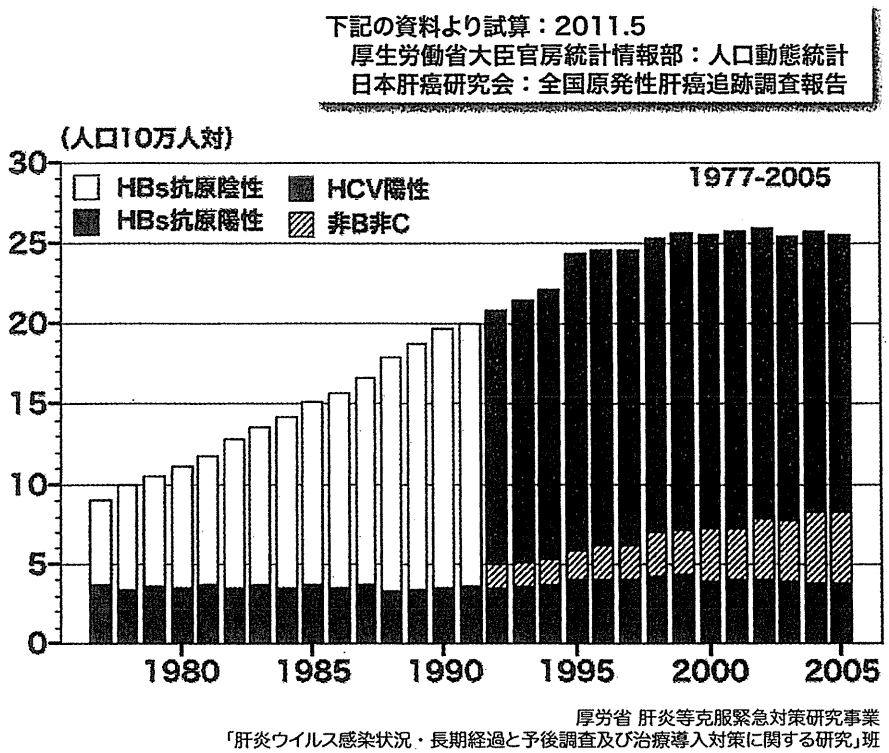

図2　病因別にみた肝がんによる死亡数の経年的推移

たことがわかる．1992年以降，HCV 感染の診断が可能となると図2のようにそのうちの約90％が HCV の持続感染に起因するものであったことが見て取れる．一方，2000年以降，非 B 非 C 型に由来する肝細胞癌による死亡割合が増加傾向にあることが明らかとなり，その原因や動向について NASH(Non-alcoholic steatohepatitis）との関連性が示唆されている．しかし，わが国の肝細胞がんによる死亡の約７割は HCV の持続感染に起因するものであり，肝癌対策を構築する上でも，HCV 持続感染者（HCV キャリア）の規模の把握や HCV 感染予防対策が重要と考えられる．

4. HCV キャリア率の把握（Prevalence）

4-1. 一般集団における HCV キャリア率

HCV 持続感染者（HCV キャリア）の規模の把握を試みるために，2000年以後に得られた大規模集団，すなわち初回供血者集団と節目検診受診者集団から一般集団における年齢階級別にみた HCV キャリア率（prevalence）を算出し示す．

日本赤十字血液センターの献血時のスクリーニング検査は，輸血用血液の安全性確保のために行われるものであり，全国一律の基準，同一の試薬を用いて精度を維持し判定されている．また，2002年から５ヶ年計画で実施に移された節目・節目外検診は，老人保健法の住民検診に組み込まれた形で，公的補助により肝炎ウイルス検査（C 型肝炎ウイルス検査，B 型肝炎ウイルス検査）が行われたものであり，全国統一の検査手順に従って判定されたものである．

．いずれも，自身が肝炎ウイルスに感染していることがわかっている場合は，献血や検診の対象者にはならないと考えられることから，この２つの集団から得られた HCV キャリア率は，感染を知らずにいる感染者の割合を示している．

また，初回供血者集団はその約85％が40歳未満の年齢であり，また，節目検診受診対象者は40歳以上の年齢層であることから，40歳未満の年齢層における HCV キャリア率については初回供血者集団の資料を，40歳以上の年齢層における HCV キャリア率は節目検診受診者集団の資料を用いた．

