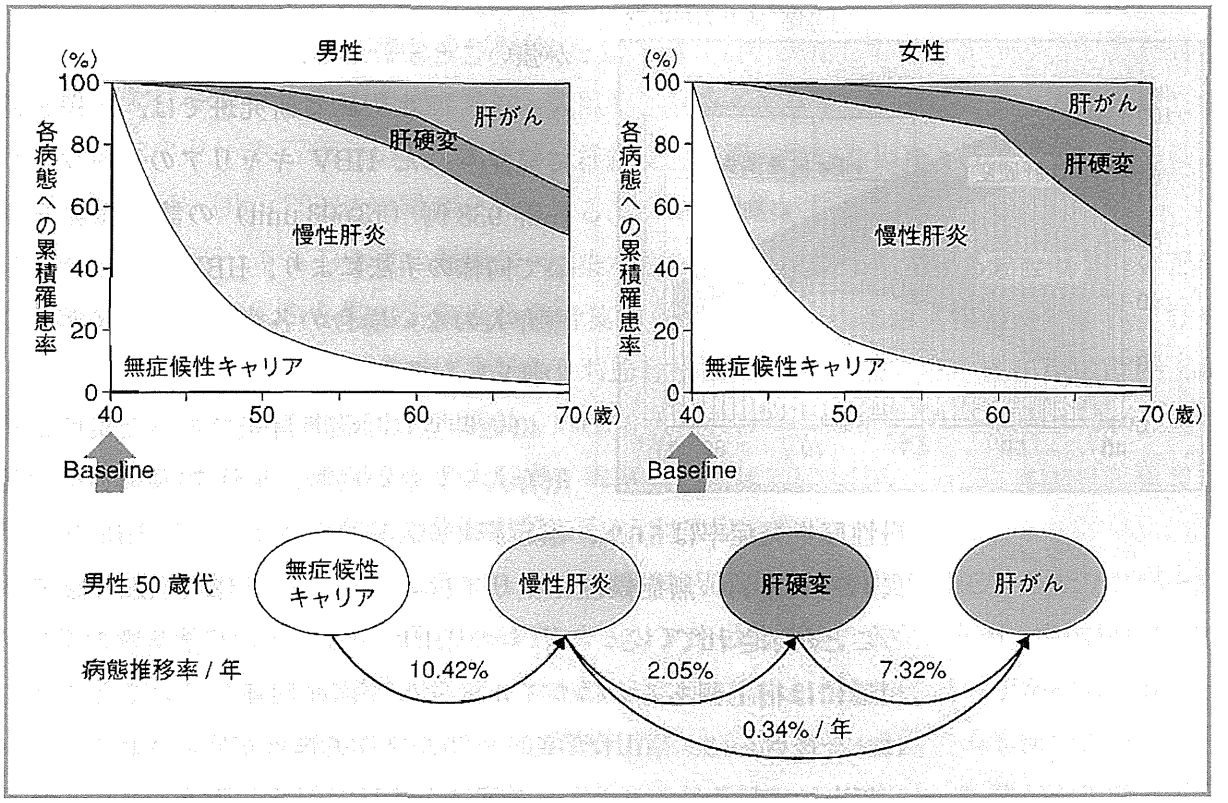
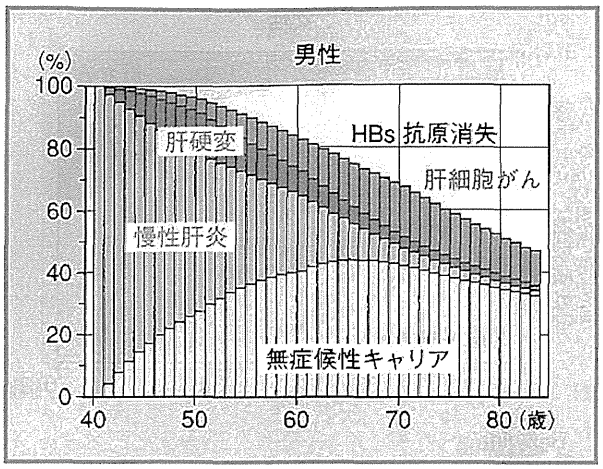
図4 HCV キャリアの病態推移：マルコフモデルによる数理疫学的推定ー抗ウイルス療法を行わない場合ー
（文献[8]より引用改変）



