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mentioned in the recent editorial by McColl and Gillen (4). Howden and Kahrilas state that, "in summary, there is no clinical- or clinical trial-evidence of undue difficulty in reducing or discontinuing PPI treatment in GERD patients, apart from those with erosive esophagitis" (2). This is not correct as a placebo-controlled trial of discontinuation of PPIs in patients on long-term therapy was performed a few years ago (5). Most patients participating had gastroesophageal reflux disease (GERD) as the indication for the PPI and GERD patients had significantly more difficulties discontinuing PPI therapy as compared with patients with other indications (5). Exclusion criterion for participation was erosive esophagitis (5). In the articles mentioned above (2,3) there is no disagreement with the last paragraph of the editorial, that "PPI treatment remains an important, valuable and safe intervention for a multitude of patients with appropriate indications" (1). Finally, it is somewhat surprising that authors that are chosen to write the editorial of a study showing that PPI therapy can induce dyspeptic symptoms have strong and multiple conflicts of interest with the pharmaceutical companies producing PPIs (1).

CONFLICT OF INTEREST

Guarantor of the article: Einar Bjornsson MD, PhD.

Financial support: None.

Potential competing interests: None.

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[1]Divison of Gastroenterology and Hepatology, Landspitali University Hospital, Reykjavik, Iceland.

Correspondence: Einar Bjornsson, MD, PhD, Divison of Gastroenterology and Hepatology, Landspitali University Hospital, Reykjavik, Iceland. E-mail: einarsb@landspitali.is

Withdrawing PPI Treatment

Colin W. Howden, MD, FACG[1] and Peter J. Kahrilas, MD, FACG[1]

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To the Editor: Our editorial (1) sought to highlight both the strengths and limitations of the study by Niklasson *et al.* (2), of which Dr Bjornsson was a co-author. We presume that Dr Bjornsson would agree that the results of controlled studies should not be overinterpreted, which is precisely what we had observed with the previously reported study of Reimer *et al.* (3) and what we caution of here. We do not dispute that some (although by no means all) investigators have shown rebound acid hypersecretion following PPI withdrawal and that such an effect is biologically plausible. Rather, our concern regards the clinical relevance of this phenomenon. Furthermore, although the observations of Reimer *et al.* (3) and Niklasson *et al.* (2) might be explained on the basis of rebound acid hypersecretion, readers should understand that neither study actually measured this phenomenon.

Regarding our stated conflicts of interest, readers are free to make of them what they choose. We welcome and adhere to the Journal's policy of making a declaration of all relevant financial relationships mandatory ("strong" and otherwise); this helps to ensure transparency and objectivity. However, a thoughtful reading of our editorial would conclude that we advocate minimizing and withdrawing PPI treatment whenever appropriate, hardly a viewpoint steeped in bias. To re-state our main argument, this is generally easily accomplished in clinical practice.

CONFLICT OF INTEREST

Dr Howden has been a consultant for Takeda Pharmaceuticals North America, Takeda Global Research and Development, Santarus, XenoPort, Schering-Plough Healthcare, Novartis Consumer Health, Novartis Oncology, Procter & Gamble, Eisai, Otsuka, and Boehringer Ingelheim. He has received speaking honoraria from Takeda Pharmaceuticals North America, Otsuka, and Novartis. He has received research support for an investigator-initiated project from AstraZeneca. Dr Kahrilas has been a consultant for AstraZeneca, Xenoport, ARYx Therapeutics, Eisai, EndoGastric Solutions, Novartis, Movetis, and Revalesio. He has received research support for investigator-initiated studies from the National Institutes of Health and Reckitt Benckiser Group plc.

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[1]Division of Gastroenterology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA. Correspondence: Colin W. Howden, MD, FACG, Division of Gastroenterology, Northwestern University Feinberg School of Medicine, 676 N St Clair Street, Suite 1400, Chicago, Illinois 60657, USA. E-mail: c-howden@northwestern.edu

Double-Contrast Ultrasound: A Novel Surveillance Tool for Hepatocellular Carcinoma

Masatoshi Kudo, MD[1], Kinuyo Hatanaka, MD[1], Takashi Kumada, MD[2], Hidenori Toyoda, MD[2] and Toshifumi Tada, MD[2]

This letter underwent AJG editorial review.

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To the Editor: Hepatocellular carcinoma (HCC) is the third most common cause of

Table 1. Results of surveillance by double contrast US: B-mode US vs. double contras US