すなわち，2001年から2006年の全供血者のうち「初回供血者」3,748,422人の資料を抽出し，20～39歳（2005年時点の年齢換算）のHCV抗体陽性率に70％を乗じた値をHCVキャリア率とした．また，厚生労働省「肝炎ウイルス検診」の「節目検診受診者」6,204,968人の成績を用いて40～74歳のHCVキャリア率を算出した(図3)[5]．

全国8地域別，5歳刻みの年齢階級別HCVキャリア率を図3に示す．HCVキャリア率は，8地域ともに高年齢層において高い値を示し，20歳代以下の若年層では0.2％以下の極めて低い値を示す傾向が認められている．また，肝発がん年齢と考えられる60歳以上の高年齢集団のHCVキャリア率は，関東以西の地域，すなわち北陸東海(1.9％)，近畿(2.1％)，中国(1.7％)，四国（2.0％）の地域では，北海道（1.1％）や東北地域（0.9％）と比較して高い値を示していることがわかる．

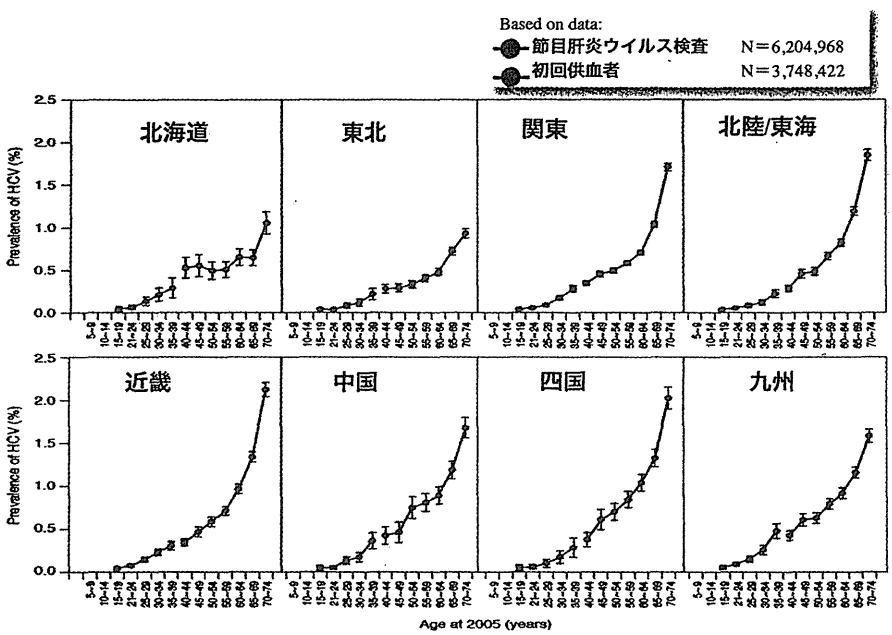

図3　地域別年齢階級別にみたHCVキャリア率

表1　出生年別にみた小学生でのHCV感染率

岩手県予防医学協会

出生年	対象数	HCV抗体陽性数(%)	小計
1978	2,429	4 (0.16)	
1979	4,180	4 (0.10)	
1980	3,538	6 (0.17)	HCV抗体陽性数
1981	2,512	3 (0.12)	24/26,996(0.09)
1982	1,591	1 (0.06)	
1983	1,088	0 (0.00)	HCV RNA陽性数
1984	5,991	4 (0.07)	Not Done
1985	5,667	2 (0.04)	
1986	6,775	2 (0.03)	HCV抗体陽性数
1987	6,505	6 (0.09)	26/32,049(0.08)
1988	6,310	10 (0.16)	
1989	6,436	5 (0.08)	HCV RNA陽性数
1990	6,023	3 (0.06)	7/32,049(0.02)
合計	59,045	50 (0.08)	

4-2. 児童におけるHCVキャリア率

岩手県予防医学協会がとりまとめた小学校入学時の調査成績を表1に示す．HCV抗体陽性率は，いずれの出生年においても0.1%あるいは0.1%以下の極めて低い値を示していることがわかる．ただし，節目検診の成績からみたHCVキャリア率を都道府県別にみると，岩手県は全国でも低率の県にあたることから，岩手県の調査成績がそのまま全国の児にあてはまるとはいえない．しかし，20歳以下の年齢層におけるHCVキャリア率は前項で示したように全国いずれの地域においても低いことから，他の地域における児童のHCVキャリア率も同様に低い値であることが推察される．

