 ± 97	<0.001
GGT (IU/L)	73 ± 79	83 ± 97	0.196
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.4	0.883
Triglyceride (mg/dL)	161 ± 105	99 ± 51	<0.001
Total cholesterol (mg/dL)	211 ± 33	176 ± 38	<0.001
FPG (mg/dL)	104.1 ± 10.5	95.8 ± 9.3	<0.001
FPG (DM/pre-DM /normal)	208/330/1062	184/276/1140	<0.001
Platelet ($\times 10^4$/mm^3)	22.1 ± 6.5	15.8 ± 5.8	<0.001
AFP (ng/mL)	3.4 ± 2.4	10.8 ± 10.0	<0.001
Follow-up period (year)	8.2 ± 3.8	8.2 ± 3.9	0.928

†Data are number of patients or mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus, FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease.

NAFLD group and the HCV group in both males and females.

Predictive factors for the development of malignancies

The factors associated with the development of malignancies in the NAFLD group and HCV group are shown in Tables 4 and 5. In the NAFLD group, multivariate Cox proportional hazards analysis shows that malignancies occurred when patients had an age of ≥70 years (hazard ratio [HR] : 2.10; 95%CI = 1.38–3.17; $P < 0.001$), current smoking (HR : 1.64; 95%CI = 1.18–2.27; $P = 0.003$), and elevated glucose level (HR : 1.32; 95%CI = 1.08–1.61; $P = 0.007$).

On the other hand, in HCV group, multivariate Cox proportional hazards analysis shows that malignancies development rate was high with statistical significance when patients had elevated AFP (HR : 2.52; 95%CI = 1.94–3.44; $P < 0.001$), elevated glucose level (HR : 1.35; 95%CI = 1.18–1.59; $P < 0.001$), elevated AST level (HR : 1.75; 95%CI = 1.13–2.70; $P = 0.010$), hypoalbuminemia (HR : 1.51; 95%CI = 1.15–1.97; $P = 0.002$), male (HR : 1.49; 95%CI = 1.16–1.94; $P = 0.002$), and thrombocytopenia (HR : 1.49; 95%CI = 1.14–1.96; $P = 0.002$).

DISCUSSION

THE DEVELOPMENT INCIDENCE of malignancies in elderly patients with NAFLD or HCV has been described in the present study. The reason for selecting elderly patients is that development of malignancies in patients with age of ≥60 years occur frequently compared with young patients. Thus, it is likely that the difference between NAFLD and HCV patients tends to become clear.

The present study shows several findings with regard to the development of malignancies in elderly Japanese patients with NAFLD or HCV. First, HCC in the NAFLD group accounted for approximately 6% of the cause of malignancies. The four malignancies of the stomach, colon, prostate, and lung accounted for about 60% in the NAFLD group. Matsuda *et al.* have reported the cancer incidence in Japan.[32] According to their report, the outbreak of malignancies in a Japanese male popu-

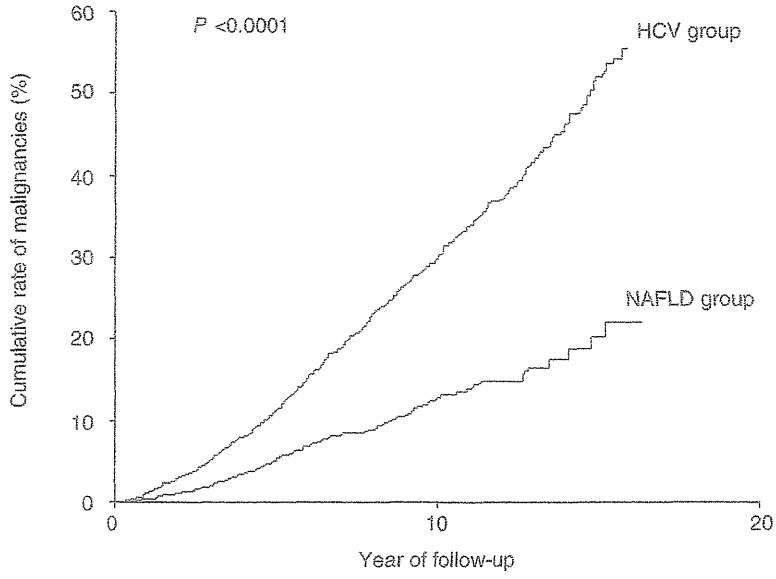

Figure 1 Cumulative development rate of malignancies in non-alcoholic hepatic diseases (NAFLD) or hepatitis C virus (HCV) patients.

lation was observed in the following order in 2005: gastric cancer 20.4% > colon cancer 16.0% > lung cancer 15.4% > prostatic cancer 10.9% > HCC 7.4%. On the other hand, the outbreak of malignancies in a Japanese female population was observed in the following order in 2005: mammary cancer 18.0% > colon cancer 16.2% > gastric cancer 13.6% > lung cancer 9.3% > uterine cancer 6.8%. The incidence of prostate cancer in NAFLD was greater than that in a total Japanese population. Renehan *et al.* showed that body mass index is connected with prostate carcinogenesis relative to other tumours.[33] NAFLD patients might tend to have

Table 2 Development rate of each malignancy in the non-alcoholic fatty liver disease (NAFLD) group and the hepatitis C virus (HCV) group†

Malignancies	NAFLD group		HCV group		*P*‡
	n (%)†	1000 person years	*n* (%)†	1000 person years	
Total	167 (100%)	12.96	395 (100%)	30.88	<0.001
Hepatocellular carcinoma	10 (6.0%)	0.78	267 (67.9%)	20.86	<0.001
Gastric cancer	34 (20.4%)	2.66	28 (7.1%)	2.19	0.522
Colon cancer	31 (18.6%)	2.42	26 (6.6%)	2.03	0.593
Prostate cancer	21 (12.6%)	1.64	14 (3.5%)	1.10	0.308
Lung cancer	17 (10.2%)	1.33	13 (3.3%)	1.02	0.583
Malignant lymphoma	1 (0.6%)	0.08	9 (2.3%)	0.70	0.021
Other cause	46 (27.5%)	3.59	31 (7.8%)	2.43	0.106
Unknown origin	6 (3.6%)	0.46	7 (1.8%)	0.55	1.000

†Data are number of patients (%) and development rates of each malignancy per 1000 person years. ‡Comparison of new development in each malignancy between both groups by log rank test.

Table 3 Development rate of Each Malignancy between the non-alcoholic fatty liver disease (NAFLD) group and the hepatitis C virus (HCV) group based on the difference of gender†

Malignancies	Male		*P*‡	Female		*P*‡
	NAFLD ($n = 1200$)	HCV ($n = 1200$)		NAFLD ($n = 400$)	HCV ($n = 400$)	
Total	13.96	34.17	<0.001	10.31	20.93	<0.001
Hepatocellular carcinoma	0.83	23.75	<0.001	0.63	10.83	<0.001
Gastric cancer	2.91	2.40	0.571	1.88	1.39	1.000
Colon cancer	2.42	2.19	0.655	1.88	1.39	1.000
Lung cancer	1.33	1.05	0.676	1.25	0.93	1.000
Malignant lymphoma	0.08	0.63	0.124	0.00	0.93	0.577
Prostate cancer	1.64	1.10	0.306			
Breast cancer				1.81	1.41	1.000
Other cause	3.59	4.38	0.604	2.43	1.71	0.577
Unknown origin	0.46	0.52	1.000	0.30	0.62	1.000

†Data are development rates of each malignancy per 1000 person years. ‡Comparison of new development in each malignancy between NAFLD group and HCV group based on the difference of gender by log rank test

carcinogenesis of prostate based on obesity. Our results show that physicians in charge of NAFLD patients should pay attention to the malignancies of stomach, colon, prostate, and lung in addition to development of HCC. Moreover, aging, hyperglycemia, and smoking were dominating factors to enhance the development of malignancies in NAFLD group.

Second, HCC in the HCV group accounted for about two-thirds of the outbreak of malignancies. In the present study, the development rates of HCC and malignant lymphoma in the HCV group were statistically higher than those in the NAFLD group. The high incidences of HCC and malignant lymphoma have been reported by many reseachers.[15–19,34] Male, hyperglycemia, elevated AST, hypoalbuminemia, thrombocytopenia, and elevated AFP were dominating factors to enhance the development of malignancies in the HCV group. Hypoalbuminemia, thrombocytopenia,

Table 4 Predictive factors for malignancies in the non-alcoholic fatty liver disease (NAFLD) group†

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, ≥70/<70)	2.34 (1.60–3.44)	<0.001	2.09 (1.42–3.07)	<0.001
Gender (M/F)	1.11 (0.76–1.60)	0.631		
BMI (≥25/<25)	0.74 (0.52–1.04)	0.079		
Hypertension (−/+)	1.27 (0.88–1.84)	0.197		
Smoking (+/−)	1.62 (1.18–2.24)	0.003	1.64 (1.18–2.27)	0.003
AST (IU/L, ≥34/<34)	1.03 (0.62–1.70)	0.973		
ALT (IU/L, ≥36/<36)	1.27 (0.76–2.08)	0.357		
GGT (IU/L, ≥109/<109)	1.26 (0.79–2.01)	0.350		
Albumin (g/dL, <3.9/≥3.9)	1.41 (0.90–2.04)	0.145		
Triglyceride (mg/dL, ≥150/<150)	1.20 (0.85–1.69)	0.282		
Total cholesterol (mg/dL, ≥220/<220)	1.39 (0.87–2.23)	0.170		
Glucose (DM/ pre-DM/non-DM)	1.39 (1.14–1.69)	0.001	1.32 (1.08–1.61)	0.007
Platelet ($\times 10^4$/mm^3, <15/≥15)	1.41 (1.02–1.96)	0.036		
AFP (ng/mL, ≥10/<10)	1.11 (0.35–3.48)	0.338		

†Data are number of patients or mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase.