No.	Sex	Age	Virus	Location	Size (mm)	B-mode US	CEUS Kupffer phase	Double-contrast US	Pathological diagnosis
1	M	64	HCV	S6	6×6	Not detected	Defect	Positive	HCC
2	M	53	HCV	S8	7×7	Not detected	Defect	Positive	HCC
3	M	76	HCV	S6	8×8	Not detected	Defect	Positive	HCC
4	F	72	HCV	S7	8×7	Not detected	Defect	Positive	HCC
5	M	68	HBV	S5	8×8	Not detected	Defect	Positive	HCC
6	M	72	HCV	S2	9×8	Not detected	Defect	Positive	HCC
7	M	71	HCV	S3	10×9	Not detected	Defect	Positive	HCC
8	M	70	HBV	S8	10×10	Not detected	Defect	Positive	HCC
9	M	68	HCV	S2	10×7	Not detected	Defect	Positive	HCC
10	F	75	HCV	S6	11×11	Not detected	Defect	Positive	HCC
11	M	67	HCV	S6	11×10	Not detected	Defect	Positive	HCC
12	M	73	HBV	S7	12×11	Not detected	Defect	Positive	HCC
13	M	74	HCV	S5	12×11	Not detected	Defect	Positive	HCC
14	F	69	HCV	S2	12×10	Not detected	Defect	Positive	HCC
15	M	70	HCV	S6	12×11	Not detected	Defect	Positive	HCC
16	M	76	HCV	S8	13×12	Not detected	Defect	Positive	HCC

CEUS, contrast-enhanced US; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; US, ultrasound.

cancer death worldwide. Practice guidelines in the West (1) and East (2) recommend ultrasound (US) surveillance as a first-line test. However, despite the performance of periodic surveillance, some HCCs are still detected at advanced stages because of the coarse liver parenchyma. Furthermore, even HCCs detected at early stages, such as single nodular HCCs smaller than 3 cm, still show high annual recurrence rates (15–20%) after resection or ablation (3). These phenomena are attributed to the tumor biology of HCCs, which frequently metastasize via the portal vein even when they are less than 2 cm (4). Detection of much smaller HCC nodules that do not yet have microsatellites or vascular invasion is an urgent clinical need.

In 2007, Sonazoid, a second-generation US contrast agent, was approved for routine clinical use in Japan. The most important property of this agent is that it allows very stable Kupffer phase imaging for at least 60 min, which is tolerable for multiple scanning in addition to real-time imaging. From December 2007 to November 2009, Kupffer phase surveillance was performed for 292 consecutive patients with hepatitis B- or C-related cirrhosis, who are at very high risk for HCC. At the outpatient clinic, 0.01 ml/kg of Sonazoid was injected, followed by entire liver scanning at the Kupffer phase. Among the 292 patients, 27 Kupffer defects that were not detected by B-mode US were detected by Kupffer phase surveillance. Of these defects, 16 hypervascular nodules (5.5%) were confirmed as HCC by re-injecting Sonazoid at the Kupffer phase (double-contrast US) (5). All 16 nodules were proven to be HCC histologically, with a size range of 6–13 mm (**Table 1**). After resection ($n=2$) or radiofrequency ablation ($n=14$), none of these nodules showed local recurrence or intrahepatic recurrence during a median follow-up period of 2.3 years. Only one HCC nodule located at the subphrenic region was missed during detection by double-contrast US. The sensitivity of detecting B-mode US-undetectable hypervascular HCC was 94% using double-contrast US.

In conclusion, Kupffer phase surveillance of the cirrhotic liver followed by re-injection of Sonazoid (double-contrast US) is a novel technique in the surveillance program for detecting small hypervascular HCCs that are in a completely curable state. Based on these findings, a prospective randomized phase III multicenter controlled trial comparing B-mode and double-contrast US surveillance for virus-related cirrhotic patients is now ongoing (http://www.clinicaltrials.com; NCT 00822991).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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[1]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama,

Japan; [2]Department of Gastroenterology, Ogaki Municipal Hospital, Gifu, Japan. Correspondence: Masatoshi Kudo, MD, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Ohno-Higashi, Osaka-Sayama 589-8511, Japan. E-mail: m-kudo@med.kindai.ac.jp

Incidence Reduction Following Colonoscopic Polypectomy

Sidney J. Winawer, MD[1] and Ann G. Zauber, PhD[2]

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To the Editor: In Dr Sandler's editorial (1) in which he reviewed the current controversy in screening colonoscopy, he stated that the National Polyp Study (NPS) finding that colonoscopic polypectomy reduces colorectal cancer (CRC) incidence has not been replicated (2). This is an inaccurate statement. An incidence and mortality reduction similar to that observed by NPS was replicated in two other studies of post polypectomy patients that showed a 67% incidence reduction and an 88% mortality reduction, respectively (3,4). The studies that he cited as having a similar design to the NPS in fact had different designs with respect to the initial colonoscopy that identified the adenoma patients. In the NPS, all patients referred to participating clinical centers for initial colonoscopy prospectively had a protocol colonoscopy that reached the cecum, all polyps detected were removed, and all colonoscopies were performed by experienced endoscopy investigators. Those patients identified as having adenomas at this initial examination were eligible for the NPS. The studies cited by Sandler (1) had adenomas identified from community-based practices and then, 1 year later, had a clearing colonoscopy performed by experienced endoscopy investigators. Interval cancers attributable to missed lesions are not uncommon in community-based practice (5). When the missed cancers of the first non-protocol colonoscopy were excluded, the post-polypectomy CRC rate dropped from 1.8 to 0.96 per 1000 person years of follow-up, which is very similar to that of the NPS (0.6 per 1000). The CRC incidence reduction observed in the NPS compared with a simulated cohort of adenoma patients without their adenomas removed (90%) and compared with the general population Surveillance, Epidemiology and End Results rate (76%) was probably achieved as a result of the NPS design and methodology, which included rigorous baseline clearing with a 13% repeat for inadequate preparation.