HCV キャリアの治療介入のない場合の各病態への累積罹患率の成績を図4に示す．抗ウイルス療法などの積極的治療が行われていなかった1990年代に通院したC型慢性肝疾患患者942人（診断情報総数2,251例）について，病態年推移確率をマルコフ過程モデルにあてはめたものである[8]．積極的治療を行わない場合には，40歳時点には無症候性HCVキャリアの状態であった集団は，男性では50歳，女性では60歳を過ぎる頃から肝発がん率が上昇し始め，60歳時点の男性の13％が肝硬変に，10％が肝がんに移行し，女性の11％，5％がそれぞれ移行することが推定されている．最終的には，70歳時点の男性の約35％が肝がん，15％が肝硬変，女性では20％，33％がそれぞれに移行し累積すると推定されている．

肝硬変から肝がんへの病態推移率は，50歳代の男性では年率7.32％，女性では2.60％であるが，60歳代では，男女いずれも高い推移率になることが示されている（同10.67％，4.71％）．一方，慢性肝炎から肝硬変への病態推移率をみると，60歳代の女性では年率5.26％と，

図5 HBVキャリアの病態推移：マルコフモデルによる推理疫学的推定ー治療介入なしー
(文献[9]より引用改変)



男性（2.86％）より高い値を示し，女性は肝がんへの移行より肝硬変にとどまる傾向が強いことが分かる．

一方，厚生労働省研究班では，住民検診で発見された HBV キャリアの長期フォロー群 938 例（13,603 unit）の診療情報を用いて同様の手法により，HBV キャリアの治療介入のない場合の各病態への累積罹患率の推定[9]を試みている（図5）．

40 歳時点には慢性肝炎であった集団が治療介入をしない場合，男性 70 歳時点の累積慢性肝炎発症率は 5.6％，累積肝発がん率は 16.2％，累積 HBs 抗原消失率は 32.6％，無症候性キャリアは 42.6％と，多様な病態が混在することが示されている．これらの HBV キャリアの自然病態の進展の相違には宿主側あるいはウイルス側の要因が関連していると考えられ，今後さらに，臨床疫学的，ウイルス学的解析が期待されている．

以上の成績から考えると，感染を知らずに社会に潜在している肝炎ウイルスキャリアを正しい検査で見いだし，肝がんに至る前段階で適切な治療や健康管理を行うことが重要であることが理解できる．特に，HBV キャリアでは，多様な病態への進展を適宜とらえて診断し，適切な治療介入を行うための継続受診の必要性が，さらに高まっていると言える．

感染を知らないまま社会に潜在する肝炎ウイルスキャリア数の推計

肝炎ウイルスに持続感染している人（肝炎ウイルスキャリア）がどのくらいいるのかを把握し，さらに，肝がんへ進行する可能性のある人数規模や地域年齢偏在を把握することは，治療戦略や肝がん対策の基礎資料になる．

しかし，肝炎ウイルスに感染している人のほとんどは自覚症状がなく，肝臓の状態が進行してもなかなか自覚症状が現われないという特性を持っているため，その数を正確に把握することは困難である．厚生労働省研究班では，これまでの疫学的調査成績や患者調査・数理疫

学手法などを用いて，肝炎ウイルスに持続感染しているキャリア数を“社会における存在状態4群別”に把握することを試みている[10]．この4群とは，“感染を知らないまま潜在しているキャリア”，“患者としてすでに通院・入院しているキャリア”，“感染を知ったが受診しないでいる，あるいは継続受診に至っていないキャリア”，“新規感染によるキャリア”である．