なお，HBV母子感染防止事業は1986年以後に出生したすべての児を対象に全国規模で実施されているが，HCV抗体陽性率/HCVキャリア率に関しては1986年を境にした前後の2つの期間に出生した児の集団間の差は認められていない．

5. HCV感染のリスク（Incidence）

感染の広がりを示すprevalenceについては，地域別あるいは年齢別のHCVキャリア率あるいはHCV抗体陽性率からその概要を示した．次に，感染のリスクを示すincidenceについて，これまでの疫学的調査結果をもとに，水平感染と母子感染の項を分けて示す．

5-1. 水平感染について

水平感染によるHCV新規発生について前向き調査を行った成績を表2に示す．

供血者集団を対象とした調査では，広島県赤十字血液センターにおける1994年6月から2004年4月までの供血者418,269人（総献血本数1,409,465本）を対象として前向きに観察し[6]，新たな感染の有無について解析を行ったところ，期間内に複数回献血をした218,797人（861,842人年）のうち新たなHCV感染が確認されたのは16例であり，人年法による解析でHCV新規発生率は10万人年あたり1.86人（95% CI：1.06～3.01人/10万人年）と示された．この成績は，同様の調査を1992年から3年間の観察期間で行った結果（1.8/10万人年，95% CI:0.4～5.2人/10万人年）とほぼ同じ値であった[7]．一方，女性のHCV新規発生率は2.77人/10万人年（95% CI：1.38～4.95人/10万人年）と，統計学的な有意差は認められなかったが，男性（1.08人/10万人年（0.35～2.51人/10万人年））

表2　HCV感染の新規発生率　1988～2004

	対象者	新規感染	観察人年	新規感染率　95% CI Incidence Rate
●供血者【広島】				
1992～1995	114,266	3	168,479	1.8/10万人年 0.4～5.2
1994～2004	218,797	16	861,842	1.9/10万人年 1.1～3.0
●供血者【大阪】				
1992～1997	448,020	59 ※抗体陽転	1,095,668	5.4/10万人年 4.1～7.0
●定期健康診断受診者【広島】				
1992～1995	3,079	0	5,786	0/10万人年 0～0.6
●障害者・老人福祉施設入所者【静岡】				
1988～1992	678	0	2,712	0/10万人年 0～1.3
●血液透析施設【広島】				
1999～2003	2,744	16	4,893	3.3/1000人年 1.7～4.9

と比較して高い値を示していた．年代別の検討では，女性の20歳代（3.21人：0.87～8.22人/10万人年），50歳代（6.02人：1.64～15.42人/10万人年）の新規感染率が他の世代と比較してやや高い傾向があったが，その理由については不明である．また，大阪の供血者集団を対象とした1990年代前半の調査では，HCV抗体陽転率[8]は5.4人/10万人年（4.1～7.0人/10万人年）であり，抗体陽性の70％をHCVキャリアと換算しても，広島の同集団と比較するとやや高く，地域により新規感染率の多寡に相違がある可能性が示唆される．

一方，1990年代の同時期に行われた定期健康診断受診集団や障害者・老人福祉施設入所者集団を対象とした血清疫学的調査からは，新規感染者は見いだされていない．

次に，観血的処置を頻回に受ける血液透析患者を対象とした多施設前向き調査を行った成績[9]では，3ヶ月以上の観察が可能であった2,114人のうちHCVキャリアの新規発生数は16例あり，これを人年法により推計すると，HCV新規感染率は1,000人年あたり3.3人（95％CI：1.7～4.9人/1,000人年）となった．

上記に示した成績は，現在のわが国の一般集団においてはHCV感染の新規発生はごく稀であることを示している一方，血液を介した感染の可能性がある集団等における新規発生率は，供血者集団と比較して10^2倍程度高い値を示すことから，引き続きHCV感染防止対策は重要であることが示唆されている．

5-2. 垂直感染，母子感染について

わが国におけるHCVの母子感染が白木[10]によって初めて報告された時点では，HCVの母子感染予防対策が公衆衛生上，社会において必要であるかが検討課題であった．

当時，広島と愛媛における34の病・医院の産科・小児科の協力により，健康な妊婦16,714人を対象に行ったHCV母子感染に関する前向き調査[11]の成績を示す（図4）．1990～1993年の観察期間に，追跡可能かつ協力が得られた84人