Table 5 Predictive factors for malignancies in the hepatitis C virus (HCV) group†

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, ≥70/<70)	1.41 (1.11–1.78)	0.003		
Gender (M/F)	1.78 (1.4692.10)	<0.001	1.49 (1.16–1.94)	0.002
BMI (≥25/<25)	1.85 (0.71–4.85)	0.201		
Hypertension (+/−)	1.20 (1.01–1.44)	0.045		
Smoking (+/−)	1.71 (1.43–2.10)	<0.001		
AST (IU/L, ≥36/<36)	2.26 (1.73–3.01)	<0.001	1.75 (1.13–2.70)	0.010
ALT (IU/L, ≥30/<30)	1.69 (1.33–2.16)	<0.001		
GGT (IU/L, ≥109/<109)	1.99 (1.53–2.58)	0.014		
Albumin (g/dL, <3.9/≥3.9)	2.07 (1.65–2.56)	<0.001	1.51 (1.15–1.97)	0.002
Triglyceride (mg/dL, ≥150/<150)	1.15 (0.56–2.41)	0.789		
Total cholesterol (mg/dL, ≥220/<220)	0.51 (0.19–1.35)	0.159		
Glucose (DM/pre-DM/non-DM)	1.37 (1.23–1.55)	<0.001	1.35 (1.18–1.59)	<0.001
Platelet ($\times 10^4/mm^3$, <15/≥15)	2.28 (1.81–2.92)	<0.001	1.49 (1.14–1.96)	0.002
AFP (ng/mL, ≥10/<10)	3.10 (2.46–4.11)	<0.001	2.50 (1.94–3.44)	<0.001

†Data are number of patients or mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, GGT, gamma-glutamyltransferase.

and elevated AFP indicate the advanced liver fibrosis: it is probable that these factors enhance the HCC development as reported before.[35] Our result shows that HCV positive males with hyperglycemia, hypoalbuminemia, elevated AST, thrombocytopenia, and elevated AFP should be carefully checked for HCC.

Third, there were no significant differences in the development of each malignancy between males and females in the NAFLD group. On the other hand, rare development of HCC in males was statistically higher than that of females. However, there are no significant differences in the development of each malignancy except for HCC between males and females in the HCV group. This result suggests that development differences based on gender except for HCC in HCV group might be not important.

Cirrhotic NASH enhances the liver-related events such as HCC and liver failure. However, most patients with NAFLD do not have NASH. According to Japanese annual health check reports, 9–30% of Japanese adults demonstrate evidence of NAFLD by US. Since it is known that about 10% of individuals with NAFLD have NASH, the prevalence of NASH is estimated to be 1–3% of the adult Japanese population.[14] In patients with cirrhotic NASH, HCC and liver failure are the main causes of morbidity and mortality (5-year cumulative HCC development rate 11.3%, 5-year survival rate 75.2%, respectively). However, in the present study, most NAFLD was thought to be non-NASH. Our results suggest that patients with NAFLD before progression to NASH should be followed up to closely check the malignancies other than HCC in addition to HCC. On the other hand, patients with HCV should be followed up to take care to check liver-related disease containing HCC

The present study was limited that most of the NAFLD patients were not undergoing histological or morphological assessment by peritoneoscopy or liver biopsy before the starting time of follow up owing to their advanced age on the day of the first consulting or normal transaminase. Another limitation was that there are several differences in clinical background such as liver fibrosis between the NAFLD and HCV groups. This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up with a large number of patients included.

Our results indicate the following: (i) Physicians in charge of NAFLD patients should pay attention to the carcinogenesis development of stomach, colon, prostate, and lung containing HCC; and (ii) physicians in charge of HCV patients should closely check for HCC.

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REFERENCES

1 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; 346: 1221–31.
2 Williams R. Global changes in liver disease. *Hepatology* 2006; 44: 521–6.
3 Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology* 2008; 134: 1682–98.
4 Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. *Hepatology* 2009; 49: 306–17.
5 Fan JC, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. *J Hepatol* 2009; 50: 204–10.
6 Watanabe S, Yaginuma R, Ikejima K, Miyazaki A. Liver diseases and metabolic syndrome. *J Gastroenterol* 2008; 43: 509–18.
7 Vega GL, Chandalia M, Szczepaniak LS, Grundy SM. Metabolic correlates of nonalcoholic fatty liver in women and men. *Hepatology* 2007; 46: 716–22.
8 van der Poorten D, Milner KL, Hui J *et al.* Visceral fat a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; 48: 449–57.
9 Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; 30: 1356–62.
10 Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP. Identification of individuals with insulin resistance using routine clinical measurements. *Diabetes* 2005; 54: 333–9.
11 Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. *Hepatology* 2004; 39: 909–14.
12 Kheirandish-Gozal L, Sans Capdevila O, Kheirandish E, Gozal D. Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. *Chest* 2008; 133: 92–9.
13 Arase Y, Suzuki F, Ikeda K, Kumada H, Tsuji H, Kobayashi T. Multivariate analysis of risk factors for the development of type 2 diabetes in nonalcoholic fatty liver disease. *J Gastroenterol* 2009; 44: 1064–70.
14 Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011; 46 (Suppl 1): 63–9.
15 Simonetti RG, Camma C, Fiorello F *et al.* Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case control study. *Ann Intern Med* 1992; 116: 97–102.
16 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; 2: 1394–402.
17 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; 129: 94–9.
18 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: 653–9.
19 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 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–30.
20 Yasuda K. Early gastric cancer: diagnosis, treatment techniques and outcomes. *Eur J Gastroenterol Hepatol* 2006; 18: 839–45.
21 Van Gossum A. Guidelines for colorectal cancer screening – a puzzle of tests and strategies. *Acta Clin Belg* 2010; 65: 433–6.
22 Tsukada K, Takada T, Miyazaki M *et al.* Japanese Association of Biliary Surgery; Japanese Society of Hepato-Biliary-Pancreatic Surgery; Japan Society of Clinical Oncology. Diagnosis of biliary tract and ampullary carcinomas. *J Hepatobiliary Pancreat Surg* 2008; 15: 31–40.
23 Cascinu S, Falconi M, Valentini V, S J, Guidelines ESMO. Working Group. Pancreatic cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; 21 (Suppl 5): v55–8.
24 Currie GP, Kennedy AM, Denison AR. Tools used in the diagnosis and staging of lung cancer: what's old and what's new? *QJM* 2009; 102: 443–8.
25 Maresh EL, Mah V, Alavi M *et al.* Differential expression of anterior gradient gene AGR2 in prostate cancer. *BMC Cancer* 2010; 10: 680–7.
26 Harris NL, Jaffe ES, Stein H *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; 84: 1361–92.
27 Genuth S, Alberti KG, Bennett P *et al.* Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003; 26: 3160–7.
28 Lonardo A, Bellini M, Tartoni P, Tondelli E. The bright liver syndrome. Prevalence and determinants of a "bright" liver echopattern. *Ital J Gastroenterol Hepatol* 1997; 29: 351–6.
29 Harrington DP, Fleming TR. A class of rank test procedures for censored survival data. *Biomedaica* 1983; 62: 205–9.
30 Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J Am Stat Assoc* 1958; 53: 457–81.
31 DR Cox. Regression models and life tables. *J R Stat Soc* 1972; 34: 248–75.
32 Matsuda T, Marugame T, Kamo KI, Katanoda K, Ajiki W, Sobue T. The Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2005:

based on data from 12 population-based cancer registries in the monitoring of cancer incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 2011; 41: 139–47.
33 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; 371: 569–78.
34 Kawamura Y, Ikeda K, Arase Y *et al.* Viralimination reduces incidence of malignant lymphoma in patients with hepatitis C. *Am J Med* 2007; 120: 1034–41.
35 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; 28: 930–8.

Effect of Type 2 Diabetes on Risk for Malignancies Includes Hepatocellular Carcinoma in Chronic Hepatitis C

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The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for malignancies after the termination of interferon (IFN) therapy in Japanese patients for hepatitis C virus (HCV). A total of 4,302 HCV-positive patients treated with IFN were enrolled. The mean observation period was 8.1 years. The primary outcome was the first onset of malignancies. Evaluation was performed using the Kaplan-Meier method and Cox proportional hazard analysis. A total of 606 patients developed malignancies: 393 developed hepatocellular carcinoma (HCC) and 213 developed malignancies other than HCC. The cumulative development rate of HCC was 4.3% at 5 years, 10.5% at 10 years, and 19.7% at 15 years. HCC occurred significantly ($P < 0.05$) when the following characteristics were present: advanced histological staging, sustained virological response not achieved, male sex, advanced age of ≥50 years, total alcohol intake of ≥200 kg, and presence of type 2 diabetes (T2DM). T2DM caused a 1.73-fold enhancement in HCC development. In patients with T2DM, HCC decreased when patients had a mean hemoglobin A1c (HbA1c) level of <7.0% during follow-up (hazard ratio, 0.56; 95% confidence interval, 0.33-0.89; $P = 0.015$). The cumulative development rate of malignancy other than HCC was 2.4% at 5 years, 5.1% at 10 years, and 9.8% at 15 years. Malignancies other than HCC occurred significantly when patients were of advanced age of ≤50 years, smoking index (package per day × year) was ≥ 20, and T2DM was present. T2DM caused a 1.70-fold enhancement in the development of malignancies other than HCC. *Conclusion:* T2DM causes an approximately 1.7-fold enhancement in the development of HCC and malignancies other than HCC in HCV-positive patients treated with IFN. In T2DM patients, maintaining a mean HbA1c level of <7.0% reduces the development of HCC. (HEPATOLOGY 2012;000:000-000)

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease worldwide. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20%-50% of cases over a period of 10-30 years.[1,2] In addition, HCV is a major risk factor for hepatocellular carcinoma (HCC).[3-7]

On the other hand, the prevalence of patients with type 2 diabetes mellitus (T2DM) is increasing in many nations, including Japan.[8] Thus, the management of T2DM patients who are chronically infected with HCV is one of the most important issues confronted by physicians. Few studies have reported relationships between T2DM and total malignancies, including HCC in HCV patients. In addition, it is not clear whether the stringent control of T2DM is necessary for protecting the development of malignancies in HCV patients. This issue needs to be confirmed via long-term follow-up of a large cohort of patients at high risk of developing malignancy.

