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Characteristics of elderly hepatitis C virus-associated hepatocellular carcinoma patients

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Key words
alanine aminotransferase (ALT), alpha-fetoprotein (AFP), average integration value of ALT, elderly patient, hepatitis C virus (HCV), hepatocellular carcinoma (HCC), platelet count, propensity score.

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Abstract

Background and Aim: The average age of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) patients has been rising in Japan. We evaluate characteristics of HCV-positive patients who develop HCC in older age to determine an optimal surveillance strategy.

Methods: A total of 323 patients with three or more years of follow-up before HCC diagnosis and 323 propensity-matched controls without HCC were studied. HCC patients were classified into four groups according to age at the time of HCC diagnosis: group A (≤ 60 years, $n = 36$), group B (61–70 years, $n = 115$), group C (71–80 years, $n = 143$), and group D (> 80 years, $n = 29$). Clinical and laboratory data were compared.

Results: Platelet counts were significantly higher in the older groups at HCC diagnosis ($P < 0.0001$). The rate of platelet counts decline was lower in older groups ($P = 0.0107$). The average integration value of serum alanine aminotransferase (ALT) in groups A, B, C, and D were 80.9 IU/L, 62.3 IU/L, 59.0 IU/L, and 44.9 IU/L, respectively ($P < 0.0001$). In older patients (≥ 65 years old), cirrhosis and average integration value of ALT were significantly associated with hepatocarcinogenesis, but platelet count was not.

Conclusion: Elderly HCV-positive patients (≥ 65 years old) with low ALT values developed HCC regardless of their platelet counts. These findings should be taken into account when designing the most suitable HCC surveillance protocol for this population.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies, particularly in southern and eastern Asia. In Japan, HCC is the third leading cause of cancer death in men, behind lung and stomach cancer. In women, HCC is the fifth leading cause of cancer death during the past decade, behind colon, stomach, lung, and breast cancer.[1] Hepatitis C virus (HCV) infection accounts for approximately 75–80% of cases. Each year, HCC develops in 6–8% of patients with HCV-associated cirrhosis.[2]

In Japan, screening the blood supply for HCV, which commenced in November 1989 and began using second-generation enzyme immunoassays in February 1992, decreased the risk of post-transfusion hepatitis from more than 50% in the 1960s to virtually zero presently.[3] The age of Japanese patients diagnosed with HCC has been steadily increasing. Up to 1999, the majority of HCC mortalities occurred in patients under 69 years of age, but in 2000 more than half of HCC patients were over the age of 70.[1] This aging trend is also observed in HCV patients undergoing interferon-based therapy in Japan.[4] In contrast, HCV infection in the United States and other western countries is most prevalent among persons 30 to 50 years of age,[5] and the incidence of HCV-associated HCC is expected to rise. As a country with more experience with HCV-associated HCC, Japan's long-term experience can be helpful in planning strategies to contain HCV infection and to cope with its long-term sequelae worldwide.

The aim of this study is to evaluate characteristics of HCV-positive patients who develop HCC in older age and to determine an optimal surveillance strategy for these patients.

Materials and methods

Study population. This study cohort was comprised of 6740 consecutive HCV-positive patients (1019 patients with HCC and 5721 patients without HCC) referred to the Department of Gastroenterology at Ogaki Municipal Hospital from January 1990 to December 2006.

There were 323 patients who fulfilled the following inclusion criteria out of 1019 HCC patients: (i) detectable HCV-RNA for at least six months, (ii) no evidence of hepatitis B virus infection; (iii) other possible causes of chronic liver disease were ruled out

(no history of hepatotoxic drug use, and negative tests for autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); (iv) a follow-up period of greater than three years before HCC diagnosis; (v) no interferon therapy within the last 12 months; and (vi) serum alanine aminotransferase (ALT) measurements taken more than twice yearly. The patients were classified into four groups according to age at the time of HCC diagnosis: group A (≤ 60 years, $n = 36$), group B (61–70 years, $n = 115$), group C (71–80 years, $n = 143$), and group D (> 80 years, $n = 29$).

Of the 5721 patients who have not developed HCC, 3275 patients fulfilled the same inclusion criteria. To reduce the confounding effects of covariates, we used propensity scores to match HCC patients with unique control patients based on age, sex, Child-Pugh classification at the start of follow-up, and follow-up duration. We were able to match 323 patients with HCC to 323 patients without HCC. The patients were classified into four groups according to age at the end of follow-up: group A' (≤ 60 years, $n = 30$), group B' (61–70 years, n = 114), group C' (71–80 years, $n = 136$), and group D' (> 80 years, $n = 43$).

The start of follow-up was defined as the date a patient first visited our hospital and ended on the date of HCC diagnosis for the HCC patients, or the date of the last visit at our hospital or December 31, 2010, whichever occurred earlier, in control patients.

Histological examinations were performed in 234 out of 646 patients. Cirrhosis was diagnosed pathologically in 120 patients. The remaining 412 patients were evaluated with ultrasonography (US) and biochemical tests.[6–8] Patients who did not satisfy the criteria for cirrhosis were classified as having chronic hepatitis for the purposes of this study. All together, 288 out of 646 patients were diagnosed with chronic hepatitis, and 358 were diagnosed with cirrhosis.

The study protocol was approved by the Ethics Committee at Ogaki Municipal Hospital in January 22, 2009 and complied with the Helsinki Declaration. Each patient provided written informed consent.

Laboratory test for liver disease and virologic markers. Platelet counts, prothrombin time, and serum levels of ALT, albumin, total bilirubin, alpha-fetoprotein (AFP), *lens culinaris* agglutinin–reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP) were determined at the start of follow-up. ALT is expressed as an average integration value.[6] Serum AFP concentration was determined with a commercially available kit. AFP-L3 was measured by lectin-affinity electrophoresis and antibody-affinity blotting with the AFP Differentiation Kit L (Wako Pure Chemical Industries. Ltd, Osaka, Japan).[9] DCP was quantified with the Picolumi PIVKA-II kit (Eisai Co., Ltd, Tokyo, Japan).[10] HCV genotype was determined by PCR using genotype-specific primers, and HCV-RNA was quantified (before November 2007; COBAS Amplicor HCV monitor test and after December 2007; COBAS AmpliPrep/COBAS TaqMan HCV test, Roche Diagnostics K.K., Tokyo, Japan).

Alcohol exposure. Past alcohol exposure was estimated based on chart review of drinking patterns over five years. Patients were categorized as either "excessive" or "moderate" alcohol consumers. Excessive alcohol consumers drank over 50 g daily for five years.

Methods of follow-up. All patients received medical examinations at least every six months at our institution. Imaging studies, either US, computed tomography (CT), or magnetic resonance imaging (MRI), were performed at least every six months. When patients were considered to have developed cirrhosis by laboratory data or imaging findings, imaging was performed at three-month intervals.[11]

Diagnosis and treatment of HCC. The diagnosis of HCC was made based on either pathological or clinical and radiological criteria. Histological examination of resected hepatic tumors or US-guided needle biopsy specimens confirmed HCC in 165 patients (resected specimens: 111 patients; biopsy specimens: 54 patients). In the remaining 158 patients, the diagnosis of HCC was made using clinical criteria and imaging findings obtained from B-mode US, CT, MRI, and CT angiography.[12,13]

Tumor staging was performed according to the American Joint Committee on Cancer (AJCC) classification system.[14] In cases where pathologic evaluation was not available, vascular invasion was assessed by dynamic CT and angiography.

Treatment for each patient was individualized according to evidence-based clinical practice guidelines for HCC in Japan.[14] Hepatic resection was performed on 111 patients. Percutaneous ethanol injection therapy was performed in 16 patients. Radiofrequency ablation therapy was performed in 104 patients. Transcatheter arterial chemoembolization was performed in 62 patients. Thirty patients did not undergo treatment because of the patient's wishes or impaired liver function.

Statistical analyses. Statistical analysis was performed with the Statistical Program for Social Science (SPSS ver.18.0 for Windows; SPSS Japan Inc., Tokyo, Japan). Continuous variables are represented as medians (range). The non-parametric Jonckheere–Terpstra test was used to assess continuous variables. The Steel–Dwass or Shirley–Williams multiple comparisons method was applied if the Jonckheere–Terpstra test yielded significant results. The Cochran–Armitage test or the chi-square test was used to assess categorical variables. Actual survival was estimated using the Kaplan–Meier method,[15] and differences were tested with the log-rank test.[16] The Cox proportional hazards model and forward selection method were used to estimate the relative risk of HCC development associated with age, sex, cirrhosis, alcohol consumption, diabetes mellitus, effect of prior interferon therapy, platelet count, AFP at the start of follow-up, and average integration value of ALT. and the annual rate of platelet count decline. Statistical significance was set at $P < 0.05$.

Results

Clinical features at baseline. The clinical profiles of the HCC patients at the start of follow-up are shown in Table 1. There was a higher proportion of women diagnosed with HCC at a later age ($P = 0.0016$): the percentage of women in groups A, B, C, and

Table 1 Profile of HCV-infected HCC patients at the start of follow-up

	Group A ($n=36$)	Group B ($n=115$)	Group C ($n=143$)	Group D ($n=29$)	P
Sex (female/male)	5/31	43/72	63/80	15/14	0.0016
Age at the start of follow-up† (years)	49 (36–57)	59 (47–66)	66 (52–75)	74 (64–80)	< 0.0001
Duration of observation period until HCC diagnosis† (years)	6.4 (3.1–16.7)	6.9 (3.0–15.8)	8.0 (3.0–17.7)	9.3 (3.0–15.7)	0.0003
Alcohol consumption (≥ 50 g per day/< 50 g per day)	9/27	24/91	26/117	2/27	0.0873
History of blood transfusion (present/absent)	6/30	26/89	35/108	2/27	0.8247
Diabetes mellitus (present/absent)	24/12	40/75	51/92	5/24	0.0008
Prior interferon therapy (SVR/non-SVR/absent)	3/17/16	12/32/71	0/15/128	0/1/28	< 0.0001

†Expressed as median (range).
Group A, diagnosis of HCC at age ≤ 60 years; Group B, 61–70 years; Group C, 71–80 years; Group D, > 80 years.
HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SVR, sustained virologic response.

Table 2 Profile of control patients with HCV infection at the start of follow-up

	Group A' ($n=30$)	Group B' ($n=114$)	Group C' ($n=136$)	Group D' ($n=43$)	P
Sex (female/male)	7/23	48/66	56/80	20/23	0.1175
Age at the start of follow-up† (years)	48 (40–56)	58 (48–67)	66 (54–75)	74 (65–82)	< 0.0001
Duration of observation period until the end of follow-up† (years)	7.0 (3.0–15.5)	7.8 (3.0–18.7)	8.5 (3.0–17.7)	8.5 (3.6–19.1)	0.0064
Alcohol consumption (≥ 50 g per day / < 50 g per day)	8/22	27/87	20/116	3/40	0.0630
History of blood transfusion (present/absent)	5/25	29/85	40/96	2/41	0.1939
Diabetes mellitus (present/absent)	7/23	38/76	47/89	12/31	0.0758
Prior interferon therapy (SVR/non-SVR/absent)	4/15/11	8/34/72	3/20/113	0/1/42	< 0.0001

†Expressed as median (range).
Group A', age ≤ 60 years at the end of follow-up; Group B', 61–70 years; Group C', 71–80 years; Group D', > 80 years.
HCV, hepatitis C virus; SVR, sustained virologic response.

D was 13.9, 37.4, 44.1, and 51.7, respectively. As the patient's age at HCC diagnosis increased, the patient's age at the start of follow-up and the duration of the observation period until HCC diagnosis increased ($P < 0.0001$ and $P = 0.0003$, respectively). Patients who received a diagnosis of HCC at a more advanced age have a significantly decreased incidence of diabetes mellitus and prior interferon therapy ($P = 0.0008$ and $P < 0.0001$, respectively). The clinical profiles of the control patients at the start of follow-up are shown in Table 2. The same tendency between HCC patients and control patients was observed.