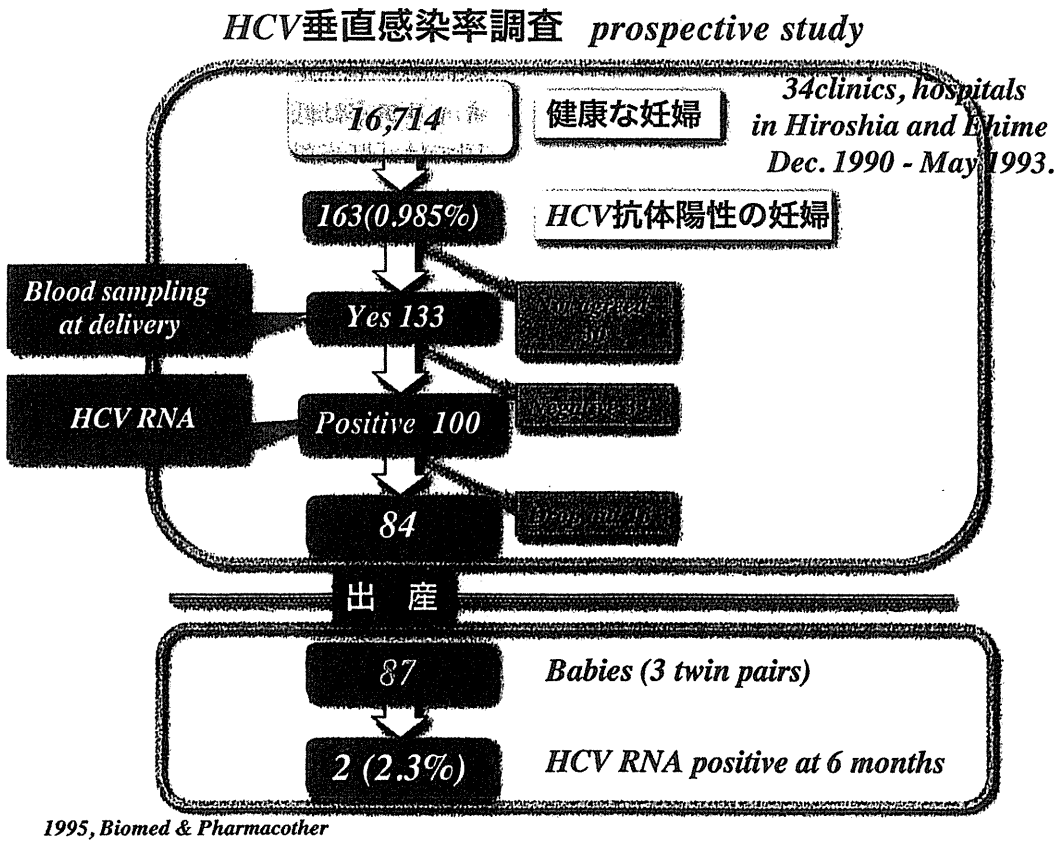

図4　HCV垂直感染率調査

のHCVキャリア妊婦から生まれた87児のうち，６ヶ月時点で感染が確認されたのは２例（2.3%）であった．２例の母親の出産時のHCV RNA量は1.0×10^7 Eq /ml(bDNA)，2.3×10^7 Eq /ml(bDNA）と高く，genotypeは母子共にそれぞれ2a，1bであり，児は24ヶ月，12ヶ月時点でHCV RNAが検出され感染が確認されている．

一方，HCV母子感染率の頻度に関する他の調査成績から報告された値は，調査地域や対象妊婦の背景因子の相異などにより２～10%と幅が大きい[12,13]．また，感染が確認された児の同胞すべてが感染成立したとはいえず，分娩方法や児の免疫能，出産時の母体のHCV RNA量などが関与していることが示唆されている．諸外国における調査報告からは，母親がHIV-HCV重複感染の場合のHCV母子感染率は高いことが明らかとなっているが，HCV単独感染の場合の母子感染率は低いことから，わが国では公的補助によるHCVの母子感染予防措置は行われていない．

6. 感染症法によるC型急性肝炎の発生状況について（相崎）

わが国では1999年４月に施行された感染症法により，急性のウイルス性肝炎を診断した医師は全例保健所へ届け出ることが必要になった．C型急性肝炎は，５類感染症に分類されており，届け出に基づいた集計解析は国立感染症研究所において行われている．