Abbreviations: CH, chronic hepatitis; CI, confidence interval; HbA1c, hemoglobin A1c; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IFN, interferon; LC, liver cirrhosis; SVR, sustained virological response; T2DM, type 2 diabetes mellitus; TAI, total alcohol intake.

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With this background in mind, the present study was initiated to investigate the cumulative incidence and risk factors of malignancies, including HCC after prolonged follow-up in HCV patients treated with interferon (IFN) monotherapy or combination therapy of IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Patients and Methods

Patients. The number of patients who were diagnosed with chronic HCV infection and treated for the first time with IFN monotherapy or combination therapy between September 1990 and March 2009 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan, was 7,205. Of these, 4,302 patients met the following enrollment criteria: (1) no evidence of malignancies by physical examination, biochemical tests, abdominal ultrasonography, gastrofiberscope (or gastrography), or chest X-ray (or computed tomography); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy within 1 year before the initiation of IFN therapy; (3) positivity for serum HCV-RNA before the initiation of IFN therapy; (4) period of $\geq$1 month to $\leq$1 year of IFN therapy; (5) negativity for hepatitis B surface antigens, antibody to hepatitis B core, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay, or indirect immunofluorescence assay; (6) age of $\geq$30 years to $\leq$80 years; (7) no underlying systemic disease, such as systemic lupus erythmatosus or rheumatic arthritis; and (8) repeated annual examinations during follow-up. Annual examinations included biochemical tests, tumor marker (carcinoembryonic antigen, alpha-fetoprotein, and prostate-specific antigen [only in men]), and abdominal ultrasonography. Patients with were excluded from the study if they had illnesses that could seriously reduce their life expectancy or if they had a history of carcinogenesis.

The primary outcome was the first development of malignancy. The development of malignancies was diagnosed by clinical symptoms, tumor marker, imaging (ultrasonography, computed tomography, or magnetic resonance imaging), and/or histological examination.[9-15] All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The physicians in charge explained the purpose, method, and side effects of IFN therapy to each patient and/or the patient's family. In addition, the physicians in charge received permission for the use of serum stores and future use of stored serum. Informed consent for IFN therapy and future use of stored serum was obtained from all patients. The study was approved by the Institutional Review Board of our hospital.

Medical Evaluation. Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and body mass index was calculated as kg/m^2. All patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and heath-related habits, including questions on alcohol intake and smoking history.

The value for hemoglobin A_{1C} (HbA_{1C}) was estimated as a National Glycohemoglobin Standardization Program equivalent value (%). Patients were defined as having T2DM when they had a fasting plasma glucose level of $\geq$126 mg/dL and/or HbA_{1C} level of $\geq$6.5%.[16]

Patients were regarded as hypertensive when systolic blood pressure was $\geq$140 mm Hg and/or diastolic blood pressure was $\geq$90 mm Hg for at least three visits. Smoking index (packs per day $\times$ year) and total alcohol intake (TAI) were evaluated by the sum of before, during, and after the IFN therapy.

Laboratory Investigation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Anti-HCV was detected using an enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). HCV genotype was examined via polymerase chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported.[17] HCV-RNA was determined using the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The serum samples stored at -80°C before IFN therapy were used. The linear dynamic range of the assay was 1.2-7.8 log IU/mL, and the undetectable samples were defined as negative. A sustained virological response (SVR) was

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Table 1. Clinical Backgrounds at Initiation of Follow-up in Enrolled Patients

Variable	Total	HCC Group	Non-HCC Malignancy Group	Without Events Group	P
No. of patients	4,302	393	213	3,696	
Age, years	52.0 ± 11.8	55.8 ± 7.9	57.9 ± 9.1	51.3 ± 12.1	<0.001
Sex, male/female	2528/1774	272/121	129/84	2127/1569	<0.001
Height, cm	163.0 ± 9.2	162.8 ± 8.3	163.3 ± 9.1	163.0 ± 9.3	0.772
Weight, kg	61.4 ± 13.0	62.3 ± 10.6	60.8 ± 10.1	61.3 ± 13.4	0.142
BMI	23.0 ± 4.0	23.4 ± 3.0	22.8 ± 2.8	23.0 ± 4.1	0.012
Blood pressure, mm Hg					
Systolic	128 ± 18	132 ± 19	133 ± 20	127 ± 17	<0.001
Diastolic	77 ± 13	80 ± 12	80 ± 13	77 ± 13	<0.001
TAI, kg*	95 ± 92	151 ± 101	135 ± 81	85 ± 89	<0.001
Smoking index*	6.4 ± 9.4	10.8 ± 11.1	12.5 ± 11.8	5.5 ± 8.7	<0.001
AST, IU/L	42 ± 44	64 ± 55	42 ± 31	40 ± 42	<0.001
ALT, IU/L	44 ± 53	72 ± 63	43 ± 43	42 ± 52	<0.001
GGT, IU/L	54 ± 61	63 ± 65	56 ± 45	53 ± 38	0.007
Albumin, g/dL	4.1 ± 0.3	4.1 ± 0.3	4.1 ± 0.2	4.1 ± 0.2	0.310
Triglyceride, mg/dL	101 ± 53	104 ± 54	105 ± 50	100 ± 52	0.329
Cholesterol, mg/dL	170 ± 32	165 ± 31	169 ± 33	171 ± 32	0.025
FPG, mg/dL	100 ± 22	110 ± 26	104 ± 22	98 ± 21	<0.001
HbA1c, %, NSPG	5.6 ± 1.2	5.9 ± 1.4	5.7 ± 1.4	5.5 ± 1.1	<0.001
T2DM, +/−	267/4,035	63/330	34/179	170/3,526	<0.001
Platelet count, $\times 10^4/mm^3$	17.1 ± 5.1	13.7 ± 4.9	16.5 ± 5.4	17.5 ± 5.4	<0.001
Staging, LC/non-LC	433/3,869	113/285	27/189	293/3,395	<0.001
HCV genotype, 1b/2a/2b/other	2,721/995/458 /128	283/52/20/38	121/62/18/12	2,317/881/420/78	<0.001
HCV RNA, log IU/mL	6.06 ± 1.05	6.22 ± 0.52	6.05 ± 0.86	6.04 ± 1.05	0.003
IFN monotherapy†/combination therapy‡	2,861/1,441	358/35	175/38	2,328/1,368	<0.001
Efficacy, SVR/non-SVR	1,900/2,402	44/349	88/125	1,768/1,928	<0.001

Data are presented as no. of patients or mean ± SD.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; M, male; NGSP, National Glycohemoglobin Standardization Program.

*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

†Outbreak of IFN monotherapy: recombinant IFN-α2a, n = 220, recombinant IFN-α2b, n = 183, natural IFN-α, n = 1,678, natural IFN-α, n = 691, total dose of IFN = 560 ± 164 megaunit. Outbreak of pegylated IFN monotherapy: pegylated IFN-α2a, n = 89, total dose of pegylated IFN = 7.52 ± 2.24 mg.

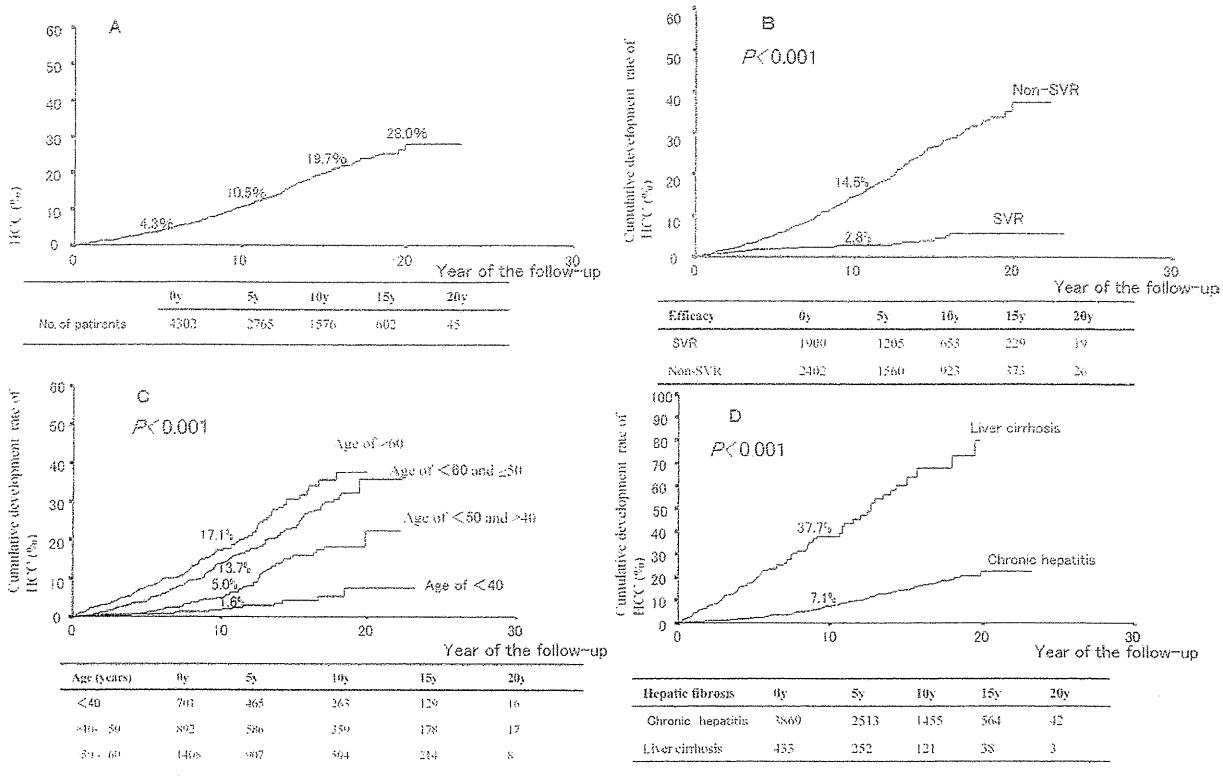
‡Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, n = 335, total dose of IFN = 508 ± 184 megaunit, total dose of ribavirin = 160 ± 68 g; natural IFN-β + ribavirin, n = 101, total dose of IFN = 502 ± 176 megaunit, total dose of ribavirin = 156 ± 67 g; pegylated IFN-α2b+ribavirin, n = 1,005 cases, total dose of pegylated IFN = 4.14 ± 1.10 mg, total dose of ribavirin = 206 ± 58 g.

defined as clearance of HCV-RNA using the COBAS TaqMan HCV test 6 months after the cessation of IFN therapy.