Laboratory data of the HCC patients at the start of follow-up are shown in Table 3. Patients diagnosed with HCC at a more advanced age had lower baseline serum ALT and AFP levels ($P < 0.0001$ and $P = 0.0043$, respectively) and higher baseline platelet counts ($P = 0.0032$). In Table 4, the oldest group of control patients had lower baseline serum ALT and AFP levels ($P < 0.0001$ and $P = 0.0261$, respectively); however, no significant differences in baseline platelet count were observed.

The results of the Cox proportional hazards model and forward selection method to test factors associated with the age-related development of HCC to patient age at the start of follow-up are shown in Table 5. Ten covariates including age, sex, cirrhosis, alcohol consumption, diabetes mellitus, effect of prior interferon therapy, platelet count, baseline AFP, average integration value of ALT, and the annual rate of platelet count decline were studied. Age, cirrhosis, average integration value of ALT, platelet count, and AFP were significantly associated with hepatocarcinogenesis. However, only cirrhosis and average integration value of ALT were selected as factors significantly associated with hepatocarcinogenesis in patients ≥ 65 or 70 years old. Platelet count was not a significant factor.

Clinical features at the time of HCC diagnosis. Platelet counts at the time of HCC diagnosis in groups A, B, C, and group D were $72 \times 10^3/mm^3$ (40–192), $84 \times 10^3/mm^3$ (28–256), $99 \times 10^3/mm^3$ (31–355), and $119 \times 10^3/mm^3$ (58–232), respectively. There is a statistically significant trend toward higher platelet counts as the age at HCC diagnosis increases ($P < 0.0001$). In contrast, platelet counts at the end of follow-up in groups A', B', C', and D' were $194 \times 10^3/mm^3$ (44–543), $172 \times 10^3/mm^3$ (40–484), $177 \times 10^3/mm^3$ (21–415), and $193 \times 10^3/mm^3$ (52–429), respectively. There is no significant difference between the four groups of control patients ($P = 0.4772$). The annual rate of decline in platelet count, calculated as [platelet count at the start of the study period—platelet count at the time of HCC diagnosis]/duration of the observation period until the diagnosis of HCC, decreased significantly as the age at HCC diagnosis increased, and the annual rate of decline in platelet count, calculated as [platelet count at the start of study period—platelet count at the end of follow-up]/duration of observation period until the end of follow-up in control patients, did not increase significantly as the age at the end of follow-up increased (Fig. 1, $P = 0.0247$ and 0.1571, respectively). The annual rate of platelet count decline was

Table 3 Baseline laboratory data of HCV-infected HCC patients

	Group A ($n = 36$)	Group B ($n = 115$)	Group C ($n = 143$)	Group D ($n = 29$)	*P*
Platelet count† ($\times 10^3$/mm^3)	104 (34–249)	114 (29–253)	125 (44–307)	124 (70–201)	0.0032
Prothrombin time† (%)	87 (52–129)	88 (24–119)	85 (22–128)	86 (45–129)	0.6062
Total bilirubin† (mg/dL)	0.8 (0.3–1.8)	0.7 (0.2–4.7)	0.7 (0.3–6.7)	0.6 (0.2–1.3)	0.4583
ALT† (IU/L)	125 (24–361)	76 (18–387)	64 (8–154)	44 (17–221)	< 0.0001
Child-Pugh classification[17] (A or B/C)	33/3	103/12	130/13	24/5	0.5512
HCV genotype‡ (1/2)	26/6	66/24	75/29	15/6	0.4083
HCV viral concentration† (log copies/mL)	5.7 (2.7–8.0)	5.0 (2.0–8.0)	5.4 (2.0–6.9)	5.5 (3.0–7.0)	0.4952
AFP† (ng/mL)	13.5 (1.8–163.4)	8.4 (1.9–583.4)	7.2 (1.0–372.3)	4.8 (1.2–141.5)	0.0043
AFP-L3† (%)	0 (0–56.3)	0 (0–43.6)	0 (0–15.2)	0 (0–7.0)	1.0000
DCP† (mAU/mL)	19 (10–154)	19 (10–367)	17 (10–745)	15 (10–182)	0.0958
Cirrhosis (present/absent)	31/5	95/20	112/31	21/8	0.0903

†Expressed as median (range).
‡Data were unavailable for 76 patients.
AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin-reactive fraction of AFP; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; Group A, diagnosis of HCC at age ≤ 60 years; Group B, 61–70 years; Group C, 71–80 years; Group D, > 80 years; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

Table 4 Baseline laboratory data of control patients with HCV infection

	Group A′ ($n = 30$)	Group B′ ($n = 114$)	Group C′ ($n = 136$)	Group D′ ($n = 43$)	*P*
Platelet count† ($\times 10^3$/mm^3)	204 (58–375)	180 (40–540)	187 (51–484)	196 (52–418)	0.4301
Prothrombin time† (%)	100 (52–138)	96 (38–153)	96 (48–144)	95 (47–145)	0.3435
Total bilirubin† (mg/dL)	0.5 (0.2–1.2)	0.4 (0.2–5.3)	0.4 (0.2–5.3)	0.3 (0.2–1.5)	0.6298
ALT† (IU/L)	53 (12–131)	46 (5–490)	35 (8–484)	22 (2–199)	< 0.0001
Child-Pugh classification[17] (A or B/C)	30/0	103/11	128/8	40/3	0.1088
HCV genotype‡ (1/2)	15/10	60/23	66/25	12/5	0.0869
HCV viral concentration† (log copies/mL)	5.9 (2.7–6.6)	5.7 (2.7–7.3)	5.8 (2.0–7.0)	5.1 (3.0–6.6)	0.1130
AFP† (ng/mL)	4.3 (0.8–156.3)	3.1 (0.8–170.3)	3.1 (0.8–219.2)	2.0 (0.8–29.2)	0.0261
AFP-L3† (%)	0 (0–26.9)	0 (0–34.2)	0 (0–41.4)	0 (0–5.2)	1.0000
DCP† (mAU/mL)	22 (10–122)	19 (10–487)	19 (10–503)	16 (10–30)	0.2549
Cirrhosis (present/absent)	5/25	35/79	48/88	11/32	0.1201

†expressed as median (range).
‡Data were unavailable for 107 patients.
AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin-reactive fraction of AFP; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; Group A′, age ≤ 60 years at the end of follow-up; Group B′, 61–70 years; Group C′, 71–80 years; Group D′, > 80 years; HCV, hepatitis C virus.

Table 5 Factors associated with the development of HCC according to the age at start of follow-up in multivariate analysis

		All patients ($n = 646$) hazard ratio (95% CI)	≥ 60 years ($n = 428$) hazard ratio (95% CI)	≥ 65 years ($n = 255$) hazard ratio (95% CI)	≥ 70 years ($n = 92$) hazard ratio (95% CI)
Age (years)	≤ 60	1			
	> 60, ≤ 70	1.600 (1.240–2.064)			
	> 70	2.738 (1.858–4.036)			
Cirrhosis	Absent	1	1	1	1
	Present	2.165 (1.575–2.978)	2.269 (1.554–3.311)	2.734 (1.724–4.336)	2.962 (1.200–7.310)
Average integration value of ALT (IU/L)	≤ 20	1	1	1	1
	> 20, ≤ 40	4.239 (1.336–13.800)	4.885 (1.179–20.249)	5.243 (1.253–22.020)	12.162 (1.549–95.496)
	> 40, ≤ 60	5.518 (1.725–17.648)	6.661 (1.619–23.397)	6.739 (1.610–28.250)	6.797 (0.854–54.080)
	> 60, ≤ 80	7.182 (2.230–23.130)	9.362 (2.268–38.641)	12.265 (2.867–56.471)	11.183 (1.400–89.317)
	> 80	10.211 (3.175–33.031)	12.249 (2.494–50.884)	13.087 (2.962–57.815)	11.052 (0.964–126.671)
Platelet count ($\times 10^3$/mm^3)	≥ 150	1	1		
	< 150	1.644 (1.237–2.186)	1.728 (1.240–2.408)		
AFP* (ng/mL)	≤ 10	1			
	> 10, ≤ 20	1.406 (1.002–1.971)			
	> 20	1.609 (1.214–2.132)			

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma.

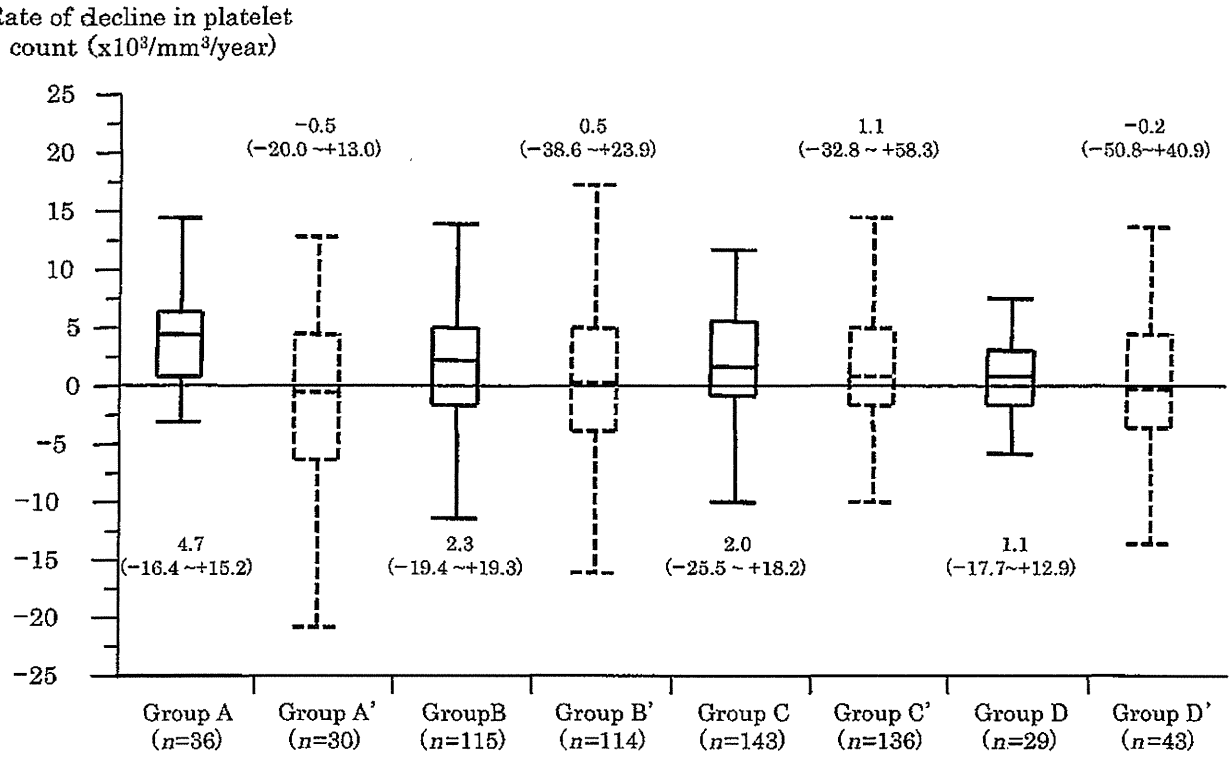

Figure 1 Rate of decline in platelet count prior to hepatocellular carcinoma (HCC) diagnosis in HCC patients and prior to the end of follow-up in control patients. The annual rate of platelet count decline in the period prior to HCC diagnosis was lower in the groups that were older at the time of HCC diagnosis. In control patients, there was no trend toward higher annual rates of platelet count decline in the period prior to the end of follow-up when the patients were classified by age ($P = 0.0247$ and 0.1571, respectively, Jonckheere-Terpstra Test). Group A, HCC diagnosed at age ≤ 60 years; group B, 61–70 years; group C, 71–80 years; group D, > 80 years. group A', control patients ≤ 60 years old at the end of follow-up; group B', 61–70 years; group C', 71–80 years; group D', > 80 years. The annual rate of platelet count decline was significantly lower in group A' than in group A ($P = 0.0039$); however, there were no significant differences when HCC patients in other age groups were compared to their respective matched controls.

lower in group A' than in group A ($P = 0.0039$), and there were no significant differences between group B and group B', group C and group C', and group D and group D'.