1999年４月から2009年12月までに届け出されたC型急性肝炎723例について[14]まとめたものを紹介する．1999年以来，急性C型肝炎と診断され報告された年別の患者数は，1999年136症例，2000年119症例，2001年65症例と2001年までは減少傾向が認められたが，それ以降2009年まで年間約30～70症例でほぼ横ばいに転じており，男女別に相異は認められていない．年齢階級別にみた報告数の分布では，30代前半及び50代後半の２つのピークが認められるが，14歳以下の小児または90歳以上の高齢者の報告は極めて少ない．男女別にみると，30代前半及び50代後半にみられる報告数のピークは女性で認められており，背景に感染の要因が潜在していることが推察される．都道府県別にみると，大都市部である大阪（125例），東京都（55例）等の報告数が多い一方，報告数がゼロの都道府県もありC型急性肝炎発生率には地域差が認められるが，報告義務の履行状況が地域ごとに異なる可能性もあり，発生数（率）の評価には注意が必要である．

2006年４月以降に報告されたC型急性肝炎167例について，感染の「原因不明」が全体の62%を占め，HCV感染原因は特定しにくいことが示されている．そのほかの感染原因として報告されたのは，針等刺入（22%），性的接触（11%）であった．また，報告総数は少ないが全体の22%を占める「針等刺入」の内訳では，針刺事故など医療行為に伴う感染以外に，ピアス，刺青，カミソリの共用，覚醒剤など，と報告されている．

医師の届け出義務の周知を広く徹底すると共に，得られる情報を適切に予防対策や啓蒙活動に取り入れることが求められている．

7. おわりに

わが国の社会生活全般における水平感染の発生要因が急速に消滅し，新規感染が低下した結果，若い世代におけるHCV抗体陽性率/HCVキャリア率は低い値を示すに至っている．わが国では「肝炎対策基本法」(2009年12月）を基盤として，すでに感染しているキャリアへの対策，具体的には，肝炎ウイルス検査の推進，肝疾患診療ネットワークの構築，新規治療法の開発等が積極的に進められている．

さまざまな機会で肝炎ウイルス検査が行われることにより感染を知る機会が増えたことで，感染を知らないままでいるHCVキャリア数は2005年時点，約81万人と推計し[15]，2000年時の推計値と比較して減少したと示した．一方で，感染していることを知ったがさまざまな理由から医療機関への受診をしないままでいるHCVキャリアや医療機関への継続受診に至っていないHCVキャリアが増加していることが問題点として指摘されている[16]．

世界的にみても肝炎対策先進国であるわが国は，これまでの感染防止策を継続しつつ，肝炎肝がん対策の新たな局面を迎えていると考えら

れる.

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Characteristics of elderly hepatitis C virus-associated hepatocellular carcinoma patients

Takashi Kumada,* Hidenori Toyoda,* Seiki Kiriyama,* Makoto Tanikawa,* Yasuhiro Hisanaga,* Akira Kanamori,* Toshifumi Tada* and Junko Tanaka†

*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Gifu, and †Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

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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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Correspondence
Dr Takashi Kumada, Department of Gastroenterology, Ogaki Municipal Hospital, 4-86, Minaminokawa-cho, Ogaki, Gifu 503-8052, Japan. Email: hosp3@omh.ogaki.gifu.jp

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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 ($\leq$ 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 ($\geq$ 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 ($\geq$ 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 ($\leq$ 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' ($\leq$ 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.