Evaluation of Liver Cirrhosis. Status of liver was mainly determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style; Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.[18]

Follow-up. The observation starting point was 6 months after the termination of IFN therapy. After that, patients were followed up at least twice a year in our hospital. Physical examination and biochemical tests were conducted at each examination together with a regular checkup. In addition, annual examinations during follow-up were undertaken. When a patient had complaints during follow-up, the physician in charge performed additional examinations based on symptoms. Four hundred eighteen patients were lost to follow-up. The final date of follow-up in 418 patients with loss of follow-up was regarded as the last consulting day. In addition, 881 patients were retreated with IFN. The final date of follow-up in 881 patients re-treated with IFN were regarded as the time of the initiation of IFN retreatment. Thus, 418 patients with loss of follow-up and 881 patients retreated with IFN were counted censored data in statistical analysis.[19] The mean follow-up period was 6.8 (SD 4.3) years in 418 patients with loss of follow-up and 7.5 (SD 4.8) years in 881 patients retreated with IFN. Censored patients were counted in the analysis.

Statistical Analysis. Clinical differences among three groups of patients with HCC with malignancies other than HCC without events were evaluated using the Kruskal-Wallis test. The cumulative development rates of malignancies were calculated using the Kaplan-Meier technique, and differences in the curves were



	0y	5y	10y	15y	20y
No. of patients	4302	2765	1576	602	45

Efficacy	0y	5y	10y	15y	20y
SVR	1900	1205	653	229	19
Non-SVR	2402	1560	923	373	26

Age (years)	0y	5y	10y	15y	20y
<40	760	465	263	129	16
≥40 - 50	892	586	359	178	17
50 - 60	1468	987	564	284	8
≥60	1301	807	450	82	4

Hepatic fibrosis	0y	5y	10y	15y	20y
Chronic hepatitis	3869	2513	1455	564	42
Liver cirrhosis	433	252	121	38	3

Fig. 1. Cumulative development rate of HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) efficacy, (C) age, and (D) hepatic fibrosis.

tested using the log-rank test.[20,21] Independent risk factors associated with malignancies were studied using the stepwise Cox regression analysis.[22] The following variables were analyzed for potential covariates for incidence of primary outcome: (1) age, sex, T2DM, and hypertension at the initiation time of follow-up; (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy; (3) average value of body mass index, aspartate aminotransferase, alanine aminotransferase, triglyceride, total cholesterol, and platelet count during follow-up; (4) sum value of smoking and alcohol before, during, and after the IFN therapy; and (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A $P < 0.05$ was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Patient Characteristics. Table 1shows the baseline characteristics of the 4,302 enrolled patients at initiation of follow-up. The patients were divided into three groups: with HCC, with malignancies other than HCC, and without events. There were significant differences in several baseline characteristics among the three groups. The SVR rate was 34.4% (985/2,861) in IFN monotherapy and 63.5% (915/1,441) in combination therapy of IFN and ribavirin. Thus, the number of patients with SVR was 1,900. The mean follow-up was 8.1 (SD 5.0) years.

Development and Breakdown of Malignancies. As shown in Table 1, 606 of 4,302 patients developed malignancies: 393 developed HCC and 213 developed malignancies other than HCC. HCC accounted for 33.3% (44/132) of malignancies in patients with SVR and 73.6% (349/474) in patients without SVR. The breakdown of malignancies other than HCC was as follows: stomach cancer, n = 36; colon cancer, n = 35; lung cancer, n = 20; malignant lymphoma, n = 19; pancreatic cancer, n = 12; prostatic cancer, n = 16; breast cancer, n = 15; other cancers, n = 60.

Predictive Factors for the Development of HCC. The cumulative development rate of HCC was 4.3% at 5 years, 10.5% at 10 years, 19.7% at 15 years, and 28.0% at 20 years (Fig. 1A). The factors associated with the development of HCC are shown in Table 2. Multivariate Cox proportional hazards analysis

Table 2. Predictive Factors for Development of HCC in Enrolled Patients

	Univariate Analysis		Cox Regression Analysis	
Variable	HR (95% CI)	P	HR (95% CI)	P
Age, years (per 10)	1.84 (1.64-2.06)	<0.001	1.97 (1.71-2.28)	<0.001
Sex, male/female	1.47 (1.18-1.83)	<0.001	1.67 (1.24-2.23)	0.001
BMI, ≥22/<22	1.37 (1.12-1.66)	0.002		
T2DM, +/−	2.77 (2.13-3.60)	<0.001	1.73 (1.30-2.30)	<0.001
Hypertension, +/−	1.32 (1.02-1.71)	0.036		
Smoking index, ≥20/<20*	1.43 (1.14-1.79)	0.002		
TAI, kg, ≥200/<200*	2.13 (1.74-2.61)	<0.001	1.45 (1.11-1.88)	0.007
AST, IU/L, ≥34/<34	3.00 (2.40-3.89)	<0.001		
ALT, IU/L, ≥36/<36	2.74 (2.16-3.42)	<0.001		
GGT, IU/L, ≥109/<109	1.79 (1.19-2.46)	0.039		
Albumin, g/dL, <3.9/≥3.9	1.92 (1.37-2.55)	0.015		
Triglyceride, mg/dL, ≥100/<100	1.14 (0.94-1.37)	0.179		
Cholesterol, mg/dL, <150/≥150\	1.38 (1.10-1.72)	0.004		
Platelet count, $\times 10^4/mm^3$, <15/≥15)	3.27 (2.56-4.17)	<0.001		
Histological diagnosis, LC/non-LC	7.09 (5.59-9.01)	<0.001	5.01 (3.92-6.40)	<0.001
Combination of ribavirin, +/−	0.66 (0.45-0.97)	0.033		
Type of IFN, α/β	1.10 (0.85-1.41)	0.474		
Total dose of IFN, MU, ≥500/<500	1.12 (0.91-1.38)	0.291		
HCV genotype, ½	1.67 (1.30-2.14)	<0.001		
HCV-RNA, log IU/mL, ≥5/<5	1.02 (0.98-1.05)	0.315		
Efficacy, non-SVR/SVR	4.78 (3.47-6.59)	<0.001	4.93 (3.53-6.89)	<0.001

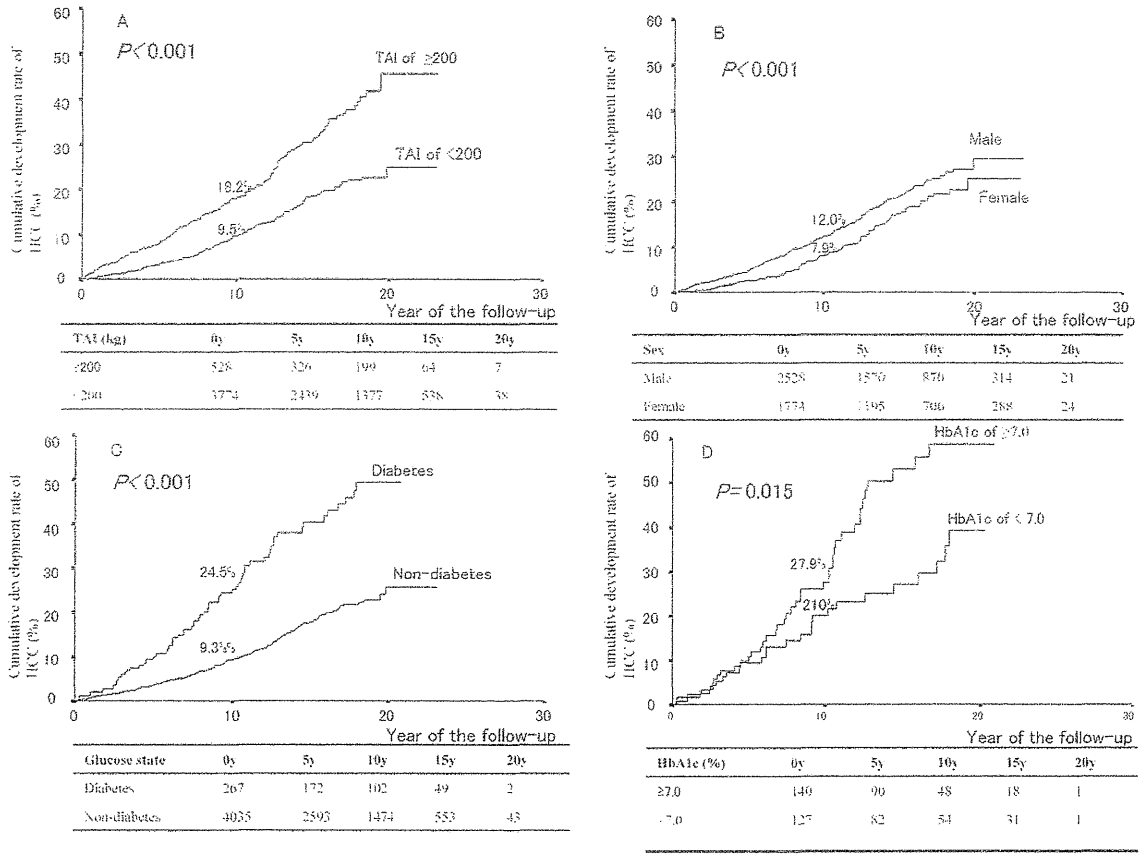
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein.