The average integration value of ALT in groups A, B, C, and D was 80.9 IU/L (25.3–179.3), 62.3 IU/L (14.5–167.9), 59.0 IU/L (9.9–134.1), and 44.9 IU/L (22.7–91.9), respectively. The average integration value of ALT was significantly lower in patients diagnosed with HCC at an older age (Fig. 2, $P < 0.0001$). There was a similar trend among control patients (Fig. 2, $P < 0.0001$). The average integration values of ALT in groups A', B', C', and D' were significantly lower than in groups A, B, C, and D, respectively ($P < 0.0001$).

Patient profiles at the time of HCC diagnosis are shown in Table 6. There were no significant differences in tumor characteristics and levels of tumor markers among the age groups. Fewer patients in Group D underwent hepatic resection ($P = 0.0293$).

Survival rates according to age at HCC diagnosis. Five and 10-year cumulative survival rates of groups A, B, C, and D were 44.2%, 58.2%, 44.3%, and 33.3% and 22.7%, 31.2%, 26.6%, and not available, respectively (Fig. 3). There were no significant differences in the cumulative survival rate among the four groups.

Discussion

In Japan, the average age of patients with chronic hepatitis, cirrhosis, or HCV-associated HCC is increasing. The number of deaths due to these diseases is also increasing. The age-specific prevalence of HCV seropositivity in the USA is about 30 years below that in Japan; thus, a majority of patients in the USA with chronic HCV infection will reach an advanced age in the near future.[3]

In our study, elderly HCC patients have high platelet counts and low ALT values. In addition, multivariate analysis using propensity-matched control patients revealed that the presence of cirrhosis and high ALT levels (> 20 IU/L) are significantly associated with the development of HCC. However, platelet count is not significantly associated with hepatocarcinogenesis in elderly HCV carriers (≥ 65 years). Physicians should be aware that patients aged 65 years or older could develop HCC regardless of their platelet count.

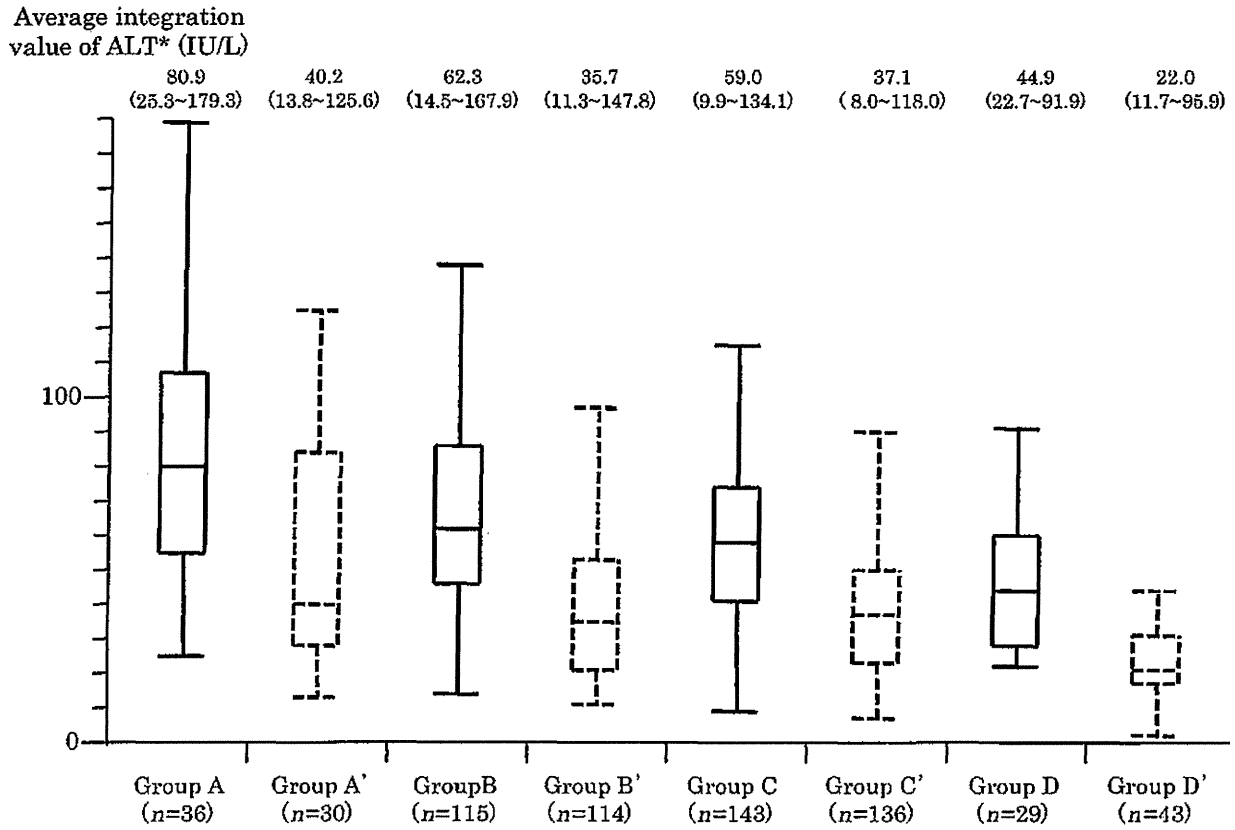

Figure 2 Average integration values of alanine aminotransferase (ALT) prior to HCC diagnosis in HCC patients and prior to the end of follow-up in control patients. Patients who were older at the time of HCC diagnosis had lower average integration values of ALT in the period prior to HCC diagnosis. In control patients, the average integration values of ALT in the period prior to the end of follow-up were lower in the groups that were older at the end of follow-up ($P < 0.0001$ and < 0.0001, respectively, Jonckheere-Terpstra Test). Average integration values of ALT in groups A', B', C', and D' were significantly lower than in groups A, B, C, and D, respectively ($P < 0.0001$).

Table 6 Profile of HCV-infected HCC patients at the time of HCC diagnosis

	Group A ($n = 36$)	Group B ($n = 115$)	Group C ($n = 143$)	Group D ($n = 29$)	P
AFP† (ng/mL)	23.9 (0.8–500)	19.8 (0.6–10500)	12.8 (0.8–12680)	17.8 (0.8–99720)	0.2347
AFP-L3† (%)	0 (0–89)	0 (0–87.2)	0 (0–81.0)	0 (0–40.7)	1.0000
DCP† (mAU/mL)	36 (10–36164)	35 (10–5941)	32 (10–50904)	24 (10–6229)	0.5650
Tumor size† (cm)	2.0 (0.8–10.0)	2.0 (0.3–8.8)	2.0 (0.6–11.4)	2.3 (1.0–9.0)	0.3754
Number of tumors†	1 (1–6)	1 (1–8)	1 (1–10)	1 (1–4)	1.0000
Portal thrombus (present/absent)	2/34	3/112	6/137	0/29	0.3293
Stage (I/II/III/IV)	14/15/5/2	41/53/21/0	50/61/29/3	10/12/7/0	0.4957
Initial treatment (HR/PT/TACE/none)	9/18/4/5	47/44/16/8	51/47/33/12	4/11/9/5	0.0293

†Expressed as median (range).

AFP, α-fetoprotein; AFP-L3, *lens culinaris* agglutinin–reactive fraction of AFP; DCP, des-γ-carboxy prothrombin; Group A, diagnosis of HCC at age ≤ 60 years; Group B, 61–70 years; Group C, 71–80 years; Group D, > 80 years; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hepatic resection; PT; percutaneous treatment including ethanol injection therapy, microwave coagulation therapy, and radiofrequency ablation therapy; TACE, transcatheter arterial chemoembolization.

The male-to-female ratio of HCC patients in Japan has decreased from 4.5 in 1984–1985 to 2.5 in 2002–2003.[1] It is well known that the mean age of female HCC patients with HCV infection is higher than that of males.[18,19] The increased proportion of female patients is considered a result of more older patients with HCV-related HCC. In our study, the proportion of female patients was the highest in group D. Further investigation of the role of sex in hepatocarcinogenesis is needed.

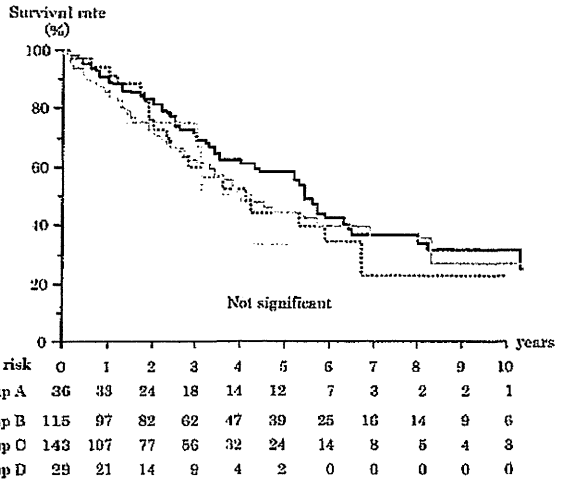

Figure 3 Cumulative survival rate of groups A, B, C, and D according to age at hepatocellular carcinoma (HCC) diagnosis. Kaplan-Meier curves showing the survival rate stratified by age at HCC diagnosis. There were no significant differences in the survival rate among the four groups. ——, A group (≤ 60 years, $n = 36$); ·····, B group (61–70 years, $n = 115$); ·, C group (71–80 years, $n = 143$); , D group (> 80 years, $n = 29$).

We previously reported that the average integration value of ALT was associated with the cumulative incidence of hepatocarcinogenesis and that minimizing ALT is necessary for the prevention of hepatocarcinogenesis.[20] In addition, we demonstrated a 6.242-fold higher (95% confidence interval: 1.499–25.987) cumulative incidence of hepatocarcinogenesis in patients with average ALT integration values between 20 and 40 IU/L (within the current normal range) than in patients with 20 IU/L or below.[21] In this study, the average integration value of ALT significantly decreased as the age at HCC diagnosis increased. Especially in group D, the average integration value of ALT was 44.9 IU/L (range, 22.7–91.9 IU/L), which is near the upper limit of the conventional reference range of ALT (40 IU/L). There was the same tendency in control patients; however, average integration values of ALT were lower in control patients than HCC patients in each corresponding age group. These data suggest close surveillance for HCC is important even if older patients (≥ 65 years) have low ALT values.

It is likely that low platelet counts account for a large proportion of patients with cirrhosis, consistent with the theory that HCC develops in patients with progressive or advanced liver disease. Cirrhosis is an established risk factor for HCC in patients with HCV.[22,23] It is generally accepted that platelet count is a surrogate marker of liver fibrosis.[24,25] Platelet counts were highest in group D, both at the start of follow-up and at the time of HCC diagnosis. In contrast, there were no differences in platelet counts among control patients without HCC. It is particularly worth noting that group D had the smallest annual decline in platelet count, at levels comparable to the control patients. A previous report showed that the rate of progression of fibrosis to cirrhosis was accelerated by aging.[24] The precise mechanism of this discrepancy is uncertain. Probably, differences in patient selection might account for this discrepancy. We hypothesize that in our study, the increased rate of annual decline in platelet count may be linked to accelerated carcinogenesis occurring in the younger patients. Group D also had the lowest values of AFP, which is considered a marker of hepatic regeneration as well as a HCC tumor marker in viral hepatitis.[26] Taken together, this suggests a weaker inflammatory response in older patients. Further investigation is necessary.

Why do elderly patients progress to HCC even though liver function appears stable? Aging is associated with a number of events at the molecular, cellular, and physiological level that influence carcinogenesis and subsequent cancer growth.[22] Age may be considered as a progressive loss of stress tolerance due to declines in the functional reserve of multiple organ systems.[27] It has been hypothesized that age-associated declines in DNA repair[28] contribute to the development of HCC. The precise relationship between aging and hepatocarcinogenesis remains uncertain. Further assessment of the role of aging in the progression of HCV is needed.

We found no difference in tumor stage among the four groups. The younger groups A and B tended to receive curative therapy more often than the older groups C and D. However, there were no significant differences in survival. We hypothesize that this is due to the aggressive multiple treatments received by elderly patients with good liver function.

One limitation of our study is that histological confirmation was available in only 234 patients (36.2%). However, it is not practical to perform biopsies on all patients because of potential complications. Lu *et al.* reported that the best cutoff platelet count for the diagnosis of cirrhosis is 150×10^3 /mm^3.[29] Therefore, we employed platelet count as a surrogate marker of liver fibrosis in this study.