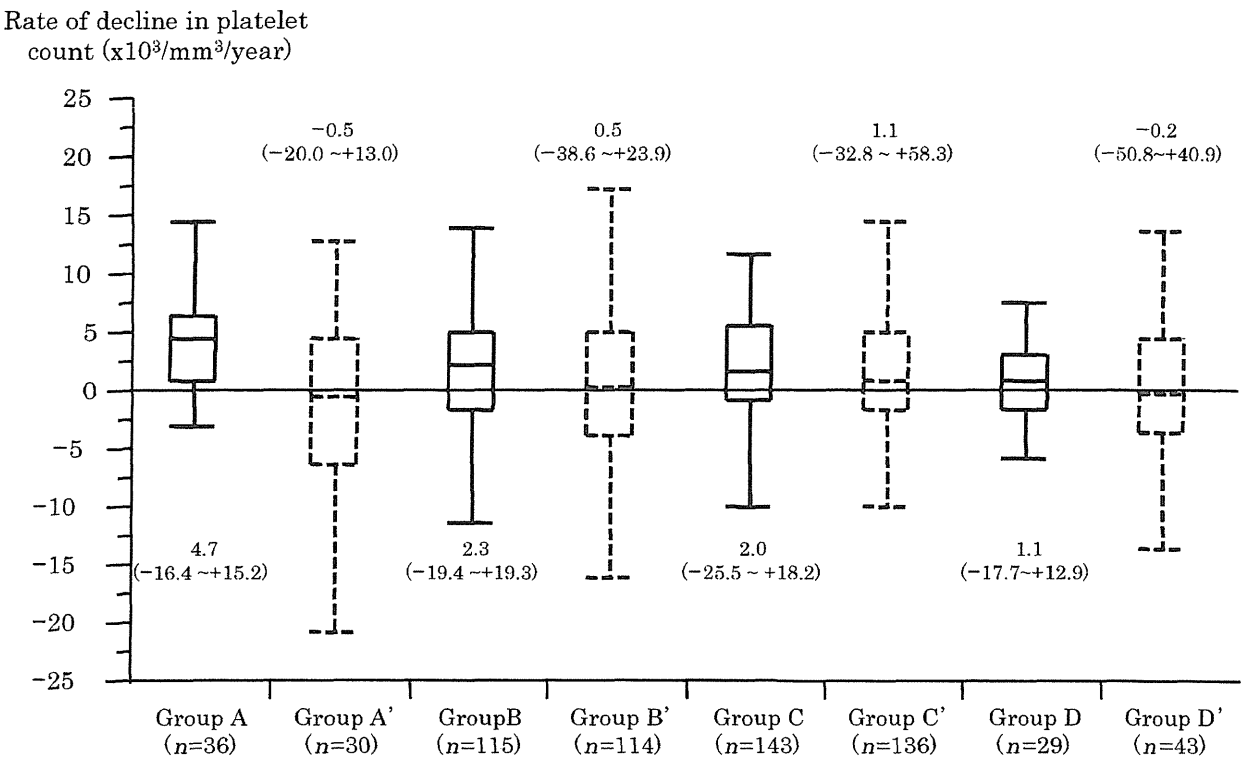


Figure 1 Rate of decline in platelet count prior to hepatocellular carcinoma (HCC) diagnosis in HCC patients and prior to the end of follow-up in control patients. The annual rate of platelet count decline in the period prior to HCC diagnosis was lower in the groups that were older at the time of HCC 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.