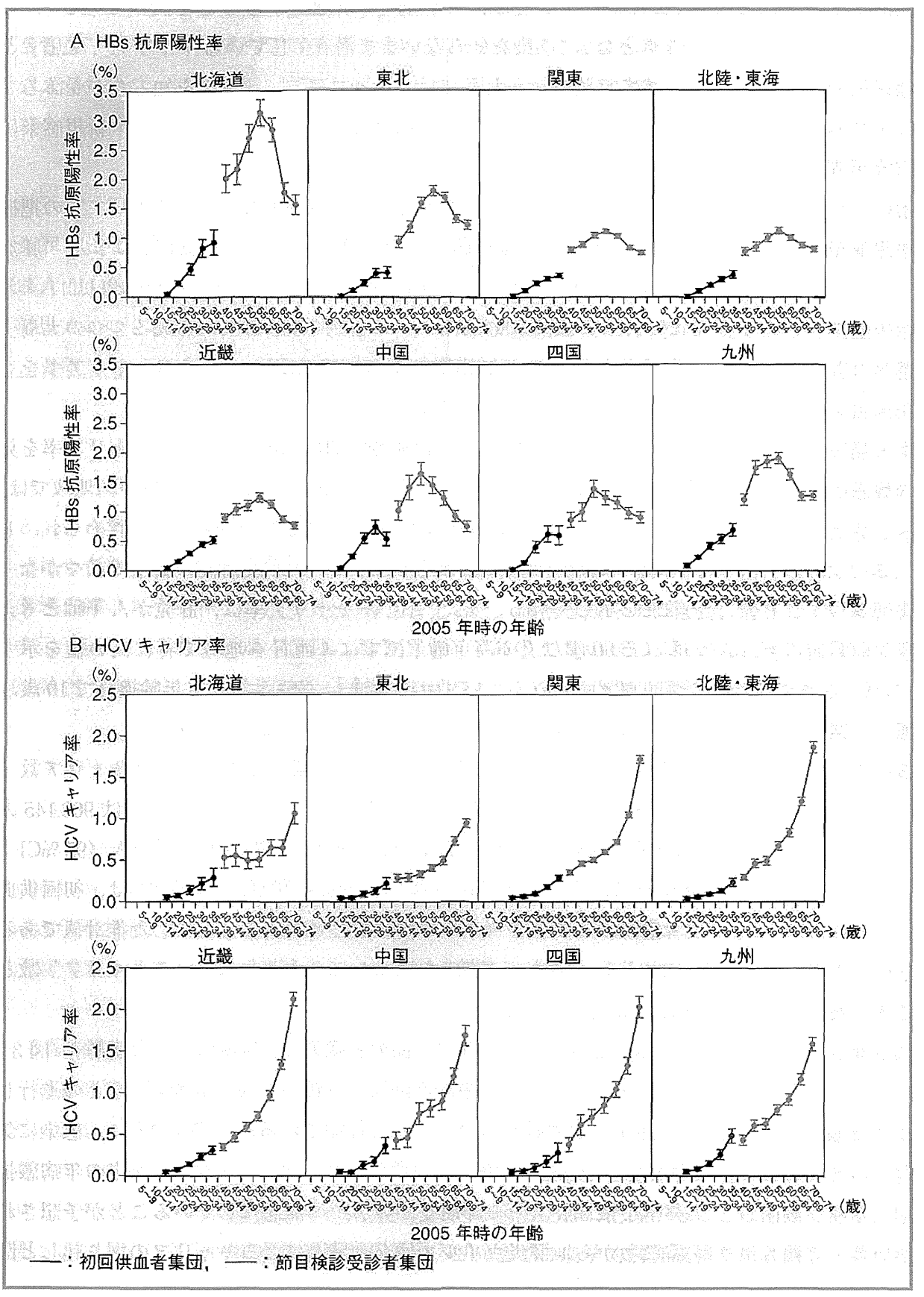
まず，自身が“感染を知らないまま潜在しているキャリア”の把握として，初回供血者集団（3,748,422人）と老人保健法による“肝炎ウイルス検診”のうち節目検診受診者集団（HBV検査6,280,111人およびHCV検査6,304,276人）の計約1,000万人から成る2つの大規模集団をもとにした年齢階級別地域別のHBV・HCVキャリア率をもとにした推計が報告[11]されている．

推計に用いられた年齢階級別地域別のHBV・HCVキャリア率を見ると（図6），HBVキャリア率は北海道，東北，九州，中国地域では，全国平均（55～59歳前後：1.4％）よりもやや高い値が認められ，8地域共に団塊の世代と考えられる55～59歳前後の集団で緩やかな一峰性を示している．また，HCVキャリア率は，肝発がん年齢と考えられる60歳以上の高年齢集団では，西日本地域で特に高い値を示す傾向が認められる．いずれの地域も，2005年時点年齢換算で19歳以下では極めて低いHBV・HCVキャリア率を示している．

これらのキャリア率をもとに，我が国全体のHBVキャリア数・HCVキャリア数を推計したところ，HBVキャリア数は903,145人（95％CI：83.7～97.0万人），HCVキャリア数は807,903人（95％CI：68.0～97.4万人）と算出された（2005年時点）．この値は，初回供血者集団および肝炎ウイルス検診受診者集団をもとにした推計値であることから，自身が“感染を知らないまま潜在しているキャリア”数と考えることができる．