In conclusion, we demonstrated that elderly HCV-positive patients (≥ 65 years old) with low ALT values developed HCC regardless of their platelet counts. This finding should be taken into account when designating the most suitable HCC surveillance protocol. The optimal screening interval for HCV-infected patients aged 65 years older should be three to four months like cirrhotic patients even in the absence of cirrhosis.

References

1 Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *J. Gastroenterol.* 2009; 44 (Suppl. 19): 102–7.
2 Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; 127 (Suppl. 1): S17–26.
3 Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. *Oncology* 2002; 62 (Suppl. 1): 8–17.
4 Honda T, Katano Y, Urano F *et al.* Efficacy of ribavirin plus interferon-alpha in patients aged >or =60 years with chronic hepatitis C. *J. Gastroenterol. Hepatol.* 2007; 22: 989–95.
5 El-Serag HB. Hepatocellular carcinoma and hepatitis C in the United States. *Hepatology* 2002; 36 (Suppl. 1): S74–83.
6 Shen L, Li JQ, Zeng MD, Lu LG, Fan ST, Bao H. Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. *World J. Gastroenterol.* 2006; 28: 1292–5.
7 Iacobellis A, Fusilli S, Mangia A *et al.* Ultrasonographic and biochemical parameters in the non-invasive evaluation of liver fibrosis in hepatitis C virus chronic hepatitis. *Aliment. Pharmacol. Ther.* 2005; 22: 769–74.

8 Caturelli E, Castellano L, Fusilli S *et al.* Coarse nodular US pattern in hepatic cirrhosis: risk for hepatocellular carcinoma. *Radiology* 2003; **226**: 691–7.
9 Shimizu K, Katoh H, Yamashita F *et al.* Comparison of carbohydrate structures of serum α-fetoprotein by sequential glycosidase digestion and lectin affinity electrophoresis. *Clin. Chim. Acta* 1996; **254**: 23–40.
10 Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer* 1998; **82**: 1643–8.
11 Makuuchi M, Kokudo N, Arii S *et al.* Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol. Res.* 2008; **38**: 37–51.
12 Kudo M. Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. *Semin. Liver Dis.* 1999; **19**: 297–309.
13 Torzilli G, Minagawa M, Takayama T *et al.* Accurate preoperative evaluation of liver mass lesions without fine-needle biopsy. *Hepatology* 1999; **30**: 889–93.
14 Vauthey JN, Lauwers GY, Esnaola NF *et al.* Simplified staging for hepatocellular carcinoma. *J. Clin. Oncol.* 2002; **20**: 1527–36.
15 Kaplan EL, Meier P. Non parametric estimation for incomplete observation. *J. Am. Stat. Assoc.* 1958; **53**: 457–81.
16 Petro R, Pike MC. Conservation of the approximation (0-E2)/E in the log rank test for survival data on tumor incidence data. *Biometrics* 1973; **29**: 579–84.
17 Pugh RNH, Murray-Lyon IM, Dawson JL *et al.* Transection of the oesophagus for bleeding oesophageal varices. *Br. J. Surg.* 1973; **60**: 646–9.
18 Miki D, Aikata H, Uka K *et al.* Clinicopathological features of elderly patients with hepatitis C virus related hepatocellular carcinoma. *J. Gastroenterol.* 2008; **43**: 550–7.
19 Saneto H, Kobayashi M, Kawamura Y *et al.* Clinicopathological features, background liver disease, and survival analysis of HCV-positive patients with hepatocellular carcinoma: differences between young and elderly patients. *J. Gastroenterol.* 2008; **43**: 975–81.
20 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**: 738–9.
21 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**: 729–35.
22 Oka H, Kurioka N, Kim K *et al.* Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. *Hepatology* 1990; **12**: 680–7.
23 Ikeda K, Saitoh S, Koida I *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.
24 Hamada H, Yatsuhashi H, Yano K *et al.* Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. *Cancer* 2002; **95**: 331–9.
25 Yatsuhashi H, Yano M. Towards control of hepatitis C in the Asia-Pacific region. *J. Gastroenterol. Hepatol.* 2000; **15** (Suppl.): E111–16.
26 Chu CW, Hwang SJ, Luo JC *et al.* Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J. Clin. Gastroenterol.* 2001; **32**: 240–4.
27 Balducci L, Extermann M. Management of cancer in the older person: a practical approach. *Oncologist* 2000; **5**: 224–37.
28 Goukassian D, Gad F, Yaar M, Eller MS, Nehal US, Gilchrest BA. Mechanisms and implications of the age-associated decrease in DNA repair capacity. *FASEB J.* 2000; **14**: 1325–34.
29 Lu SN, Wang JH, Liu SL *et al.* Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. *Cancer* 2006; **107**: 2212–22.

Research Article

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Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: A propensity score analysis

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Background & Aims: Some patients with chronic hepatitis B virus (HBV) infection progress to hepatocellular carcinoma (HCC). However, the long-term effect of nucleos(t)ide analogue (NA) therapy on progression to HCC is unclear.

Methods: Therefore, we compared chronic hepatitis B patients who received NA therapy to those who did not, using a propensity analysis.

Results: Of 785 consecutive HBV carriers between 1998 and 2008, 117 patients who received NA therapy and 117 patients who did not, were selected by eligibility criteria and propensity score matching. Factors associated with the development of HCC were analyzed. In the follow-up period, HCC developed in 57 of 234 patients (24.4%). Factors significantly associated with the incidence of HCC, as determined by Cox proportional hazards models, include higher age (hazard ratio, 4.36 [95% confidence interval, 1.33–14.29], $p = 0.015$), NA treatment (0.28 [0.13–0.62], $p = 0.002$), basal core promoter (BCP) mutations (12.74 [1.74–93.11], $p = 0.012$), high HBV core-related antigen (HBcrAg) (2.77 [1.07–7.17], $p = 0.036$), and high gamma glutamyl transpeptidase levels (2.76 [1.49–5.12], $p = 0.001$).

Conclusions: NA therapy reduced the risk of HCC compared with untreated controls. Higher serum levels of HBcrAg and BCP mutations are associated with progression to HCC, independent of NA therapy.

Introduction

An estimated 350 million individuals worldwide are chronically infected with hepatitis B virus (HBV), of whom 1 million die annually from HBV-related liver disease [1]. Chronic HBV infection is recognized as a major risk factor for the development of hepatocellular carcinoma (HCC) [1,2]. Hepatitis B surface antigen (HBsAg)-positive patients have a 70-fold increased risk of developing HCC compared to HBsAg seronegative counterparts [3,4]. HBV infection is endemic in Southeast Asia, China, Taiwan, Korea, and sub-Saharan Africa, where up to 85–95% of patients with HCC are HBsAg positive [5]. HCC is the third and fifth leading cause of cancer death in men and women, respectively, and the number of deaths and the mortality rate from HCC have greatly increased in Japan since 1975 [6]. Hepatitis C virus (HCV)-related HCC accounts for 75% of all HCCs in Japan and HBV-related HCC accounts for 15% [6].

In 2004, Liaw *et al.* reported a significant reduction in HCC in 651 adults receiving lamivudine after adjustment for baseline variables (hazard ratio, 0.49 [95% confidence interval (95% CI), 0.25–0.99], $p = 0.047$) [7]. However, the results were not significant after exclusion of 5 patients who developed HCC within 1 year of randomization (0.47 [0.22–1.00], $p = 0.052$). Therefore, in 2009, the National Institutes of Health Consensus Development Conference concluded that there was insufficient evidence to assess whether nucleos(t)ide analogue (NA) therapy can prevent the development of HCC [8].

The long-term use of lamivudine has not been recommended because of tyrosine–methionine–aspartate–aspartate (YMDD) mutations, which have occasionally been associated with severe and even fatal flares of hepatitis [9,10]. Therefore, adefovir dipivoxil should be added immediately in patients with virological or biochemical breakthroughs or no response. Currently, there are 2 nucleoside agents (lamivudine, entecavir) and 1 nucleotide agent (adefovir dipivoxil) available for treatment of HBV infection in Japan. The agent with the higher genetic barrier to resistance, entecavir, is considered the initial drug of choice [11]. Recently, 3 studies on lamivudine suggested that long-term sustained viral suppression was associated with a reduced likelihood of developing HCC [12–14].

In this study, we sought to determine if NA therapy was associated with a reduction in the development of HCC. Since the validity of treatment effects in observational studies may be limited by selection bias and confounding factors, we performed a propensity analysis [15].

Keywords: HBcrAg; BCP; Gamma-GTP; Average integration value; HBV DNA.
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Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBcrAg, HBV core-related antigen; BCP, basal core promoter; gamma-GTP, gamma glutamyl transpeptidase.

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Materials and methods

Patient selection

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

Between 1998 and 2008, 1220 consecutive HBsAg-positive patients, who visited the Department of Gastroenterology and Hepatology at Ogaki Municipal Hospital, were prospectively enrolled in our HCC surveillance program. Of these, 785 patients met the following inclusion criteria: HBsAg positive for more than 6 months, no evidence of HCV co-infection, exclusion of other causes of chronic liver disease (alcohol consumption >80 g/day, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, or Wilson's disease), follow-up duration of greater than 3 years, no evidence of HCC for at least 1 year from the start of the follow-up period, receiving no interferon treatment, and receiving NA therapy for more than 1 year before the detection of HCC (Fig. 1). In patients on NA therapy, the date of NA therapy initiation was considered the starting point of the follow-up period.

Of these 785 patients, 148 received NA therapy (NA group) and 637 patients did not receive NA therapy (non-NA group) during the follow-up period. To reduce the confounding effects of covariates, we used propensity scores to match NA patients to unique non-NA patients. Six covariates including age, sex, HBV DNA concentration, hepatitis B e antigen (HBeAg), platelet count, and alanine aminotransferase (ALT) activity were taken into account at the start of follow-up. We computed the propensity score by using logistic regression with the independent variable including age (≤40 years or >40 years), sex (female or male), HBV DNA concentration (≤5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), platelet count (>150 × 10^3/m^3 or ≤150 × 10^3/m^3), and ALT activity (≤40 IU/ml or >40 IU/ml), as shown in previous reported cut-off values according to the indication for NA therapy [16–19]. This model yielded a *c* statistic of 0.85 (95% confidence interval [CI], 0.82–0.88), indicating very good ability of the propensity score model to predict treatment status. We sought to match each patient who received NA therapy to a patient who did not receive NA therapy, having a propensity by using greedy 5–1 digit matching [20]. Once this threshold was exceeded, a patient with NA therapy was excluded. This score ranged from 0.09198 to 0.98967 and, in effect, represented the probability that a patient would be receiving NA. We were able to match 117 patients with NA therapy to 117 unique patients without NA therapy. The follow-up period ended on 31 December, 2011 or the date when HCC occurrence was identified.

Surveillance and diagnosis

All patients were followed up at our hospital at least every 6 months. During each follow-up examination, platelet count, ALT, gamma glutamyl transpeptidase (gamma-GTP), total bilirubin, alkaline phosphatase (ALP), albumin, and alpha-fetoprotein (AFP) levels were measured. We used commercially available kits to test blood samples for HBsAg, HBeAg, and anti-HBe (Abbott Japan Co., Ltd., Tokyo, Japan). Before November 2007, the serum HBV DNA concentration was monitored by a polymerase chain reaction assay (COBAS Amplicor HBV monitor test, Roche Diagnostics K. K., Tokyo, Japan) with a lower detection limit of approximately 2.6 log copies/ml, and after December 2007, it was monitored with another polymerase chain reaction assay (COBAS AmpliPrep-COBAS TaqMan HBV Test, Roche Diagnostics K. K.), with a lower detection limit of approximately 2.1 log copies/ml. HBV genotyping was performed as described previously [21]. Serum levels of HBV core-related antigen (HBcrAg) were measured using a chemiluminescence enzyme immunoassay (CLEIA) as described previously [22,23]. Precore nucleotide 1896 and basal core promoter (BCP) dinucleotide 1762/1764 were determined using the line probe assay (INNO-LiPA HBV PreCore assay; Innogenetics NV) [24,25]. The probes were designed to determine the nucleotides at position 1896 (G *vs.* A) in the precore region and positions 1762 (A *vs.* T) and 1764 (G *vs.* A and G *vs.* T) in the BCP region. A line probe assay was used to identify any emergence of YMDD mutations (INNO-LiPA HBV DR assay; Innogenetics NV).