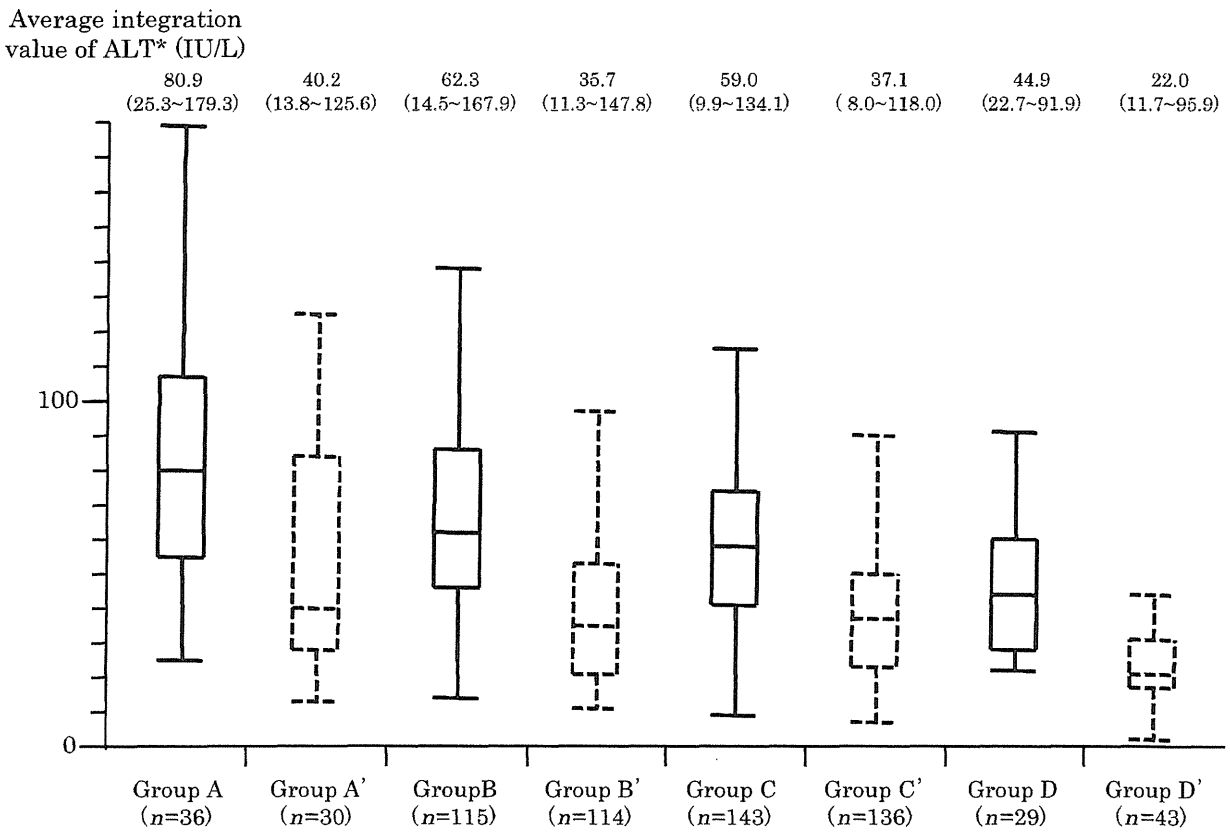


Figure 2 Average integration values of alanine aminotransferase (ALT) prior to HCC diagnosis in HCC patients and prior to the end of follow-up in control patients. Patients who were older at the time of HCC diagnosis had lower average integration values of ALT in the period prior to HCC diagnosis. In 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 ($P < 0.0001$).

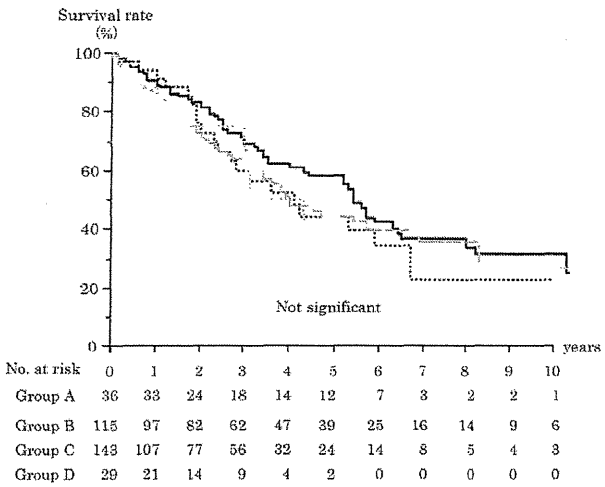
Table 6 Profile of HCV-infected HCC patients at the time of HCC diagnosis

	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 (1/2/3/4)	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.



No. at risk	0	1	2	3	4	5	6	7	8	9	10
Group A	36	33	24	18	14	12	7	3	2	2	1
Group B	115	97	82	62	47	39	25	16	14	9	6
Group C	143	107	77	56	32	24	14	8	5	4	3
Group D	29	21	14	9	4	2	0	0	0	0	0

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.

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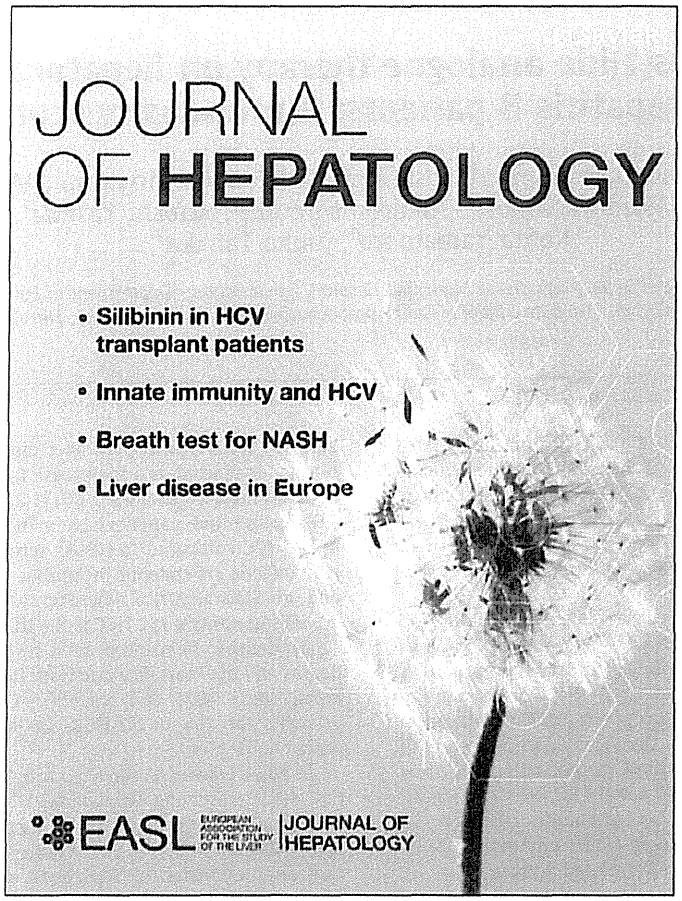