*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

showed that HCC occurred when patients had liver cirrhosis (hazard ratio [HR], 5.01; 95% confidence interval [CI], 3.92-6.40; $P < 0.001$), non-SVR (HR, 4.93; 95% CI, 3.53-6.89; $P < 0.001$), age increments of 10 years (HR, 1.97; 95% CI, 1.71-2.28; $P < 0.001$), T2DM (HR, 1.73; 95% CI, 1.30-2.30; $P < 0.001$), male sex (HR, 1.67; 95% CI, 1.24-2.23; $P = 0.001$), and TAI of ≥ 200 kg (HR, 1.45; 95% CI, 1.11-1.88; $P = 0.007$). Fig. 1B-D and Fig. 2A-C show the cumulative development rates of HCC based on difference of IFN efficacy, age, hepatic fibrosis, TAI, sex, and T2DM. The 10-year cumulative rates of HCC after IFN therapy was determined to be 7.1% in 3,869 patients with chronic hepatitis and 37.7% in 433 patients with cirrhosis by using the Kaplan-Meier Method (Fig. 1D). Fig. 2D shows the development rates of HCC in T2DM patients according to difference of mean hemoglobin A1c (HbA1c) level during follow-up. HCC decreased when T2DM patients had a mean HbA1c level of <7.0% during follow-up (HR, 0.56; 95% CI, 0.33-0.89; $P = 0.015$). The development of HCC was reduced by 44% in T2DM patients with a mean HbA1c level of <7.0% compared with those with a mean HbA1c level of ≥7.0%.

Table 3 shows the development rate of HCC and risk factors in four groups classified by the difference of hepatic fibrosis and efficacy of IFN therapy. The development rate of HCC per 1,000 person years was 1.55 in patients with chronic hepatitis (CH) at baseline and SVR (CH+SVR), 18.23 in patients with liver cirrhosis (LC) at baseline and SVR (LC+SVR), 13.53 in patients with chronic hepatitis at baseline and non-SVR (CH+non-SVR), and 50.43 in patients with LC at baseline and non-SVR (LC+non-SVR). The risk of HCC development in the CH+SVR group was advanced age, male sex, TAI of ≥200 kg, and T2DM. T2DM enhanced the development of HCC with statistical significance in three groups of CH+SVR, CH+non-SVR, and LC+non-SVR.

Predictive Factors for Development of Malignancies Other than HCC. The cumulative development rate of malignancies other than HCC was 2.4% at 5 years, 5.1% at 10 years, 9.8% at 15 years, and 18.0% at 20 years (Fig. 3A). The factors associated with the development of malignancies other than HCC are shown in Table 4. Malignancies other than HCC occurred when patients had age increments of 10 years (HR, 2.19; 95% CI, 1.84-2.62; $P < 0.001$), smoking index of ≥20 (HR, 1.89; 95% CI, 1.41-2.53; $P < 0.001$), and T2DM (HR, 1.70; 95% CI, 1.14-2.53; $P = 0.008$). Fig. 3B-D shows the cumulative development rates of malignancies other than HCC based on difference of age, smoking index, and T2DM. Fig. 3E shows the risk of malignancies other than HCC in T2DM patients according to mean HbA1c level during follow-up. The HR of HCC development in



TAI (kg)	0y	5y	10y	15y	20y
≥200	528	326	199	64	7
<200	3774	2439	1377	538	38

Sex	0y	5y	10y	15y	20y
Male	2528	1576	870	314	21
Female	1774	1195	706	288	24

Glucose state	0y	5y	10y	15y	20y
Diabetes	267	172	102	49	2
Non-diabetes	4035	2593	1474	553	43

HbA1c (%)	0y	5y	10y	15y	20y
≥7.0	140	90	48	18	1
<7.0	127	82	54	31	1

Fig. 2. Cumulative development rate of HCC based on the difference of (A) TAI, (B) sex, (C) diabetic state, and (D) mean HbA1c level during follow-up in T2DM patients.

patients with mean HbA1c level of <7.0% versus those with mean HbA1c level of ≥7.0% was 0.62 (95% CI, 0.31-1.23; $P = 0.170$). There was no significant difference in development of malignancies other than HCC based on the difference of mean HbA1c level during follow-up. Table 5 shows the impact based

Table 3. Development Rate of HCC Based on Hepatic Fibrosis and Efficacy of IFN Therapy

Variable	CH + SVR	LC + SVR	CH + Non-SVR	LC + Non-SVR
No. of patients	1,751	149	2,118	284
Age, years	51.7 ± 12.1	56.9 ± 9.8	51.5 ± 11.7	57.2 ± 9.9
Sex, male/female	1,082/669	91/58	1,190/928	165/119
HbA1c (%, NSPG)	5.5 ± 0.7	5.8 ± 0.8	5.7 ± 0.7	6.1 ± 0.8
TAI, kg	86 ± 91	104 ± 99	97 ± 90	129 ± 102
Patients with T2DM	74	13	133	47
Patients with HCC	22	22	233	116
1,000 person years of HCC	1.55	18.23	13.53	50.43
Age, years (per 10)*	2.60 (1.48-4.58)	1.83 (0.95-3.55)	2.07 (1.75-2.46)	1.09 (0.87-1.37)
P value	0.001	0.070	<0.001	0.477
Sex, male/female*	3.42 (1.01-11.63)	3.41 (1.00-11.63)	1.34 (0.99-1.81)	1.93 (1.25-3.00)
P value	0.049	0.050	0.058	0.003
TAI, kg, ≥200/<200*	2.68 (1.14-6.34)	3.84 (1.83-9.85)	2.21 (1.65-2.95)	1.54 (1.03-2.31)
P value	0.024	0.004	<0.001	0.038
T2DM, +/−*	4.76 (1.60-14.10)	2.48 (0.57-10.86)	2.53 (1.76-3.65)	1.87 (1.16-3.01)
P value	0.005	0.228	<0.001	0.010

Abbreviations: CH + Non-SVR, patients with CH at baseline and non-SVR 6 months after IFN therapy; CH + SVR, patients with CH at baseline and SVR 6 months after IFN therapy; LC + Non-SVR, patients with LC at baseline and non-SVR 6 months after IFN therapy; LC + SVR, patients with LC at baseline and SVR 6 months after IFN therapy.

*Hazard ratio (95% confidence interval) and *P* value by Cox proportional hazards analysis.

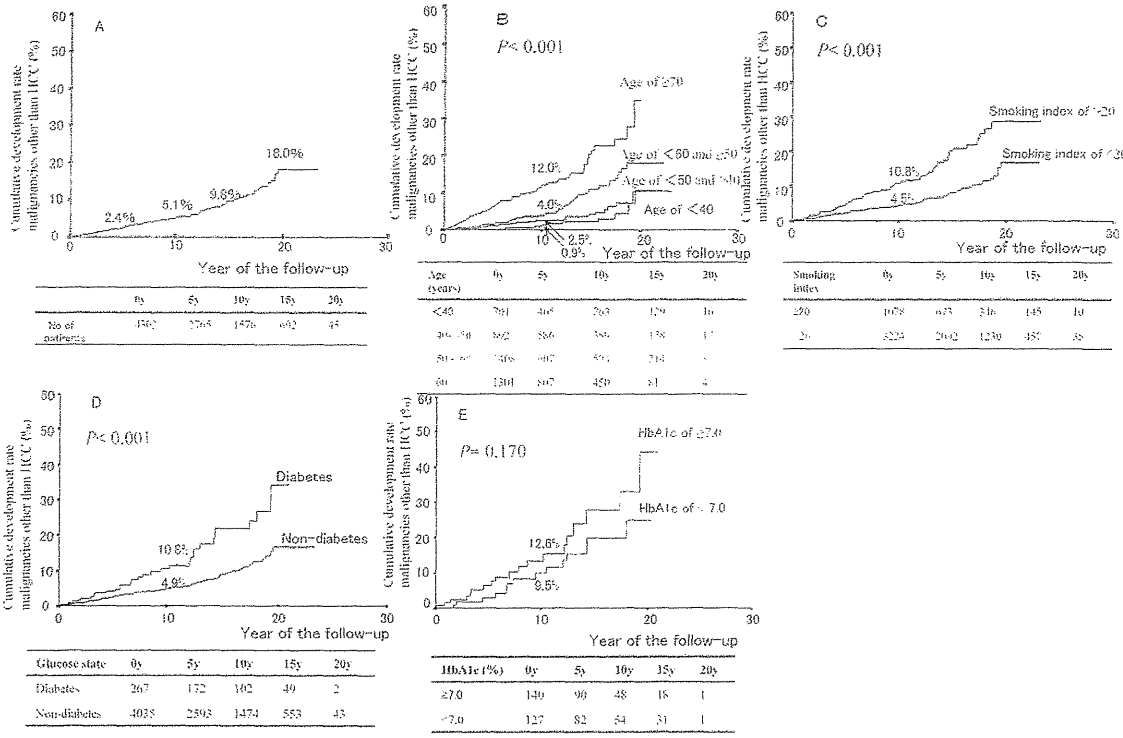


Fig. 3. Cumulative development rate of malignancies other than HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) age, (C) smoking index, (D) diabetic state, and (E) mean HbA1c level during follow-up in T2DM patients.

on three factors of age, smoking index, and T2DM for the development of each malignancy other than HCC by using Cox regression analysis. Aging enhanced carcinogenesis of stomach, colon, lung, prostate, breast, and pancreas with statistical significance. Smoking enhanced lung cancer and colorectal cancer