Platelet count, ALT, gamma-GTP, total bilirubin, ALP, albumin, AFP, and HBV DNA values were expressed as average integration values [26,27] after the start of follow-up.

According to the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan [28], we performed ultrasound (US) and monitoring of 3 biomarkers (AFP, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein [AFP-L3], and des-gamma-carboxy prothrombin [DCP]) every 3–4 months, and dynamic magnetic resonance imaging (MRI) every 12 months, for patients with cirrhosis under surveillance. For patients with chronic hepatitis, we performed US and monitoring of the 3 biomarkers every 6 months. Histological examinations were performed in 91 out of 234 patients. Among them, cirrhosis was diagnosed in 32 patients. In the remaining 143 patients, the diagnosis of cirrhosis was made according to typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [29–31]. Patients who did not satisfy these criteria were classified as having chronic hepatitis. One hundred and forty-two patients were diagnosed with chronic hepatitis and 92 patients with cirrhosis. For diagnostic confirmation of HCC, patients underwent dynamic MRI. A histological diagnosis of HCC was made in 28 patients (surgical specimen, 23 patients; US-guided needle biopsy specimen, 5 patients). The remaining 29 patients were diagnosed with HCC based on typical dynamic MRI findings, including hypervascularity in the arterial phase with washout in the portal venous or delayed phase [32].

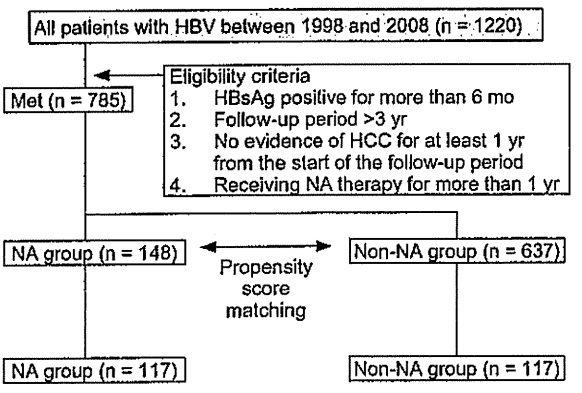

Fig. 1. Flowchart of the patient selection process.

Treatments

In the NA group, 117 patients received NA therapy including 18 patients with lamivudine, 28 patients with lamivudine and adefovir dipivoxil, and 71 patients with entecavir. The indications for NA therapy followed the guidelines of the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), or the Asian Pacific Association for the Study of the Liver (APASL) [33–35]. In contrast, of the 117 patients not on NA therapy, 104 did not receive treatment before NA was not yet approved in Japan and the remaining 13 patients declined NA therapy.

Statistical analysis

Continuous variables are expressed as medians (range). The Mann–Whitney *U* test was used for continuous variables, and the Chi-square test with Yates' correction or Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed using the Kaplan–Meier method, and differences were tested with the log-rank test. The Cox proportional hazards model and the forward selection method were used to estimate the relative risk of HCC associated with age (≤40 years or >40 years), sex (female or male), treatment (NA or no NA), HBsAg (≤3.0 log IU/ml or >3.0 log IU/ml), HBV DNA level (≤5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), precore region (wild type or mutant), BCP (wild type or mutant type), HBcrAg (≤3.0 logU/ml or >3.0 logU/ml), platelet count (>150 × 10^3/m^3 or ≤150 × 10^3/m^3), ALT (≤40 IU/ml or >40 IU/ml), total bilirubin, gamma-GTP, ALP, albumin, and AFP (≤10 ng/ml or >10 ng/ml) for univariate and multivariate analyses. We used the minimum or maximum of the reference values at our institution as cut-off values for total bilirubin, gamma-GTP, ALP, and albumin. We conducted a sensitivity analysis to determine the magnitude of an unmeasured confounder [36].

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

Table 1. Baseline characteristics of all patients.

	NA group (n = 148)	Non-NA group (n = 637)	*p* value	Standardized difference in %
Age (yr)	53 (26-81)	48 (4-85)	<0.0001	40.6
Sex (female/male)	60/88	285/352	0.5378	6.1
Genotype (A/B/C/D/F/n.d.)	2/5/137/0/1/2	24/60/389/2/0/162	<0.0001	37.6
HBsAg (log_{10} IU/ml)	3.5 (-0.1-5.5)	3.3 (-1.3-7.9)	<0.0001	53.8
HBV DNA (log_{10} copies/ml)	7.0 (2.6-9.6)	3.8 (2.3-9.9)	<0.0001	99.9
HBeAg (±)	76/72	151/486	<0.0001	62.8
Precore region (W/M/n.d.)	30/109/9	88/381/168	0.4652	0.0
BCP (W/M/n.d.)	33/123/10	135/279/205	0.0074	27.3
HBcrAg (log_{10} U/ml)	5.9 (2.9-7.0)	3.0 (2.9-7.0)	<0.0001	96.7
Platelet count (x10^3/m^3)	150 (32-388)	188 (37-503)	<0.0001	-59.7
ALT (IU/ml)	65 (7-1088)	26 (5-3410)	<0.0001	44.1
AFP (ng/ml)	3.9 (0.8-3363)	2.9 (0.8-3686)	0.0062	-6.2
Cirrhosis (presence/absence)	62/86	91/546	<0.0001	59.1
Child-Pugh classification (A/B)	132/16	618/19	0.0002	32.7
Follow-up duration (yr)	12.8 (3.1-19.6)	13.7 (3.1-20.0)	0.1565	-16.9
Administration period (yr)	6.5 (1.5-11.0)	-	-	-
Propensity score	0.58093 (0.09198-0.98686)	0.95253 (0.12913-0.98967)	<0.0001	-132.3

NA, nucleos(t)ide analogue; n.d., not done; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; W, wild type; M, mutant type; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; Child-Pugh classification, reference no [50], Standardized difference in%; $100(\bar{X}_{NA} - \bar{X}_{non\text{-}NA})/([S^2_{NA} + S^2_{non\text{-}NA}]/2)^{1/2}$, where for each covariate X_{NA} and $X_{non\text{-}NA}$ are the sample means in NA and non-NA groups, respectively, and S^2_{NA} and $S^2_{non\text{-}NA}$ are the corresponding sample variances.

Results

Patient characteristics

Table 1 shows baseline characteristics of all 785 patients before propensity matching. There were significant differences in age, HBV genotype, HBsAg, HBV DNA concentration, presence of HBeAg, BCP mutations, HBcrAg, platelet counts, ALT level, AFP level, presence of cirrhosis, and Child-Pugh classification. The baseline characteristics of the 234 study patients after propensity matching are summarized in Table 2. There are no significant differences in age, sex, HBV genotype, HBsAg, HBV DNA concentration, presence of HBeAg, precore region mutations, BCP mutations, platelet counts, ALT concentration, Child-Pugh classification, and follow-up duration. HBcrAg concentration was significantly higher in the NA group than in the non-NA group. NA was administered a median of 6.1 years (range: 1.5–10.7 years).

Factors associated with the incidence of hepatocarcinogenesis

Factors associated with the incidence of HCC as determined by the Cox proportional hazard models and the forward selection method were analyzed in all 785 patients. High age (hazard ratio, 6.43 [95% CI, 2.71–15.26], p <0.001), male sex (3.43 [1.67–7.02], p = 0.002), NA treatment (0.28 [0.21–0.85], p = 0.017), BCP mutation (19.96 [2.27–141.90], p = 0.03), high HBcrAg levels (8.21 [3.40–19.85], p <0.001), and high AFP levels (2.49 [1.43–4.34], p = 0.001) were significantly associated with the incidence of HCC.

HCC developed in 57 of 234 patients (24.4%) during follow-up after propensity matching. The 5-year, 7-year, and 10-year cumulative incidences of HCC were 9.6%, 20.4%, and 33.4%, respectively. The 5-year, 7-year, and 10-year cumulative incidences of HCC were 2.7%, 3.3%, and 3.3%, respectively, in patients on NA therapy (n = 117) and 11.3%, 26.0%, and 40.0% in patients not on NA therapy (n = 117). Hepatocarcinogenesis occurred at significantly higher rates in the non-NA group (p = 0.0094, Fig. 2). The 5-year, 7-year, and 10-year cumulative incidences of HCC were 0.0%, 0.0%, and 0.0%, respectively, in patients with wild type BCP (n = 38) and 11.0%, 25.2%, and 41.9% in patients with mutant BCP (n = 112; p = 0.0006, Fig. 3). Factors associated with the incidence of HCC as determined by the Cox proportional hazard models and the forward selection method are listed in Table 3. Higher age (hazard ratio, 4.36 [95% CI, 1.33–14.29], p = 0.015), NA treatment (0.28 [0.13–0.62], p = 0.002), BCP mutation (12.74 [1.74–93.11], p = 0.012), high HBcrAg levels (2.77 [1.07–7.17], p = 0.036), and high gamma-GTP levels (2.76 [1.49–5.12], p = 0.001) were significantly associated with the incidence of HCC. In addition, 2 patients died due to hepatic failure during the follow-up period in the non-NA group.

The sensitivity analysis found that the observed relationship between NA treatment and HCC incidence could be diminished by the unmeasured confounder that the high prevalence of the unmeasured confounder is greater in the non-NA group than in the NA group. For example, suppose a binary unmeasured confounder that increased the hazard of HCC incidence (hazard ratio, 1.50) was present in 40% of those who were treated with NA and 80% of those who were not treated with NA. Then, the study's result would become less extreme and would no longer be statistically significant (hazard ratio under sensitivity analysis, 0.48 [95% CI, 0.22–1.05]).

Follow-up data of various parameters in patients on or not on NA therapy

For this analysis, we used the average integration value during the follow-up period (Table 4). ALT, gamma-GTP, ALP, AFP, and

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Table 2. Baseline characteristics of patients on NA therapy and propensity-matched controls.

	NA group (n = 117)	Non-NA group (n = 117)	*p* value	Standardized difference in %
Age (yr)	52 (27-77)	52 (21-77)	0.9223	1.7
Sex (female/male)	44/73	45/72	0.8929	6.1
Genotype (A/B/C/n.d.)	1/4/109/3	4/7/85/21	0.1232	26.8
HBsAg (log_{10} IU/ml)	3.6 (0.9-5.5)	3.6 (0.9-7.9)	0.1440	29.9
HBV DNA (log_{10} copies/ml)	6.7 (2.6-9.6)	6.5 (2.3-9.6)	0.1273	20.5
HBeAg (±)	57/60	58/59	0.8960	2.0
Precore region (W/M/n.d.)	22/87/8	16/75/26	0.6399	5.1
BCP (W/M/n.d.)	22/88/7	17/70/30	0.9359	0.0
HBcrAg (log_{10} U/ml)	5.9 (2.9-7.0)	4.9 (2.9-7.0)	0.0022	41.2
Platelet count ($x10^3/m^3$)	143 (32-262)	146 (37-396)	0.6340	-12.1
ALT (IU/ml)	68 (7-1088)	55 (9-3410)	0.0977	1.9
AFP (ng/ml)	2.8 (0.8-402)	3.9 (0.8-1010)	0.3118	-13.5
Cirrhosis (presence/absence)	48/69	44/73	0.6882	6.1
Child-Pugh classification (A/B)	108/9	104/13	0.5024	3.1
Follow-up duration (yr)	12.3 (3.1-19.4)	11.6 (3.1-18.3)	0.7346	-4.5
Administration period (yr)	6.1 (1.5-10.7)	-	-	-
Propensity score	0.65895 (0.11449-0.96977)	0.65895 (0.12913-0.96989)	0.9931	0.0

NA, nucleos(t)ide analogue; n.d., not done; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; W, wild type; M, mutant type; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; Child-Pugh classification, reference no [50]. Standardized difference in%; $100(\bar{X}_{NA} - \bar{X}_{non\text{-}NA})/([s^2_{NA} + s^2_{non\text{-}NA}]/2)^{1/2}$, where for each covariate X_{NA} and $X_{non\text{-}NA}$ are the sample means in NA and non-NA groups, respectively, and s^2_{NA} and $s^2_{non\text{-}NA}$ are the corresponding sample variances.