この推定されたキャリア集団の肝病態は，前項に示した成績（図3）から考えると，すでに中高年齢層では慢性肝炎・肝硬変などの進行した状態がその半数を占めていると推測される．このように，感染に気づかないケース，治療介入の機会がないケースでは，一定の年病態推移率で慢性肝炎から肝硬変，肝がんへと進行していることが予想されることから，肝炎ウイルス検査の推進によるキャリアの掘り起しと医

図6　2000年以後に大規模集団から得られた地域別年齢階級別のHBs抗原陽性率とHCVキャリア率
（文献[11]より引用改変）



療機関への受診勧奨は，現時点においても，肝がん対策の面から基本的かつ重要な課題の１つと言える．

おわりに

我が国は，住民を対象とした肝炎ウイルス検診などを世界に先駆けて導入し，肝炎ウイルスの持続感染による肝炎・肝がんの対策を推進してきた．社会における存在状態別にキャリアの規模を把握し，それぞれの状態に応じた対策を講じることが有効であると言える．

人口動態統計による肝がん死亡数（率）は横ばいあるいは減少傾向にあるものの，肝硬変からの肝がんへの推移率は，50歳代男性では年率７％を超えること，しかし女性では肝がんへの病態移行確率が男性より低く肝硬変の状態でとどまっている割合が高いと推定されること，1995年以後集計可能となった肝硬変（アルコール性を除く）による死亡は，女性にやや増加傾向が見られること，などから，今後，肝がん死亡の減少と同時に肝硬変死亡の減少を意識した対策，治療戦略が必要であると考えられる．

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おける関与が示された[8]．Mathisらのグループは T 細胞を教育する胸腺髄質上皮において末梢抗原の転写を起こし，負の選択にかかわる Auto-Immune REgulator 蛋白が TRIM28 と分子会合することを示している[9]．これらのことから TRIM28 は自己反応性 T 細胞の分化や選択にも関係する可能性がある．また TRIM が分子内に共通にもつ RING motif は E3Ubiquitin-ligase 活性を有し，いくつかの TRIM 分子は RING motif を介して自然免疫系の制御を行う[10]．TRIM28 が免疫系のさまざまな局面において調節機能を有することは明らかであり，この全体像を解明するための研究が進められていくと考えている．

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消化器内科学

HCV感染と代謝異常(脂質・エネルギー)

HCV and metabolism disorders

C 型肝炎ウイルス(HCV)感染が慢性肝炎，肝硬変，肝細胞癌の原因となるだけでなく，インスリン抵抗性や脂肪肝などの糖質や脂質の代謝異常も引き起こしていることが明らかになってきている．抗ウイルス療法によりウイルスを駆除すると脂肪化が改善すること，遺伝子型 3 型のウイルスで脂肪化が顕著なことなどから，これらの代謝異常はウイルス感染による炎症よりもウイルスそのものの宿主細胞への直接作用が深くかかわっているものと考えられている．本稿では代謝物質の網羅的解析結果(メタボロミクス)を踏まえて，とくに HCV 感染が宿主代謝に与える影響について述べたい．

HCV感染が宿主脂質代謝に与える影響

これまで細胞の働きを理解しようとするとき，DNA 配列の網羅的解析(ゲノミクス)や蛋白質の網羅的解析(プロテオミクス)が行われてきた．しかし，実際の細胞内ではホメオスタシスによりゲノムレベルでの変動が表現型に一致しないことも多い．その点で，代謝産物は表現型にもっとも近いため表現型での変化が観察しやすいという特徴があり，メタボロミクスが注目されている．Roe らは，J6/JFH ウイルスを Huh-7.5 細胞に感染させ 3 日後にメタボローム解析を行ったところ，アミノ酸合成，RNA 核酸合成，ペントースリン酸経路の亢進を認めた[1]．脂質代謝に関しては HCV 感染に伴い，グリセロリン脂質，スフィンゴ脂質，コレステロール，脂肪酸などが増加していた．グリセロリン脂質，スフィンゴ脂質，コレステロールはいずれも生体膜の主要脂質成分として知られており，生活環の多くのステップで細胞の小胞体，Golgi 体，細胞膜といった生体膜脂質を利用している HCV にとって好都合な状況となっていると考えられる．とくにスフィンゴ脂質，コレステロールは脂質ラフトの構成成分であることから，HCV の感染，複製，粒子形成にも役立っているものと考えられる[2,3]．HCV による肝細胞の脂肪化の機序は，HCV コア遺伝子トランスジェニックマウスを用いた研究などで明らかになっている[4]．HCV コア蛋白は脂質合成系の転写因子 sterol regulatory element binding protein-1 の増加を介して脂肪酸合成酵素の活性を上げて脂肪酸合成を亢進させている．一方，ミトコンドリアに局在化したコア蛋白は脂肪酸のベータ酸化を抑制し，脂肪酸の消費を低