Research Article

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

Takashi Kumada[1,*], Hidenori Toyoda[1], Toshifumi Tada[1], Seiki Kiriyama[1], Makoto Tanikawa[1], Yasuhiro Hisanaga[1], Akira Kanamori[1], Takurou Niinomi[1], Satoshi Yasuda[1], Yusuke Andou[1], Kenta Yamamoto[1], Junko Tanaka[2]

[1]*Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, Ogaki, Japan;* [2]*Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*

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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* Corresponding author. Address: Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, 4-86, Minaminokawa-cho, Ogaki, Gifu 503-8052, Japan. Tel.: +81 584 81 3341; fax: +81 584 75 5715.
E-mail address: hosp3@omh.ogaki.gifu.jp (T. Kumada).

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].

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).

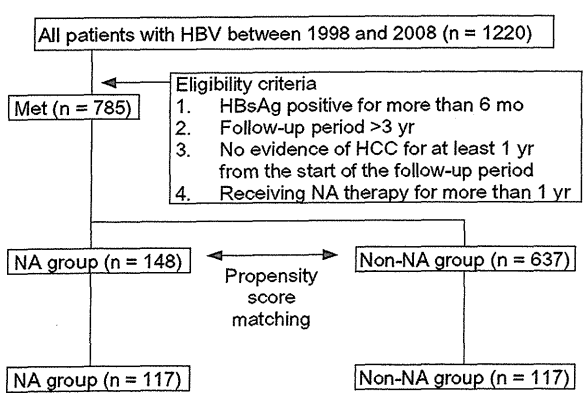

Fig. 1. Flowchart of the patient selection process.

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%; $100(X_{NA} - X_{non\text{-}NA})/([s^2_{NA} + s^2_{non\text{-}NA}]/2)^{1/2}$, 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%; $100(X_{NA} - X_{non\text{-}NA})/([S^2_{NA} + S^2_{non\text{-}NA}]/2)^{1/2}$, 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.

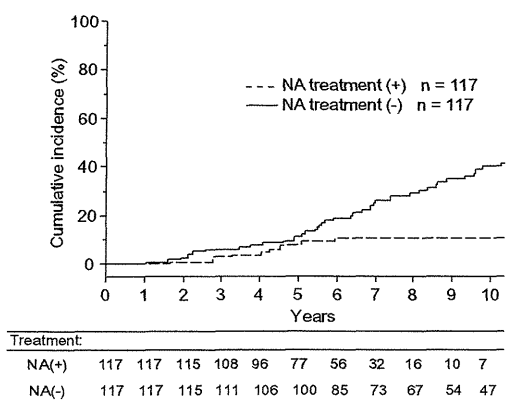

Fig. 2. Incidence of hepatocellular carcinoma (HCC) according to nucleos(t)ide analogue (NA) treatment status. The NA group had a significantly higher rate of progression to HCC than the non-NA group ($p = 0.0094$).

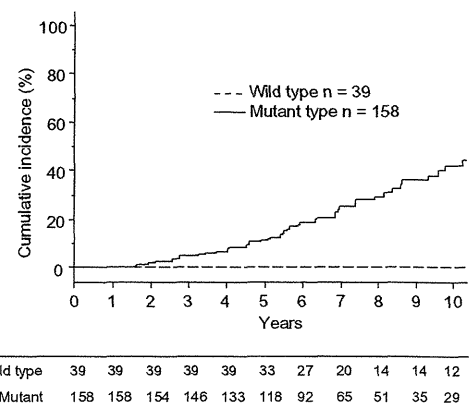

Fig. 3. Incidence of hepatocellular carcinoma (HCC) according to basal core promoter (BCP) mutations. Patients with mutant-type BCP had a significantly higher rate of progression to HCC than those with wild type BCP ($p = 0.0006$).

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,