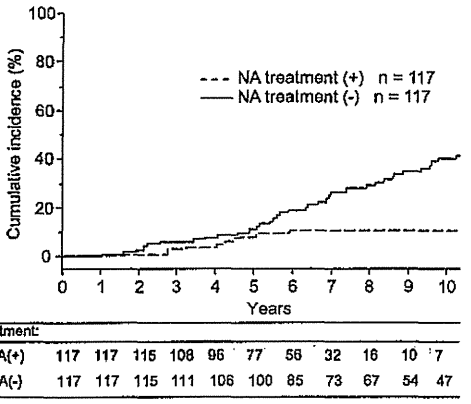

Fig. 2. Incidence of hepatocellular carcinoma (HCC) according to nucleos(t)ide analogue (NA) treatment status. The NA group had a significantly higher rate of progression to HCC than the non-NA group ($p = 0.0094$).

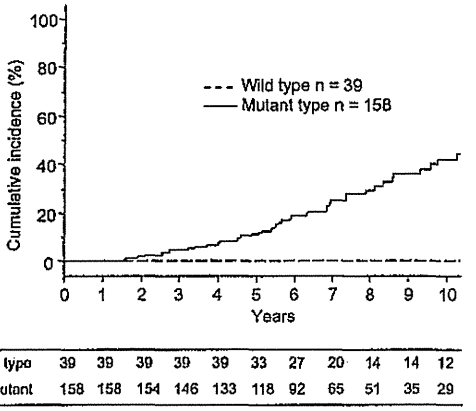

Fig. 3. Incidence of hepatocellular carcinoma (HCC) according to basal core promoter (BCP) mutations. Patients with mutant-type BCP had a significantly higher rate of progression to HCC than those with wild type BCP ($p = 0.0006$).

HBV DNA levels were significantly lower in patients on NA therapy than in patients not on NA therapy. In contrast, platelet counts and albumin levels were significantly higher in patients on NA therapy than in patients not on NA therapy.

Discussion

Our study shows that long-term NA maintenance therapy is associated with the suppression of progression to HCC. Liaw *et al.* reported that lamivudine decreased the risk of HCC in cirrhotic patients [7]. However, it is unclear whether the observed decreased risk of HCC with NA therapy was due to the short observation period in their study. It is very difficult to prove the preventive effect of NA on the development of HCC, because randomized control studies are not ethically possible. In this study, patients on NA therapy were compared to propensity score-matched untreated controls. In these control patients, NA therapy had not yet been approved or was not routinely used for chronic hepatitis B at the time, or was declined by the patient. As opposed to the entire population, these propensity-matched patients were well matched to patients on NA; significant differences included higher HBcrAg levels in the NA group.

Large community-based studies have confirmed that advanced age, male sex, HBeAg positivity, low platelet count,

Table 3. Factors associated with progression to hepatocellular carcinoma among propensity-matched patients (Cox proportional hazard model).

		Adjusted hazard ratio (95% CI)	*p* value
Age (yr)	≤40	1	0.015
	>40	4.36 (1.33-14.29)	
Treatment	no NA	1	0.002
	NA	0.28 (0.13-0.62)	
BCP	wild-type	1	0.012
	mutant-type	12.74 (1.74-93.11)	
HBcrAg (log_{10} U/ml)	≤3.0	1	0.036
	>3.0	2.77 (1.07-7.17)	
γ-GTP (IU/L)	≤56	1	0.001
	>56	2.76 (1.49-5.12)	

NA, nucleos(t)ide analogue; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; γ-GTP, gamma glutamyl transpeptidase.

higher ALT levels, elevated AFP levels, and presence of cirrhosis are factors associated with the development of cirrhosis and HCC [17,18]. Platelet count is a useful surrogate marker for the diagnosis of cirrhosis [37]. All subjects were not histologically diagnosed in this study. Therefore, we selected platelet count as a marker of hepatic fibrosis instead of cirrhosis. An elevated ALT level indicates the presence of active disease, and persistently elevated AFP levels are a reflection of an enhanced regenerative state in the liver [16]. In the REVEAL study, a high HBV DNA load was associated with an increased rate of HCC development [17]. A direct correlation was observed between baseline HBV DNA levels and the incidence of HCC, independent of serum ALT concentration. In a model that integrated baseline and follow-up HBV DNA levels, the cumulative incidence of HCC ranged from 1.3% in patients with undetectable levels of HBV DNA to 14.9% in patients with HBV DNA levels greater than or equal to 10^6 copies/ml. Therefore, we have selected factors, such as age, sex, HBeAg serostatus, HBV DNA concentration, platelet count, and ALT for propensity matching.

Although the exact mechanisms of hepatocarcinogenesis by HBV remain unclear, two mechanisms have been proposed [38,39]. One mechanism involves chronic necroinflammation of hepatocytes, cellular injury, and hepatocyte regeneration [40]. The other mechanism involves the direct carcinogenicity of HBV through chromosomal integration [41]. Complete and sustained viral suppression by NA might block both pathways and prevent the development of HCC. It is well known that the rate of HCC is significantly higher in patients with virological breakthrough or no response. In our study, when virological or biochemical breakthrough was observed and the YMDD mutation was detected in patients on lamivudine, adefovir dipivoxil was immediately added. In patients with cirrhosis, especially in the decompensated stage, sustained viral response on NA therapy was not necessarily associated with a preventative effect against the development of HCC, even though the incidence was lower than in a group not on NA [14]. It is not surprising that viral suppression decreased but did not eliminate the risk of HCC, because HBV DNA may have already integrated into the host genome before the initiation of therapy and may have resulted in genomic alternations, chromosomal instability, or both [42,43].

It is reported that patients with HBV genotype C infection have higher HBV DNA levels, higher frequency of pre-S deletions, higher prevalence of BCP T1762/A1764 mutations, and significantly higher chances of developing HCC [16,44–46]. In our study, T1762/A1764 mutations were observed in 158 (80.2%) out of 197 patients and were associated with a higher risk of developing HCC (adjusted hazard ratio, 12.740 [95% CI 1.743–93.108]), independent of NA therapy. However, the BCP T1762/A1764 mutations were detected in HCC patients from Asia and Africa, where HBV genotype C infection is predominant [16].

HBcrAg is a new HBV marker that reflects HBV load and corresponds to HBV DNA levels [21]. HBcrAg is comprised of HBV core antigen (HBcAg) and HBeAg; both are products of the precore/core gene and share the first 149 amino acids of HBcAg. The HBcrAg assay measures HBcAg and HBeAg simultaneously by using monoclonal antibodies that recognize both denatured HBcAg and HBeAg [47]. Serum HBcrAg concentration is well correlated with intrahepatic levels of covalently closed circular DNA (cccDNA) [48]. It is reported that HBcrAg is a useful marker for guiding cessation of NA therapy and evaluation of disease activity [21,49]. In our study, elevated serum HBcrAg concentration was associated with a higher risk of developing HCC (adjusted hazard ratio, 2.767 [95% CI 1.067–7.172]). This is the first report demonstrating a relationship between HBcrAg and HCC.

The present study has several limitations. The retrospective design might have introduced an unintended bias. The propensity matching method was adopted to reduce the confounding effects of covariates. Characteristics of patients who did or did not receive NA therapy were similar except for HBcrAg concentration.

Table 4. Average integration values of various parameters in patients who did or did not receive NA therapy.

	NA group (n = 117)	Non-NA group (n = 117)	*p* value
Platelet count (x10^3/m^3)	17.0 (3.3-37.2)	14.8 (3.3-296)	0.0060
ALT (IU/ml)	28.2 (8.5-88.9)	39.1 (12.2-737.5)	<0.0001
γ-GTP (IU/L)	27.0 (10.9-267.6)	36.2 (9.5-269.7)	0.0427
Total bilirubin (mg/dl)	0.7 (0.3-2.0)	0.7 (0.3-2.6)	0.1554
ALP (IU/L)	242.7 (113.5-1028.8)	265.2 (140.5-1247.6)	0.0127
Albumin (g/dl)	4.4 (3.0-5.0)	4.0 (2.4-4.8)	<0.0001
Alpha-fetoprotein (ng/ml)	2.2 (0.8-106.0)	4.5 (0.9-723.8)	<0.0001
HBV DNA (log_{10} copies/ml)	2.5 (2.1-8.9)	4.6 (2.1-9.3)	<0.0001

NA, nucleos(t)ide analogue; ALT, alanine aminotransferase; γ-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; HBV, hepatitis B virus.

Research Article

However, the non-NA group included many historical cases when NA therapy was not yet available. In addition, the HBV DNA assay used between 1998 and 2007 was not the most sensitive one.

In conclusion, NA therapy reduces the risk of HCC compared with untreated controls. Higher serum HBcrAg levels and BCP mutations are associated with development of HCC, independent of NA therapy.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] EASL Jury. EASL international consensus conference on hepatitis B, 13–14 September, 2002, Geneva, Switzerland, Consensus statement (short version). J Hepatol 2003;38:533–540.

[2] Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer 1988;61:1942–1956.

[3] Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet 1981;2:1129–1133.

[4] Szmuness W. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. Prog Med Virol 1978;24:40–69.

[5] Rustgi VK. Epidemiology of hepatocellular carcinoma. Gastroenterol Clin North Am 1987;16:545–551.

[6] Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 2004;127:S17–S26.

[7] Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521–1531.

[8] Shamliyan TA, MacDonald R, Shaukat A, Taylor BC, Yuan JM, Johnson JR, et al. Antiviral therapy for adults with chronic hepatitis B: a systematic review for a national institutes of health consensus development conference. Ann Intern Med 2009;150:111–124.

[9] Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003;36:687–696.

[10] Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 1999;30:567–572.

[11] Nguyen MH, Keeffe EB. Chronic hepatitis B: early viral suppression and long-term outcomes of therapy with oral nucleos(t)ides. J Viral Hepat 2009;16:149–155.

[12] Papatheodoridis GV, Dimou E, Dimakopoulos K, Manolakopoulos S, Rapti I, Kitis G, et al. Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. Hepatology 2005;42:121–129.

[13] Yuen MF, Seto WK, Chow DH, Tsui K, Wong DK, Ngai VW, et al. Long-term lamivudine therapy reduces the risk of long-term complications of chronic hepatitis B infection even in patients without advanced disease. Antivir Ther 2007;12:1295–1303.

[14] Eun JR, Lee HJ, Kim TN, Lee KS. Risk assessment for the development of hepatocellular carcinoma: according to on-treatment viral response during long-term lamivudine therapy in hepatitis B virus-related liver disease. J Hepatol 2010;53:118–125.

[15] Joffe MM, Rosenbaum PR. Invited commentary: propensity scores. Am J Epidemiol 1999;150:327–333.

[16] Liaw YF. Natural history of chronic hepatitis B virus infection and long-term outcome under treatment. Liver Int 2009;29:100–107.

[17] Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et alREVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;4:65–73.

[18] Yuen MF, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, et al. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. Gut 2005;54:1610–1614.

[19] Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Incidence of hepatocellular carcinoma in patients with chronic hepatitis B virus infection who have normal alanine aminotransferase values. J Med Virol 2010;82:539–545.

[20] Austin PC. Some methods of propensity-score matching had superior performance to others: results of an empirical investigation and Monte Carlo simulations. Biom J 2009;51:171–184.

[21] Kato H, Orito E, Sugauchi F, Ueda R, Gish RG, Usuda S, et al. Determination of hepatitis B virus genotype G by polymerase chain reaction with hemi-nested primers. J Virol Methods 2001;98:153–159.

[22] Kimura T, Rokuhara A, Matsumoto A, Yagi S, Tanaka E, Kiyosawa K, et al. New enzyme immunoassay for detection of hepatitis B virus core antigen (HBcAg) and relation between levels of HBcAg and HBV DNA. J Clin Microbiol 2003;41:1901–1906.

[23] Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol 2007;45:3942–3947.