下させている．また，microsomal triglyceride transfer protein を低下させるため，超低比重リポ蛋白分泌を抑制し，細胞外への脂肪酸の放出を抑制している．さらに，HCV が誘発するインスリン抵抗性による高インスリン血漿は脂肪細胞からの肝細胞への遊離脂肪酸の取込みを増加させている．

HCV感染が宿主エネルギー代謝に与える影響

著者らは，JFH ウイルスを Huh-7 細胞に感染させ，感染がすべての細胞に広がった 9 日目に細胞のライセートを質量分析法でメタボローム解析を行った．その結果，クエン酸回路，プリン・ピリミジン合成系など蛋白核酸合成などは低下し，ATP，GTP，phosphocreatine などのエネルギー供与体は減少し，一方，解糖系は著明に亢進していた．クエン酸回路や電子伝達系を有し，生体内でエネルギー供給する ATP を産生する場所がミトコンドリアである．HCV 感染した細胞を電子顕微鏡で観察したところ，ミトコンドリアのクリステ構造が破壊されていた．さらに，蛍光抗体法による観察では HCV 蛋白発現部位でミトコンドリア機能低下がみられた．このような HCV によるミトコンドリア障害はエネルギー産生低下をもたらす可能性がある．さらに，著者らは HCV 複製による細胞における ATP 消費量の変化を調べた[5]．レプリコン細胞から複製複合体を含む画分を分離し，ATP を添加しその減少量を比較したところ，オリジナルの細胞に比べて ATP 消費量が亢進していた．ATP が存在するとピーナスと CFP が結合し FRET 蛍光を発する，ATP 濃度測定プローブ ATeam を用いて生細胞内の ATP の量と局在の解析をめざした．Ateam をレプリコン RNA の NS5A の下流に挿入することにより FRET 強度から ATP の量を評価するとともに，ドット状の蛍光から複製複合体の局在を識別した．レプリコン細胞では複製複合体で強い FRET シグナルを観察した．著者らは，レプリコン細胞から複製複合体を粗精製し[2]，そこに含まれる HCV のゲノム複製に関与する宿主因子をプロテオミクスの手法を利用して探索し，creatine kinase B(CKB)を見出した[6]．CK は，エネルギーを多く必要とする，あるいは急速に必要とする組織での ATP の供給，ATP レベルの維持に重要であるとされており，CKB は NS4A との結合を介して HCV 複製複合体にリクルートされ，ATP を供給することで HCV 複製活性の維持に重要な役割を担っていると考えられた．

おわりに

HCV は感染した細胞に脂質を蓄積するという，HCV 増殖に好都合な環境をつくりだしているものと考えられる．また，感染細胞のメタボローム解析の結果，脂質代謝だけでなく，アミノ酸合成，RNA 核酸合成，TCA 回路，エネルギー産生系，糖新生・解糖系などに多彩な影響を与えている可能性が示された．このような解析は C 型肝炎患者の病態の理解につながると期待できる．

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環境衛生

カドミウムを蓄積しないコメの開発

Newly developed rice that does not accumulate cadmium

日本人の食生活の特徴は米を主食とし，魚介類を多食することである．環境汚染物質の摂取源という観点からみると，日本人はおもに米からカドミウム(Cd)を摂取し，魚介類からメチル水銀やダイオキシン類を摂取している．Cd の消化管吸収率は 5%以下と低いが，体内に取り込まれるとおもに腎に蓄積し，その生物学的半減期は 20 年以上である．40 歳以上の日本人の腎には欧米諸国の 7〜8 倍にあたる 50〜100 μg/g の Cd が蓄積している(Cd が腎障害を起こす臨界濃度は 200 μg/g とされている)．

わが国では鉱山廃水に含まれる微量の Cd が現在なお水田を汚染している．しかも平成 23(2011)年には食品衛生法による米の Cd の安全基準が 1.0 ppm から 0.4 ppm に引き下げられた．日本人の主食である米の Cd 濃度を減少させる

【雑　　誌】

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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.