Table 4. Predictive Factors for Development of Malignancies Other than HCC

	Univariate Analysis		Cox-Regression Analysis	
Variables	HR (95% CI)	P	HR (95% CI)	P
Age, years (per 10)	2.23 (1.88-2.65)	< 0.001	2.19 (1.84-2.62)	<0.001
Sex, male/female	1.06 (0.79-1.40)	0.759		
BMI, ≥22/<22	0.97 (0.75-1.24)	0.767		
T2DM, +/−	2.56 (1.76-3.72)	<0.001	1.70 (1.14-2.53)	0.008
Hypertension, +/−	2.33 (1.70-3.18)	<0.001		
Smoking index, ≥20/<20*	2.74 (2.06-3.65)	<0.001	1.89 (1.41-2.53)	<0.001
TAI, kg, ≥200/<200*	1.77 (1.33-2.37)	<0.001		
AST, IU/L, ≥34/<34	0.89 (0.65-1.20)	0.412		
ALT, IU/L, ≥36/<36	0.98 (0.72-1.34)	0.891		
GGT, IU/L, ≥109/<109	1.26 (0.79-2.01)	0.350		
Albumin, g/dL, <3.9/≥3.9	1.41 (0.90-2.04)	0.145		
Triglyceride, mg/dL, ≥100/<100	1.28 (1.03-1.60)	0.030		
Total cholesterol, mg/dL, <150/≥150	1.10 (0.82-1.46)	0.548		
Platelet count, × 10^4/mm^3, <15/≥15	1.39 (1.02-1.91)	0.038		
Histological diagnosis, LC/non-LC	1.77 (1.13-2.75)	0.012		
Combination of ribavirin, +/−	0.66 (0.44-0.97)	0.034		
Type of IFN, α/β	1.05 (0.75-1.47)	0.789		
Total dose of IFN, MU, ≥500/<500	1.31 (0.96-1.77)	0.084		
HCV genotype, ½	1.30 (0.80-2.93)	0.432		
HCV RNA, log IU/mL, ≥5/<5	0.89 (0.50-1.23)	0.612		
Efficacy, non-SVR/SVR	0.85 (0.64-1.12)	0.232		

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase.
*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

Table 5. Impact Based on Age, Smoking Index, and Diabetes for Development of Malignancies Other than HCC

	Age, Years (per 10)		Smoking Index, ≥20/<20		Diabetes, +/−	
Malignancy	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Gastric cancer (n = 36)	2.48 (1.62-3.78)	<0.001	1.69 (0.83-3.43)	0.146	2.29 (0.95-5.52)	0.065
Colorectal cancer (n = 35)	1.91 (1.28-2.86)	0.002	2.27 (1.13-4.58)	0.022	1.78 (0.68-4.66)	0.240
Lung cancer (n = 20)	2.33 (1.35-4.01)	0.002	2.90 (1.25-6.74)	0.013	1.53 (0.45-5.24)	0.496
Prostatic cancer (n = 16)	2.84 (1.32-6.13)	0.008	1.89 (0.88-3.15)	0.266	0.71 (0.09-5.47)	0.735
Breast cancer (n = 15)	2.86 (1.30-6.29)	0.009	1.29 (0.17-10.19)	0.808	1.20 (0.16-9.39)	0.859
Malignant lymphoma (n = 19)	2.21 (1.26-3.88)	0.006	1.25 (0.44-3.56)	0.671	1.39 (0.32-6.12)	0.663
Pancreatic cancer (n = 12)	3.32 (1.44-7.65)	0.005	1.41 (0.45-4.82)	0.578	3.75 (1.02-13.88)	0.046

with statistical significance. In addition, T2DM enhanced the pancreatic cancer with statistical significance and tended to enhance the gastric cancer.

Discussion

This study describes the development incidence of HCC or malignancies other than HCC after the termination of IFN therapy in HCV patients. Patients at Toranomon Hospital comprised mainly government employees, office workers, and business persons. Most patients were regularly recommended to undergo annual multiphasic health screening examinations. In the present study, patients who had undergone annual multiphasic health screening examinations were enrolled. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the development incidence and predictive factors for total malignancies after IFN therapy for HCV patients. First, the 10-year cumulative rates of HCC after IFN therapy was determined to be 7.1% in 3,869 patients with chronic hepatitis and 37.7% in 433 patients with cirrhosis using the Kaplan-Meier method. Our previous studies showed via retrospective analysis that the 10-year cumulative rates of HCC were 12.4% for 456 patients with chronic hepatitis and 53.2% for 349 patients with cirrhosis.[7,23] Although patient selection bias for IFN treatment versus no treatment had been noted in the previous studies, the results suggest the possibility that IFN therapy reduces the development of HCC in HCV patients. Several historical data in Japan suggest that IFN therapy reduces the development of HCC in HCV patients.[24-26]

Second, HCC occurred with statistical significance when the following characteristics were present: non-SVR, advanced age, cirrhosis, TAI of ≥200 kg, male sex, and T2DM. T2DM caused a 1.73-fold enhancement in HCC development. Several authors have reported an increased risk of HCC among patients with the following characteristics: non-SVR, cirrhosis, male sex, advanced age, and T2DM.[24-28] Our results show that physicians in charge of aged male patients with non-SVR, advanced fibrosis, TAI of ≥200 kg, and T2DM should pay attention to the development of HCC after IFN therapy. In addition, maintaining a mean HbA1c level of <7.0% during follow-up reduced the development of HCC. This result indicates that stringent control of T2DM is important for protecting the development of HCC.

Third, the development rate of HCC per 1,000 person years was about 1.55 in 1,751 patients with chronic hepatitis at baseline and SVR. In these patients, the risk factors associated with HCC were advanced age, male sex, TAI, and T2DM. We compared the HCC development rate in patients with chronic hepatitis at baseline and SVR to the general population. A total of 5,253 individuals without HCV antibody and hepatitis B surface antigen, who underwent annual multiphasic health screening examinations in our hospital were evaluated as controls. Individuals with either of the following criteria were excluded: (1) illness that could seriously reduce their life expectancy or (2) history of carcinogenesis. They were selected by matching 3:1 with patients who had chronic hepatitis at baseline and SVR for age, sex, T2DM, and follow-up periods. In control individuals, the mean age was 51.7 years; the prevalence (number) of male patients was 61.8% (3,246); the prevalence (number) of T2DM patients was 4.2% (222); the mean follow-up period was 8.0 years. The number of development of HCC in control individuals was only five. This result suggests that the development rate of HCC in patients with chronic hepatitis at baseline and SVR is higher than that in the general population.

Fourth, HCC accounted for 33.3% in SVR patients and 73.6% in non-SVR patients. According to Matsuda et al.,[29] the outbreak of malignancies in the Japanese male population was observed in the following order in 2005: gastric cancer 20.4% > colon

cancer 16.0% > lung cancer 15.4% > prostate cancer 10.9% > HCC 7.4%. On the other hand, the outbreak of malignancies in the Japanese female population was observed in the following order in 2005: breast cancer 18.0% > colon cancer 16.2% > gastric cancer 13.6% > lung cancer 9.3% > uterine cancer 6.8%. Our results show that HCC is the most common cause of malignancy, not only in the non-SVR group but also in the SVR group.

Finally, malignancies other than HCC occurred with statistical significance when patients were of advanced age, were smokers, and had T2DM. Our result indicates that smoking enhances lung cancer and colorectal cancer. Many authors have reported that smoking is a direct cause of cancers of the oral cavity, esophagus, stomach, pancreas, larynx, lung, bladder, kidney, and colon.[30,31] In addition, the present study indicates that T2DM enhances pancreatic cancer with statistical significance and tends to enhance gastric cancer. T2DM showed up to about 1.7-fold increase in development of malignancies other than HCC. A recent meta-analysis of cohort studies have revealed that diabetic patients increase risk of pancreatic cancer, HCC, bladder cancer, non-Hodgkin's lymphoma, colorectal cancer, and breast cancer.[32-39]

Although the role of T2DM in carcinogenesis remains speculative, the following possible mechanisms have been reported: (1) hyperglycemia increases malignancy risk via increasing oxidative stress and/or activating the rennin-angiotensin system[40]; (2) insulin resistance increases malignancy risk via down-regulation of serine/threonine kinase II to adenosine monophosphate–activated protein kinase pathway[41]; (3) reduced insulin secretion increases malignancy risk via down-regulation of sterol regulatory element-binding protein-1c with consequent up-regulation of insulin-like growth factor.[42]

T2DM is increasing dramatically worldwide over the past decades.[8] It is estimated that about 7 million people are affected by diabetes mellitus in Japan. Approximately 8%-10% of adults in Japan have T2DM. The risk factors associated with T2DM include family history, age, sex, obesity, smoking, physical activity, and HCV.[43-46] In the near future, T2DM will be increasing in HCV-positive patients.

This study is limited in that it was a retrospective cohort trial. Another limitation is that patients were treated with different types of antivirus therapy for different durations. In addition, T2DM patients were treated with different types of drugs during follow-up. Finally, our cohort contains Japanese subjects only. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

In conclusion, T2DM causes an approximately 1.7-fold enhancement in the development of HCC and malignancies other than HCC after IFN therapy. Additionally, in T2DM patients, maintaining a mean HbA1c level of <7.0% during follow-up reduced the development of HCC.

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References

1. Kiyosawa K, Furuta S. Review of hepatitis C in Japan. J Gastroenterol Hepatol 1991;6:383-391.
2. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, et al. The natural history of community acquired hepatitis C in the United States. N Engl J Med 1992;327:1899-1905.
3. Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989;2:1006-1008.
4. Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, et al. Hepatitis C-associated hepatocellular carcinoma. HEPATOLOGY 1990;12:589-591.
5. Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. Lancet 1990;335:873-874.
6. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797-1801.
7. Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. HEPATOLOGY 1993;18:47-53.
8. Waki K, Noda M, Sasaki S, Matsumura Y, Takahashi Y, Isogawa A, et al.; JPHC Study Group. Alcohol consumption and other risk factors for self-reported diabetes among middle-aged Japanese: a population-based prospective study in the JPHC study cohort I. Diabet Med 2005;22:323-331.
9. Yasuda K. Early gastric cancer: diagnosis, treatment techniques and outcomes. Eur J Gastroenterol Hepatol 2006;18:839-845.
10. Van Cossum A. Guidelines for colorectal cancer screening a puzzle of tests and strategies. Acta Clin Belg 2010;65:433-436.
11. Currie GP, Kennedy AM, Denison AR. Tools used in the diagnosis and staging of lung cancer: what's old and what's new? QJM 2009;102:443-448.
12. Maresh EL, Mah V, Alavi M, Horvath S, Bagryanova L, Liebeskind ES, et al. Differential expression of anterior gradient gene AGR2 in prostate cancer. BMC Cancer 2010;10:680.
13. Calle EE, Rodriquez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Eng J Med 2003;348:1625-1638.
14. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-1392.
15. Cascinu S, Falconi M, Valentini V, Jelic S; ESMO Guidelines Working Group. Pancreatic cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21(Suppl. 5):v55-v58.
16. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Suppl. 1):S62-S69.

17. Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. HEPATOLOGY 1994; 19:13-18.
18. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. HEPATOLOGY 1994;19:1513-1520.
19. Fleming TR, Harrington DP, O'Brien PC. Designs for group sequential tests. Control Clin Trials 1984;5:348-361.
20. Harrington DP, Fleming TR. A class of rank test procedures for censored survival data. Biomedaica 1983;62:205-209.
21. Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. J Am Stat Assoc 1958;53:457-481.
22. Cox DR. Regression models and life tables. J R Stat Soc 1972;34: 248-275.
23. Ikeda K, Saitoh S, Arase Y, K Chayama, Y Suzuki, M Kobayashi, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C; A long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. HEPATOLOGY 1999;29:1124-1130.
24. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for heparocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. HEPATOLOGY 1998:2;1394-1402.
25. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 1998:129;94-99.
26. Yoshida H, Arakawa Y, Sata M, Nishiguchi S, Ya M, Fujiyama S, et al. Interferon therapy prolonged life expectancy among chronic hepatitis patients. Gastroenterology 2002:123;483-491.
27. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. HEPATOLOGY 2008;47: 1856-1862.
28. Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, et al. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. HEPATOLOGY 2010;52:518-527.
29. Matsuda T, Marugame T, Kamo KI, Katanoda K, Ajiki W, Sobue T. The Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2005: based on data from 12 population-based cancer registries in the Monitoring of Cancer Incidence in Japan (MCIJ) Project. Jpn J Clin Oncol 2011;41:139-147.
30. Boyle P. Cancer, cigarette smoking and premature death in Europe: a review including the Recommendations of European Cancer Experts Consensus Meeting, Helsinki, October 1996. Lung Cancer 1997;17:1-60.
31. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA 2008: 300:2765-2778.
32. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A metaanalysis. JAMA 1995;273:1605-1609.
33. El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol 2006:4:369-380.
34. Larsson SC, Orsini N, Brismar K, Wolk A. Diabetes mellitus and risk of bladder cancer: a meta-analysis. Diabetologia 2006:49:2819-2823.
35. Mitri J, Castrillo J, Pittas AG. Diabetes and risk of non-Hodgkin's lymphoma: a metaanalysis of observational studies. Diabetes Care 2008:31: 2391-2397.
36. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a metaanalysis. J Natl Cancer Inst 2005;97:1679-1687.
37. Giovannucci E. Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. Am J Clin Nutr 2007;86:836S-842S.
38. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a metaanalysis. Int J Cancer 2007;121:856-862.
39. Hsing AW, Gao Y-T, Chua S, Deng J, Stanczyk FZ. Insulin resistance and prostate cancer risk. J Natl Cancer Inst 2003;95:67-71.
40. George AJ, Thomas WG, Hannan RD. The renin-angiotensin system and cancer: old dog, new tricks. Nat Rev Cancer 2010;10:745-759.
41. Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. Nat Rev Cancer 2009;9: 563-575.
42. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331-340.
43. Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, et al. Hepatitis C virus infection and incident type 2 diabetes. HEPATOLOGY 2003;38:50-56.
44. Imazeki F, Yokosuka O, Fukai K, Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. Liver Int 2008;28:355-362.
45. Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, et al. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. HEPATOLOGY 2009;49:739-744.
46. Thuluvath PJ, John PR. Association between hepatitis C, diabetes mellitus, and race. a case-control study. Am J Gastroenterol 2003;98: 438-441.

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＜特別寄稿＞

核酸アナログ薬中止に伴うリスク回避のための指針2012
—厚生労働省「B型肝炎の核酸アナログ薬治療における治療中止基準の作成と治療中止を目指したインターフェロン治療の有用性に関する研究」の報告—

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索引用語：　核酸アナログ薬　治療中止　B型肝炎　肝炎再燃
HBV cccDNA

はじめに

近年B型肝炎の治療に導入された核酸アナログ薬はHBVの増殖を強力に抑制するため，多くの症例で血中HBV DNA量は速やかに低下しALT値の正常化がもたらされる[1)]．さらに，組織学的な改善が得られ肝発癌率が低下することや[2)3)]，経口薬で副作用も少ないことから臨床的に広く使用されている．しかし，核酸アナログ薬を使用してもウイルスを完全に排除することは困難であり，本治療薬には耐性株の出現や治療中止に伴う肝炎の再燃が問題点として残されている[4)]．この原因の一つとして，血中のHBV DNA量が低下しても，HBV複製の起源となる肝細胞核内のHBV cccDNA量はほとんど減らず，これが長期に残存することが挙げられている[5)]．

B型肝炎の核酸アナログ薬治療において，同薬の中止はしばしば肝炎の再燃を伴うため，安易な中止はすべきでないとされている．しかし，中止後，いつ頃どの様な形で肝炎が再燃するかは必ずしも明らかにされてはいない．また，中止後に肝炎が再燃しない症例や再燃しても軽度で最終的に安定化する症例も少なからず存在するが，この様な症例を効率よく見分ける方法も確立されていない．

我々は，厚生労働省の科学研究費により「B型肝炎の核酸アナログ薬治療における治療中止基準の作成と治療中止を目指したインターフェロン治療の有用性に関する研究」（平成21年度～23年度）を行い，治療中止後の経過の特徴や肝炎再燃の定義，さらには再燃率の予測を検討した．本稿では，この研究成果を元に「核酸アナログ薬中止に伴うリスク回避のための指針2012」をまとめたので報告する（Table 1）．本指針は必ずしも核酸アナログ薬の中止を推奨するものではなく，様々な理由により中止を検討する必要がある場合の参考になるよう定めた．

I．本指針の目指すもの

本指針は，核酸アナログ薬の中止を検討する際に，中止成功の可能性が高い症例や逆に治療を継続すべき症例を明らかにすること，さらに，中止後の経過観察

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Table 1　核酸アナログ薬中止に伴うリスク回避のための指針　2012

I. 本指針の目指すもの

B型慢性肝炎の核酸アナログ薬治療において，同薬の中止により drug free を目指すことは重要な治療目標の一つである．しかし，同薬の中止によりしばしば肝炎が再燃し，時に重症化することがある．このため，中止に際してはその危険性に十分配慮する必要がある．

核酸アナログ薬治療は HBs 抗原の陰性化を目標とするが，必ずしも容易ではない．このため，HBs 抗原が陰性化しなくても治療の中止を考慮する場合がある．本指針は，この様な状況下で核酸アナログ薬を中止し，最終的に非活動性キャリアの状態（ALT＜30 IU/L かつ血中 HBV DNA＜4.0 log copies/ml）に落ち着くことを目標として作成した．

核酸アナログ薬の中止と継続のどちらが生命予後や肝発癌に対して有利かは現在のところ明らかではない．このため，本指針は様々な理由により中止を検討する必要がある場合の参考になるよう定めた．この際，中止成功の可能性が高い症例を見いだすことや逆に治療を継続すべき症例を明らかにすること，さらに，中止後の経過観察の指標を設定することにより，核酸アナログ薬中止に伴うリスクを極力回避することを目指した．

II. 肝炎再燃に伴う重症化のリスクを回避するための必要条件

重症化のリスクをあらかじめ想定し，これを回避するため，以下を中止の必要条件とした．

1. 核酸アナログ薬中止後には肝炎再燃が高頻度にみられ，時に重症化する危険性があることを主治医，患者共に十分理解している．
2. 中止後の経過観察が可能であり，再燃しても適切な対処が可能である．
（専門医が関与することが推奨される．）
3. 肝線維化が軽度で肝予備能が良好であり，肝炎が再燃した場合でも重症化しにくい症例である．
（肝硬変やこれに近い線維化の進行した慢性肝炎の症例では中止すべきでない．）

III. HBV 増殖能の評価と再燃のリスクを低下させるための条件

1. 核酸アナログ薬中止の必要条件

HBV 増殖能が高い症例では中止後の再燃はほぼ必発である．この様な症例で中止を行わないことが肝要であり，このための必要条件を以下に示す．

中止の必要条件
◇中止時，血中 HBV DNA（リアルタイム PCR 法）が陰性．
◇中止時，血中 HBe 抗原が陰性．

2. 核酸アナログ薬治療期間の条件

核酸アナログ薬治療期間が短いと再燃しやすいため，以下の条件を満たすことが望ましい．

治療期間の条件
◇核酸アナログ薬投与開始後2年以上経過している．

3. ウイルス抗原量のスコア化による再燃の危険性の評価

中止の必要条件（中止時 HBV DNA 陰性かつ HBe 抗原陰性）を満たす症例について，中止時の HBs 抗原量と HB コア関連抗原量をスコア化し，合計スコアから再燃のリスクを以下の3群に分けて予測することが可能である．この予測リスクを参考に中止の可否を決定することにより再燃のリスクを低下させることを目指す．

中止時 HBs 抗原量	スコア	中止時 HB コア関連抗原量	スコア
1.9 log IU/ml 未満（80 IU/ml 未満）	0	3.0 log U/ml 未満	0
1.9 ～ 2.9 log IU/ml（80 ～ 800 IU/ml）	1	3.0 ～ 4.0 log U/ml	1
2.9 log IU/ml 以上（800 IU/ml 以上）	2	4.0 log U/ml 以上	2