[24] Liu CJ, Kao JH, Lai MY, Chen PJ, Chen DS. Evolution of precore/core promoter mutations in hepatitis B carriers with hepatitis B e antigen seroreversion. J Med Virol 2004;74:237–245.

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

[26] Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, 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.

[27] Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. J Hepatol 2009;50:729–735.

[28] Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008;38:37–51.

[29] Shen L, Li JQ, Zeng MD, Lu LG, Fan ST, Bao H. Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. World J Gastroenterol 2006;28:1292–1295.

[30] Iacobellis A, Fusilli S, Mangia A, Clemente R, Festa V, Giacobbe A, et al. Ultrasonographic and biochemical parameters in the non-invasive evaluation of liver fibrosis in hepatitis C virus chronic hepatitis. Aliment Pharmacol Ther 2005;22:769–774.

[31] Caturelli E, Castellano L, Fusilli S, Palmentieri B, Niro GA, del Vecchio-Blanco C, et al. Coarse nodular US pattern in hepatic cirrhosis: risk for hepatocellular carcinoma. Radiology 2003;226:691–697.

[32] Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology 2011;53:1020–1022.

[33] Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661–662.

[34] European Association for the Study of the L. EASL clinical practice guidelines: management of chronic hepatitis B. J Hepatol 2009;50:227–242.

[35] Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008;2:263–283.

[36] Greenland S. Basic methods for sensitivity analysis of biases. Int J Epidemiol 1996;25:1107–1116.

[37] Lu SN, Wang JH, Liu SL, Hung CH, Chen CH, Tung HD, et al. Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. Cancer 2006;107:2212–2222.

[38] Brechot C, Gozuacik D, Murakami Y, Paterlini-Brechot P. Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol 2000;10:211–231.

[39] Koike K, Tsutsumi T, Fujie H, Shintani Y, Kyoji M. Molecular mechanism of viral hepatocarcinogenesis. Oncology 2002;621:S29–S37.

[40] Chisari FV, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S, et al. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell 1989;59:1145–1156.

[41] Moradpour D, Wands JR. The molecular pathogenesis of hepatocellular carcinoma. J Viral Hepat 1994;1:17–31.

[42] Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. N Engl J Med 1981;305:1067–1073.
[43] Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 2001;120: 1763–1773.
[44] Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 2003;124:327–334.
[45] Tong MJ, Blatt LM, Kao JH, Cheng JT, Corey WG. Basal core promoter T1762/ A1764 and precore A1896 gene mutations in hepatitis B surface antigen-positive hepatocellular carcinoma: a comparison with chronic carriers. Liver Int 2007;27:1356–1363.
[46] Kusakabe A, Tanaka Y, Inoue M, Kurbanov F, Tatematsu K, Nojiri S, et al. A population-based cohort study for the risk factors of HCC among hepatitis B virus mono-infected subjects in Japan. J Gastroenterol 2011;46:117–124.
[47] Kimura T, Rokuhara A, Sakamoto Y, Yagi S, Tanaka E, Kiyosawa K, et al. Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. J Clin Microbiol 2002;40:439–445.
[48] Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol 2009;81:27–33.
[49] Matsumoto A, Tanaka E, Minami M, Okanoue T, Yatsuhashi H, Nagaoka S, et al. Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy. Hepatol Res 2007;37:661–666.
[50] Pugh RNH, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646–649.

学術助成論文

血液透析患者における肝炎ウイルス感染率と生命予後

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key words：生命予後，血液透析，肝疾患，C型肝炎ウイルス，B型肝炎ウイルス

要　旨

血液透析患者集団では，肝炎ウイルス陽性率が高いことが以前より知られているが，肝発がん予防まで考慮した積極的な抗ウイルス治療は現実には進んでいない．肝炎ウイルスキャリアへの抗ウイルス療法に対して公費助成金制度が2008年より開始され，また2011年には透析患者におけるC型肝炎治療ガイドラインが策定されている．我々は，血液透析患者における肝炎ウイルスの感染とその予後との関連についての検討を行った．

広島県内9透析施設で行った肝炎ウイルス感染状況調査（1999年から2004年）に登録された3,096例を対象として，2010年に転帰，合併症の有無，原疾患などを調査するとともに，採血によるB型肝炎ウイルス（HBV）検査，C型肝炎ウイルス（HCV）検査を行い，関連性について検討した．

解析対象とした3,087例中，HBs抗原単独陽性は2.2%（68例），HCV RNA単独陽性は13.8%（425例），重複感染は0.2%（7例），両方陰性は83.8%（2,587例）であった．観察期間中の累積死亡率はそれぞれのグループで45.6%，60.2%，57.1%，47.2%であり，HCV感染で有意に高くなっていた（$p<0.001$）．また肝疾患関連死はそれぞれ死亡の9.7%，8.6%，0%，1.3%であった．

転帰日の明らかな3,064例について，死亡をエンドポイントとした生存分析により，HBVとHCV共に感染の有無による生存率の差は認められなかった．一方，原疾患が「糖尿病性腎症」，あるいは「糖尿病を合併している」と有意に生存率が低い結果であった（$p<0.0001$）．

血液透析患者では一般住民集団よりも肝炎ウイルス陽性率が高いにもかかわらず，肝疾患関連死は少ないことが示された．肝炎ウイルス感染は生存率との関連性は認められなかった．

1　はじめに

血液透析患者では，頻回の観血的処置により肝炎ウイルス感染のハイリスク集団であることがよく知られている．現在では透析施設にて院内感染防止の措置がとられ，新規の感染予防対策が行われているが，いまだに肝炎ウイルス陽性率（有病率・キャリア率）は高いままの状態である[1]．

我が国では，ウイルス性肝炎患者に対する公費医療助成が2008年より開始され，肝炎ウイルス感染者の新規受療が促進されている．血液透析患者に対しても，慢性に感染が持続するいわゆるキャリアの状態にある者に，定期的検査や抗ウイルス療法介入の推奨がなされている．2011年には，透析患者におけるC型肝炎治療ガイドラインも発表された[2]．

我々は，1999年から行っている広島県下の血液透析患者集団のコホート調査にて，肝炎ウイルス陽性率，

A Study of prevalence of hepatitis virus among hemodialysis patients and its relevance to their prognosis
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HCV罹患率，院内感染に対する予防策などについて報告してきた[3]．今回の研究は，肝炎ウイルス感染のある血液透析患者の生命予後について検討するため，1999年からの血液透析患者集団コホートにて，肝炎ウイルス感染とその生命予後に関する調査を行った．

2 対象と方法

広島県内の九つの透析施設（表1）において，1999年11月から2004年8月まで行った，肝炎ウイルス感染状況調査のさいに，登録された3,096例を対象とした．男性1,818例，女性1,278例，1999年時の平均年齢は61.3±13.2歳であった．

2-1 転帰調査

2010年に各施設にて3,096例の転帰調査を行った．予後に関する項目として，臨床経過，転帰，転帰日，死亡の場合は死因，各施設での肝炎ウイルス検査の結果，肝疾患の有無と，透析開始日，透析に至った原疾患，糖尿病の有無などについて調査を行った．

2-2 肝炎ウイルス検査

今回の調査で，採血可能な症例は同意を得て新規採血を行い，行えなかった症例では保存血清にて肝炎ウイルス検査を行った．HBV検査について，HBs抗原検出のためマイセルII HBsAg検査（凝集法）を行った．HCV検査については，HCV RNAの5'NC領域のnested PCR検査[3]にて検出を行った．観察期間中に一度でもHBs抗原が陽性，あるいはHCV RNAが陽性であるものをそれぞれ「陽性」と判定した．

2-3 予後の解析

肝炎ウイルス感染と予後の関連を見るための解析として，HBs抗原の有無，HCV RNAの有無の肝炎ウイルス感染別にみた，肝疾患関連死亡率を算出した．各群の比較は二元配置分散分析にて行った．またカプランマイヤー法による生存分析を，肝炎ウイルス感染別：HBs抗原，HCV RNAの有無，原疾患別：糸球体腎炎・糖尿病性腎症・その他，糖尿病合併の有無別で行った．

なお，この調査は連結可能匿名化データの調査として，広島大学疫学研究倫理審査委員会の承認を得ている．

3 結 果

調査対象3,096例から臨時透析2例，転帰不明3例，透析導入日不明3例，肝炎ウイルス検査結果不明1例の9例を除き，3,087例を解析対象とした．男性1,815例，女性1,272例，観察終了までの平均透析期間は10.2±7.5年であった．

全体の肝炎ウイルスの感染状況（図1）は，HBs抗原単独陽性2.2%（68/3,087），HCV RNA単独陽性13.8%（425/3,087），重複感染0.2%（7/3,087），両方陰性83.8%（2,587/3,087）であった．

3-1 肝炎ウイルス感染の有無別転帰

観察開始の1999年11月から2010年12月までの転帰をまとめると，観察期間中の死亡は対象の約半数の48.9%（1,511/3,087）であった（図2）．肝炎ウイルス感染状況別にそれぞれ転帰をみると，HCV RNA単独陽性グループの死亡率は60.2%（256/425）であった．各4群の死亡率を比較すると，二元配置分散分析ではHCVの感染により死亡率が高いことが明らかとなった（図3，$p<0.001$）．

また，全体での肝疾患関連死は，肝不全・肝硬変が1.9%，肝細胞がんが0.9%であった（図4）．肝炎ウイルス感染状況別での肝疾患関連死は，HBs抗原単独

表1 調査対象施設

特定医療法人あかね会 土谷総合病院
医療法人一陽会 原田病院，同 一陽会クリニック，同 イーストクリニック
医療法人社団仁友会 尾道クリニック
医療法人社団スマイル 博愛クリニック
医療法人辰川会 山陽病院
医療法人 中央内科クリニック
医療法人社団博寿会 山下医院
医療法人社団 博美医院
医療法人社団光仁会 フェニックスクリニック