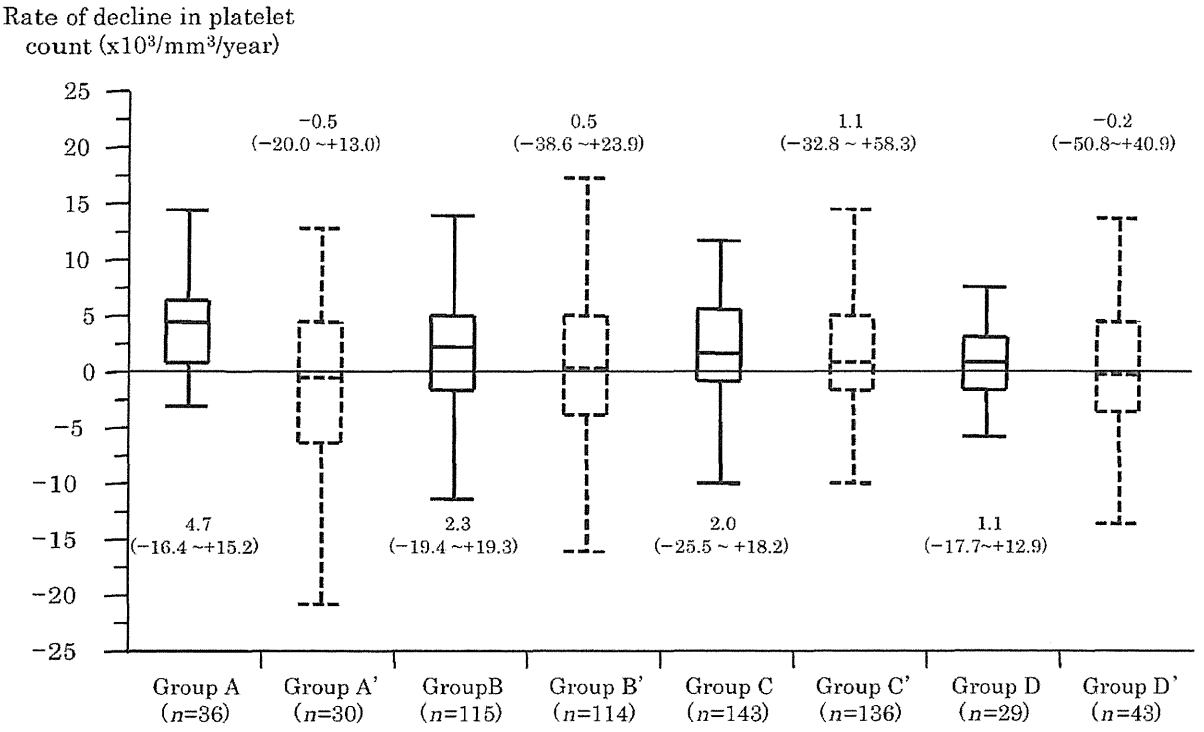

Figure 1 Rate of decline in platelet count prior to hepatocellular carcinoma (HCC) diagnosis in HCC patients and prior to the end of follow-up in control patients. The annual rate of platelet count decline in the period prior to HCC diagnosis was lower in the groups that were older at the time of HCC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