再燃リスク	総スコア	予測成功率	評価
低リスク群	0	80-90%	中止を考慮しても良い群．ただし，低リスク群でも肝炎再燃症例が存在するため，再燃に対する注意は必須である．
中リスク群	1-2	約 50%	状況によって中止を考慮しても良い群．この群では，中止の条件や方法を今後さらに検討する必要がある．
高リスク群	3-4	10-20%	治療の継続が推奨される群．ただし，35 歳未満では中止成功例が比較的高く 30-40% である．．

Table 1　核酸アナログ薬中止に伴うリスク回避のための指針　2012

Ⅳ. 中止後の経過観察方法と再治療開始の条件

1. 核酸アナログ薬中止後は定期的にHBV DNA（リアルタイムPCR法）とALTを測定し，HBVの再増殖とこれに伴う肝炎再燃に注意を払う．
2. 中止後の再燃は，中止直後から1年以内が多く，その後徐々に減少し，3年目以降はまれになる．このため，特に中止直後は再燃に対する注意が必要である．具体的には，中止後16週までは2週毎，その後は4週毎の血液検査による経過観察が望ましい．
3. 中止が成功し，最終的に非活動性キャリア状態に落ち着く症例においても，約2/3では一過性のALTまたはHBV DNAの異常値が出現する．このため，中止後の経過観察でALTまたはHBV DNAの異常値が出現しても，軽度の上昇であれば再治療を行わずに経過をみることが可能である．ただし，以下の条件では，最終的に非活動性キャリア状態に落ち着く可能性は低く，核酸アナログ薬による再治療を考慮する．

核酸アナログ薬の再投与を考慮する条件
◇中止後ALT≧80 IU/LまたはHBV DNA≧5.8 log copies/ml となる場合

Ⅴ. 注意点と今後の課題

1. 患者の状況は個々に異なる．また，中止の目的や意義も個々に異なるため，実際に中止するか否かの判断は，これらの条件を考慮し主治医が行う．また，中止を考慮する場合は肝臓専門医に相談することが推奨される．
2. 核酸アナログ薬中止後に肝炎が再燃し再投与した場合，中止しなかった場合と比較し核酸アナログ薬耐性株の出現頻度が増加するか否かについては不明である．
3. HBVキャリアでは非活動性キャリア期（HBV DNAが4.0 log copy/ml未満かつALTが30 IU/L未満）となってもまれに肝炎の再燃がみられるので，中止に成功してもキャリアとしての経過観察は継続する必要がある．また，肝発癌に関しても同様に経過観察が必要である．
4. 今後の検討課題としては，核酸アナログ薬中止基準の精度をさらに高めること，本指針で用いた基準を前向き検討で検証すること，インターフェロン併用によるシークエンシャル療法で核酸アナログ薬を積極的に中止しようとする方法の検討などが挙げられる．

の指標を設定することにより，核酸アナログ薬中止に伴うリスクを極力回避することを目指して作成した（Table 1-Ⅰ）．ここでの中止成功は，最終的に非活動性キャリアの状態，すなわちALTが30 IU/L未満かつ血中HBV DNAが4.0 log copies/ml未満に落ち着くこととした．この基準は日本のB型慢性肝炎治療ガイドラインに準拠して設定したが，このような非活動性キャリア状態になると肝病変の進行はなく発癌率も低下することが知られており[6][7]，適切なものと考えられる．

Ⅱ. 肝炎再燃に伴う重症化のリスクを回避するための必要条件

現状では，核酸アナログ薬中止後の肝炎再燃を十分高い確率で予測することはできない．このため，重症化の危険性[3]が存在することを想定し，重症化防止のための必要条件を設定した（Table 1-Ⅱ）．肝炎再燃や重症化の危険性を主治医と患者が共に理解していること，さらに，中止後の経過観察体制があり，再燃しても適切な対処が可能であることは当然の条件と考えられる．また，肝硬変やこれに近い線維化の進行した慢性肝炎症例では重症化しやすいこと，さらには将来的に発癌の危険性が高いことを考慮すると，現状では安易に中止すべきでないと判断した．

Ⅲ. HBV増殖能の評価と再燃のリスクを低下させるための条件

これまでも，核酸アナログ薬中止時にHBV DNAが十分低下しない症例またはHBe抗原陽性の症例では中止後に肝炎が高率再燃することが経験されていたが，本研究班の検討でもこれが科学的に確認された[9]．肝炎再燃を予測するHBV DNA量のcut-off値はROC解析で3.0 log copies/mlであり，これ以上の症例ではほとんど全例が1年以内に再燃したのに対し，3.0 log copies/ml未満の症例では長期に安定化する症例が30%近く存在した．さらに，HBV DNA量が3.0 log copies/ml未満の症例に限った場合，HBe抗原陽性例は1年以内に90%以上が再燃したのに対し，HBe抗原陰性例では長期に安定化する症例が少なからず存在した．この結果から，HBV DNA量の十分な低下とHBe抗原の陰性化は中止の必要条件として設定した．ここで，HBV DNA量の十分な低下の基準値については，実際の指針では3.0 log copies/ml未満ではなく，安全を考慮してリアルタイム

PCR法で陰性であることとした．

明らかに中止後の肝炎再燃が予測される症例，すなわち，核酸アナログ薬中止時にHBV DNA量が3.0 log copies/ml以上またはHBe抗原陽性の症例を除いて中止後の肝炎再燃と関連する因子をさらに解析すると，核酸アナログ薬治療期間，中止時HBs抗原量，中止時HBコア関連抗原量が有意な因子として算出された[9]．治療期間のcut-off値は16カ月と算出されたため，本指針では余裕をもって2年以上経過していることが望ましいとの条件を設定した．

中止時のHBs抗原量とHBコア関連抗原量については，ROC解析の結果からそれぞれ2つのcut-off値の存在が示唆され，HBs抗原量は1.9と2.9 log IU/ml，HBコア関連抗原量は3.0と4.0 log U/mlであった[9]．この事から，Table 1-IIIに示す如くHBs抗原量とHBコア関連抗原量をスコア化し，総スコアから低リスク群，中リスク群，高リスク群の3群を設定した．それぞれの予測成功率は低リスク群が80～90%，中リスク群が約50%，高リスク群が10～20%であった．各群の中で肝炎再燃と関連する因子をさらに検討すると，低リスク群と中リスク群では新しい因子はなかったが，高リスク群では年齢が有意な因子であった．すなわち，予測成功率が10～20%と低い高リスク群であっても，年齢が35歳未満ではこの成功率がやや高く30～40%であった．

以上の如く，治療期間やウイルスマーカーの結果から核酸アナログ薬中止後の経過を予測することが可能であり，治療中止を計画する際の指標となると考えられた．近年，HBs抗原量の測定は新しいマーカーとして注目されており，インターフェロン治療効果の予測などに有用なことが報告されている[10][11]．一方，HBコア関連抗原量は核酸アナログ薬使用下においても肝細胞核内のHBV cccDNA量を反映することが報告され[12]～[14]，この量が中止後の肝炎再燃と関連することはこれまでも報告されていた[15][16]．今回，これらの抗原量の組み合わせが中止の指針作成に有用であった点は興味深い[9]．

Ⅳ．中止後の経過観察方法と再治療開始の条件

核酸アナログ薬中止後の経過観察は，定期的にHBV DNA量（リアルタイムPCR法）とALT値を測定することにより行う．中止後の再燃は，中止直後から1年以内が多く，その後徐々に減少し，3年目以降はまれになることが今回の研究で明らかになった[9]．このため，特に中止直後は再燃に対する注意が必要であると判断した．具体的には，中止後16週までは2週毎，その後は4週毎の血液検査による経過観察が望ましいとした．

肝炎再燃をどのように定義し，中止後の経過観察をどのように行うかは本指針の要点の一つである．最終的に非活動性キャリア状態に落ち着く症例においても，約2/3では一過性のALTまたはHBV DNAの異常値が出現する．このため，中止後の経過観察でALTまたはHBV DNAの異常値が出現しても，軽度の上昇であれば再治療を行わずに経過をみることが可能である．しかし，どこまでなら経過をみて良いのかの基準はこれまで明らかにされていない．この点を明らかにするため我々は，核酸アナログ薬中止後のALT値とHBV DNA量の推移を平均値と最高値で評価した．この結果，両者とも平均値と最高値の間にきわめて強い相関があることが明らかになった[9]．ROC解析の結果より，平均ALT値の30 IU/Lは最高ALT値の79 IU/Lに，一方，平均HBV DNA量の4.0 log copies/mlは最高HBV DNA量の5.7 log copies/mlに相当することが明らかになった．すなわち，中止後にALT値が80 IU/L以上になる場合は平均値が30 IU/Lを超える可能性が高く，最終的に中止成功の基準を満足しないことが予測される．同様に，中止後のHBV DNA量が5.8 log copies/ml以上となる場合は平均値が4.0 log copies/mlを超える可能性が高く，中止成功の基準を満足しないことが予測される．これらの結果より，中止後にALT値が80 IU/L以上，またはHBV DNA量が5.8 log copies/ml以上となる場合は最終的に非活動性キャリア状態に落ち着く可能性は低く，核酸アナログ薬による再治療を考慮するとする条件を設けた．この条件設定により，より効率的で具体的な中止が可能になると考えられる．安全を考慮し，主治医の判断でこの基準をより厳しく設定することは可能である．逆に，この基準を緩く設定することも可能であるが，その場合は漫然と経過観察は行わず，何らかの方針を立てて対処することが望ましい．

Ⅴ．注意点と今後の課題

核酸アナログ薬中止の指針についてはこれまで本格的なものはなく，その意味で本指針は初めてのものとも言える．しかし，多くは後向きの検討データを基に作成したものであり，まだ不明な点も多く残されている．そのため，注意点や今後の課題を一つの項目としてまとめた（Table 1-V）．本指針では核酸アナログ薬の中止に関する判断材料を提供したが，実際に中止す