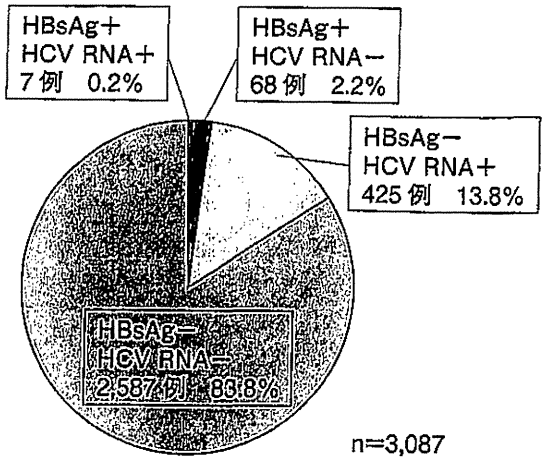

図1 透析患者3,087例の肝炎ウイルス感染状況の内訳

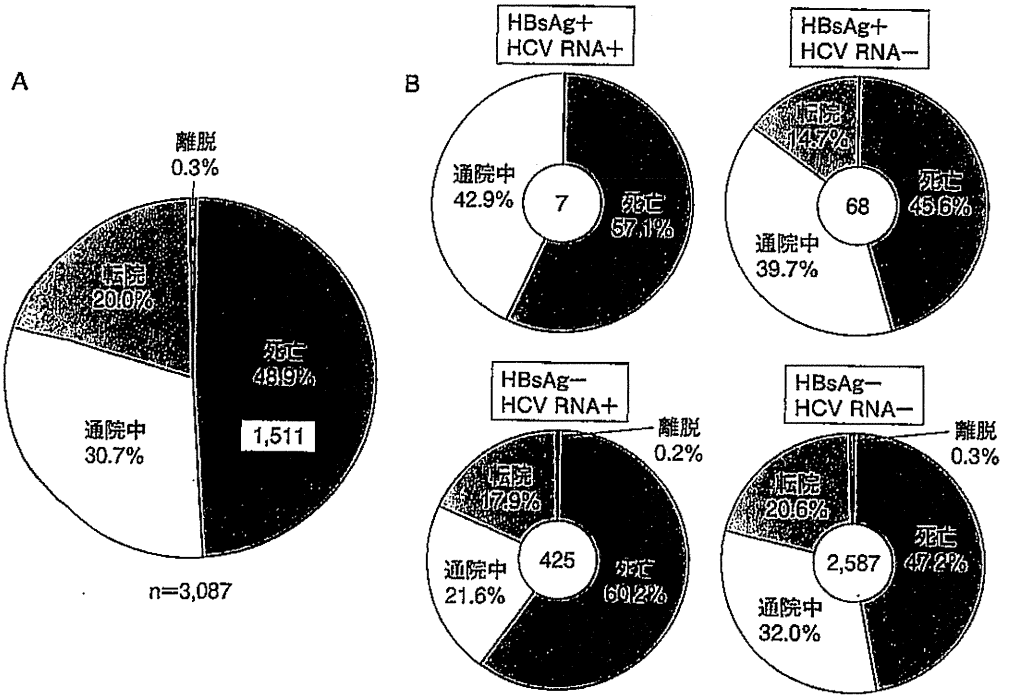

図2 観察した3,087例の転帰の内訳
(A) 全体では1,511例が死亡した．(B) 肝炎ウイルスの感染状況別にみた転帰の内訳とその割合を示す．

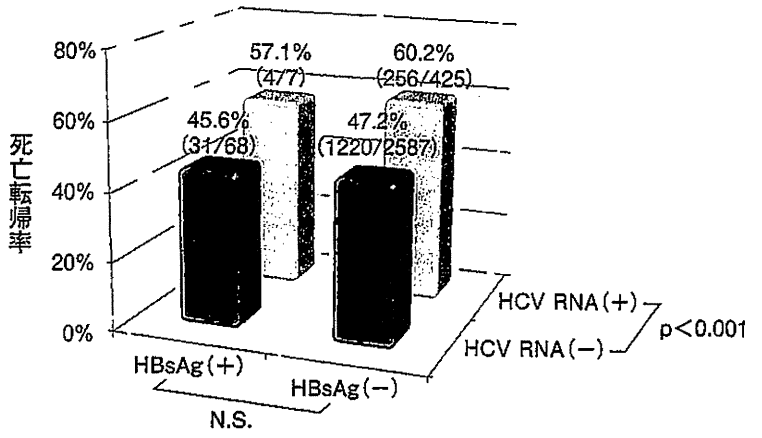

図3 肝炎ウイルス感染状況別にみた死亡転帰の割合

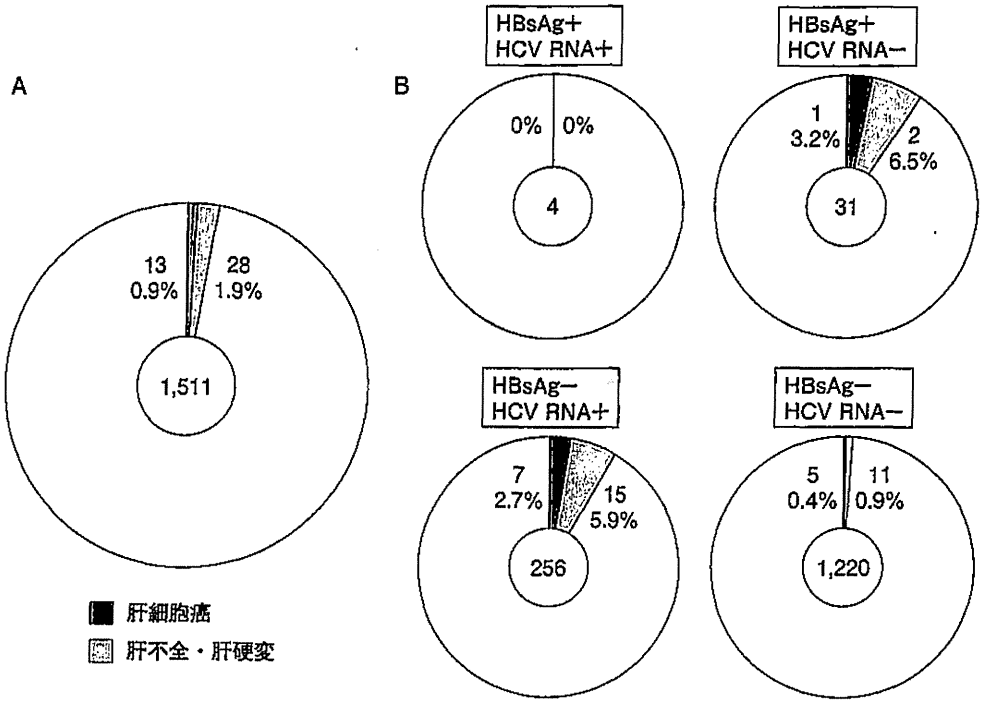

図4 観察期間中に死亡した1,511例の肝疾患関連死亡

(A) 全体と (B) 肝炎ウイルスの感染状況別を示す．死亡時の平均年齢は72.2±11.2歳(24〜99歳)．

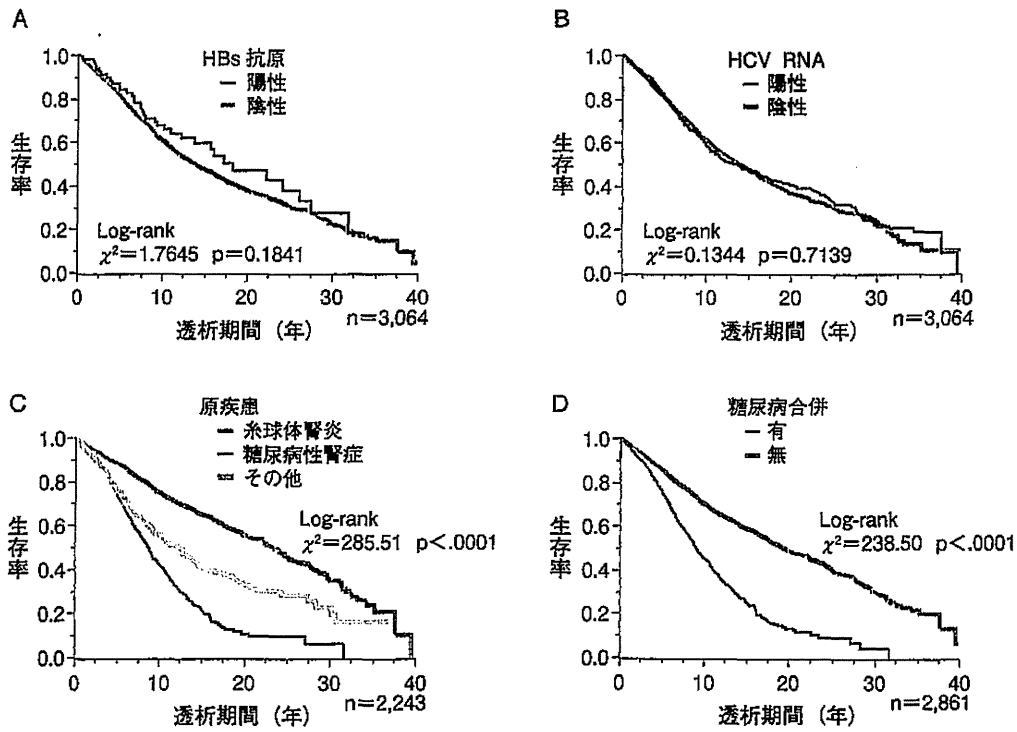

図5 カプランマイヤー法による各因子での生存時間分析

(A) HBV感染の有無，(B) HCV感染の有無では生存率に違いは無く (p=0.1841，p=0.7139)，(C) (D) 糖尿病性腎症，糖尿病の合併有で，生存率が低くなった (p<0.0001)．

陽性グループで9.7% (3/31人，肝不全・肝硬変6.5%，肝細胞がん3.2%)，HCV RNA単独陽性グループで8.6% (22/256人，5.9%，2.7%)，重複感染のグループ0%，両方陰性のグループで1.3% (16/1,220人，0.9%，0.4%) であった．

3-2 生存分析

解析対象3,087例中，転帰日の明らかな3,064例に

ついて，死亡をエンドポイントとして，生存分析を行った（図5）．各因子に関する情報が得られなかった対象を除外すると，肝炎ウイルス感染の有無については3,064例，原疾患について2,243例，糖尿病について2,861例で解析が行えた．

HBV，HCVともに感染の有無での生命予後の差は認められず，一方「原疾患が糖尿病性腎症である」こと，「糖尿病を合併している」ことで有意に生存率が低くなっていた．

4 考 察

肝炎ウイルスの感染は一般に肝発がんの大きなリスクファクターであり，また発がんしなくても，肝硬変，肝不全への肝疾患の進展もあるため，大きく死亡リスクと関与している．そのため，感染に終止符を打つ事を目的とした抗ウイルス療法が現在の肝炎治療の大きな柱となっている．

血液透析患者にとっても同様に，抗ウイルス療法に言及した，「透析患者のC型ウイルス肝炎治療ガイドライン」が2011年，日本透析医学会より発表された[2]．それによると，C型慢性肝炎に対しては，

① 生命予後の期待できるHCV感染透析患者に対しては積極的に抗ウイルス療法を推奨

② 腎移植が予定されているHCV感染透析患者にも抗ウイルス療法が推奨

とされている．

ガイドラインにも示されているが，副作用の発現頻度がやや高いこと，血液透析患者では元々ALTが低いので，肝炎が起こっていてもALTが正常値に近く重篤感に欠け，透析病院以外への受診が患者に受け入れられにくく，肝臓専門医への紹介が困難なことなどより，実際には抗ウイルス療法はまだ血液透析患者に普及しているとはいえず，これからも急速に普及が進むとは考えにくい．

血液透析患者集団のHCV抗体陽性者について，その予後はHCV抗体陰性者に比べ悪いとの報告がある[4]．しかし，本研究では，二次元配置による解析ではHCV感染で死亡率が高いことが示されたが，生存分析を行ったところ，血液透析患者における肝炎ウイルス感染とその生存率に関連は認められず，血液透析患者の予後を大きく左右しているのではないことが示された．その報告との大きな違いは，我々はHCV抗体ではなく，HCV RNAの検出を用い，C型肝炎ウイルスの感染を確定して解析したことである．そのため，HCV抗体陽性かつHCV RNA陰性の，いわゆる感染既往者を解析から除外できているため，結果に相違が出た可能性がある．

我々も以前報告したように[3]，血液透析患者のHCV感染は，院内感染として高率に起こっており，いまだに肝炎ウイルス感染のハイリスクグループとしてとらえられている．近年，一般住民検診や，初回献血者での肝炎ウイルス陽性率（キャリア率）は大変低く，HBVは0.71%，HCVは0.63%である[5]．一方，血液透析患者のHCV陽性率はこのコホートグループで2003年に報告したものでは12.9%[3]，安藤等の血液透析施設における2006年調査の報告でもHBV陽性率は2.39%，HCV陽性率は11.27%[1]と大変高くなっている．

血液透析患者の高い肝炎ウイルス陽性率（キャリア率）より，それらの死因，生命予後には肝疾患が関連するのではという仮説を考えた．しかし，肝炎ウイルスの感染状況別での死因の内訳をみると，約10年間の累積肝疾患関連死はHBV陽性のグループで9.7%，HCV陽性のグループで8.6%であった．血液透析患者集団では一般集団よりも大変キャリア率が高いにもかかわらず，肝疾患関連死は予想に反して，少ないことが特徴であることが今回の研究で示された．

この集団では，約10年間の観察期間中の累積死亡率は約50%と高く，おそらく「肝疾患を長く患う」前に，全身状態が悪化し死亡する経過と推測される．血液透析患者の死因として，2010年死亡をみると，心血管疾患である心不全，脳血管疾患，心筋梗塞と感染症，悪性腫瘍が多かった[6]．糖尿病は，動脈硬化を進展させ，易感染性を増大させるため，これら死因の上位に占める疾患に直結したものであるので，今回明らかとなった，原疾患が「糖尿病性腎症」あるいは「糖尿病の合併がある」ということが，生存率の低下と関連していたという結果は矛盾しないと考えられる．

我々が観察した血液透析患者集団において，「肝炎ウイルス感染」はHBVにしても，HCVにしても，感染の存在自体に生存率との関連性は認められなかった．さらに詳しく死因や生命予後に関連する因子についての検討が必要と考えた．

実際には血液透析患者においても肝疾患関連死が存