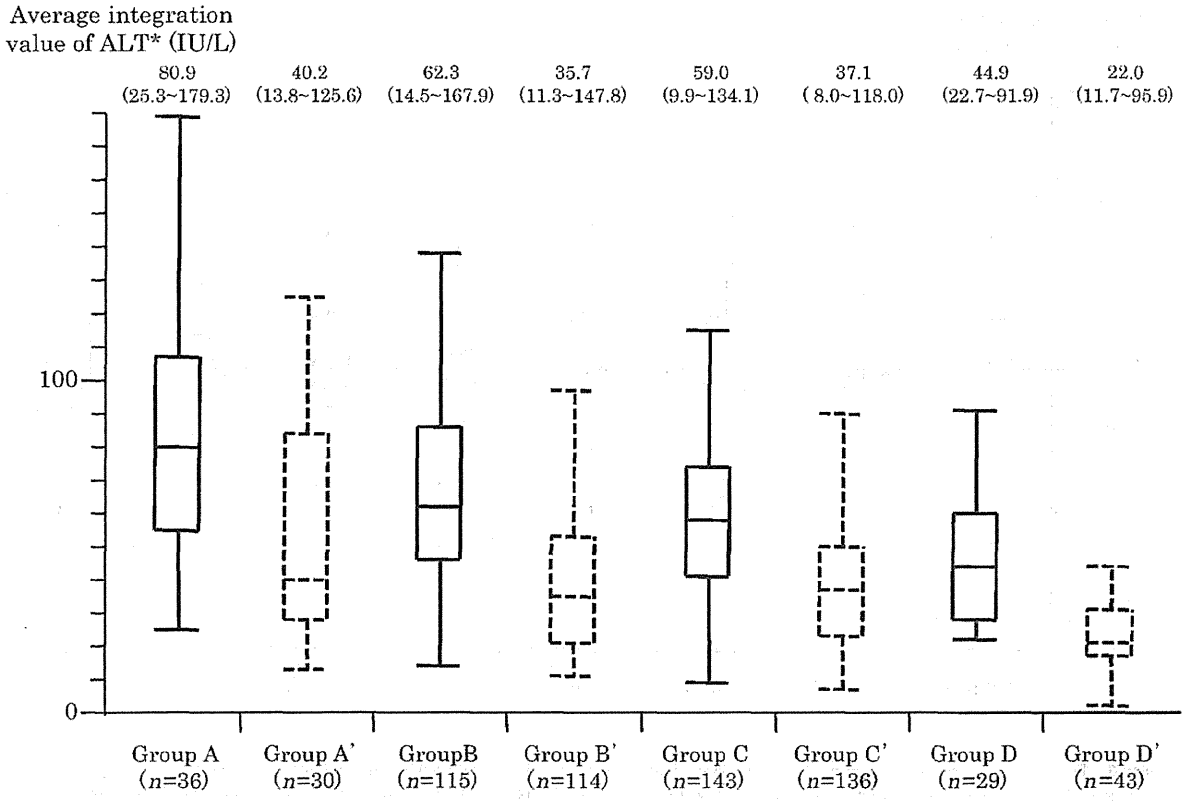

Figure 2 *Average integration values of alanine aminotransferase (ALT) prior to HCC diagnosis in HCC patients and prior to the end of follow-up in control patients.* Patients who were older at the time of HCC diagnosis had lower average integration values of ALT in the period prior to HCC diagnosis.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

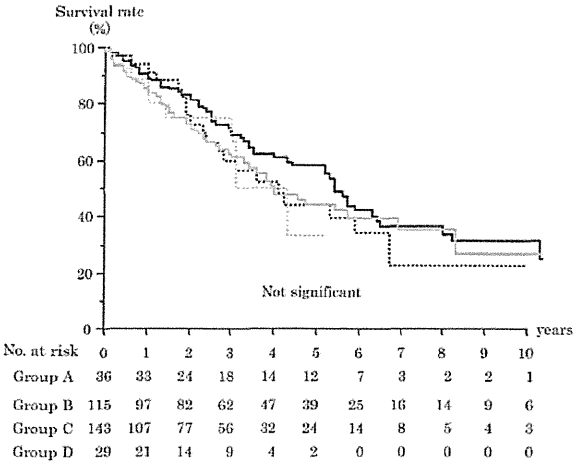

Figure 3 Cumulative survival rate of groups A, B, C, and D according to age at hepatocellular carcinoma (HCC) diagnosis. Kaplan-Meier curves showing the survival rate stratified by age at HCC diagnosis. There were no significant differences in the survival rate among the four groups. ——, A group (≤ 60 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.

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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

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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.

Research Article

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).

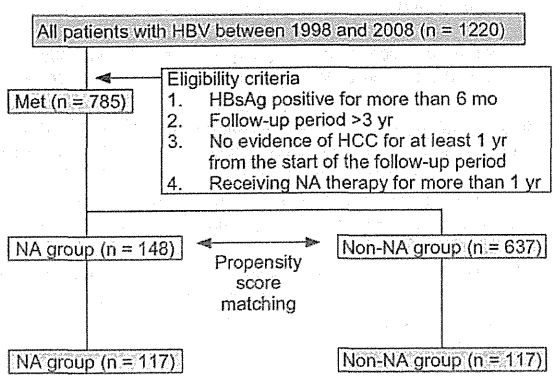

Fig. 1. Flowchart of the patient selection process.

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).

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