There are three separate but related questions: first, does removal of adenomas reduce the incidence and mortality of CRC; second, what is the precise magnitude of this reduction; and third, what is the benefit of screening colonoscopy in the general population, of whom only a proportion have adenomas. The long-standing belief in the concept of the adenoma–carcinoma sequence and that its interruption reduces CRC incidence and mortality is supported by many studies, including the NPS (2–4,6). However, the precise magnitude of the colonoscopy effect in the general population has not been clearly established, and will not be established until completion 10 or 15 years hence of the European and American screening colonoscopy randomized controlled trials (RCTs). Data from the colonoscopy RCTs will also provide a comparison of the colonoscopy effect with the recently reported sigmoidoscopy effect (6). The NPS supports the importance of finding and removing adenomas with any screening method in addition to detecting early-stage cancers. The best method to do this needs to be established.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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[1]Gastroenterology and Nutrition Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA; [2]Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York, USA. Correspondence: Ann G. Zauber, PhD, Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 44, New York, New York 10021, USA. E-mail: zaubera@mskcc.org

Appropriate Response to Influenza A (H1N1) Virus Vaccination in Patients With Inflammatory Bowel Disease on Maintenance Immunomodulator and/or Biological Therapy

Tamás Molnár, MD, PhD[1], Klaudia Farkas, MD[1], István Jankovics, MD[2], Márta Melles, MD[2], Ferenc Nagy, MD, PhD[1], Zoltán Szepes, MD, PhD[1] and Tibor Wittmann, MD, PhD[1]

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To the Editor: In April 2009 an outbreak of the novel influenza A (H1N1) virus infection occurred in Mexico and has assumed pandemic proportions soon. After initial controversial data, vaccines directed toward the influenza A (H1N1) virus have proven to be safe and efficient to prevent the complications of the infection.

Patients with inflammatory bowel diseases (IBD—Crohn's disease (CD), ulcerative colitis) on immunosuppressive therapy are at increased risk for various infections, some of which can be prevented by immunization. Inactivated influenza vaccination

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HEPATOLOGY

Characteristics and prognosis of patients with hepatocellular carcinoma after the year 2000 in Japan

Hidenori Toyoda,* Takashi Kumada,* Toshifumi Tada,* Yasuhiro Sone,† Yuji Kaneoka‡ and Atsuyuki Maeda‡

Departments of *Gastroenterology, †Radiology, and ‡Surgery, Ogaki Municipal Hospital, Ogaki, Japan

Key words
after the year 2000, early detection, hepatocellular carcinoma, liver function, prognosis, surveillance.

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Correspondence
Dr Hidenori Toyoda, Department of Gastroenterology, Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu 503-8502 Japan. Email: tkumada@he.mirai.ne.jp

Abstract

Background and Aim: The survival rate of patients with hepatocellular carcinoma (HCC) improved through the 1990s in Japan, primarily due to advances in the detection of small HCC under the establishment of surveillance systems. We investigated how the characteristics of patients with HCC changed and whether this trend is continuing after the year 2000.

Methods: The characteristics and survival rates of patients with initial HCC (not a recurrence) who were diagnosed after the year 2000 until 2008 were analyzed and compared with those of patients in whom HCC was diagnosed in the 1990s or before.

Results: In comparison to 8 years before the year 2000, the percentage of patients with better liver function at diagnosis of HCC increased after the year 2000, whereas the size of maximal HCC tumors did not change in comparison to patients before the year 2000. The survival rate of patients continued increasing after the year 2000.

Conclusions: The prognosis of patients with HCC continues to improve after the year 2000. This is not due to further improvements in the detection of small-sized HCC; the detection of small HCC had reached a plateau in the 1990s. Rather, this improvement appears to be due in part from the continued increase in the distribution of patients with better liver function at diagnosis.

Introduction

Hepatocellular carcinoma (HCC) is among the most common cancers worldwide. It is the sixth most common cancer in the world, and the third most common cause of cancer-related death.[1,2] In Japan, HCC is the third most common cause of death from cancer in men, and the fifth in women.[3] The prognosis of patients with HCC has improved due to improvements in the management of such patients, including the development of novel treatment options or techniques and increased early detection of HCC.

We previously observed the improvement of the survival rate of patients with HCC during the years 1976–2000, particularly in the 1990s.[4] In that observation, we found that the increase in the early detection of HCC associated with the establishment of surveillance systems for HCC was the strongest contributing factor in the improvement of patient survival.[4,5] However, it has not been revealed whether this trend persists after the year 2000 into the 21st century.

In the present study, we investigated how the characteristics of patients with HCC changed and whether the improvement of patient survival continues after the year 2000.

Methods

Patients and analyses

The entire protocol was approved by the hospital ethics committee and carried out in compliance with the Declaration of Helsinki. Between 1981 and 2008, a total of 2013 patients were diagnosed as having initial HCC (not a recurrence) at Ogaki Municipal Hospital (Ogaki, Japan). Diagnosis was confirmed by histological findings on the basis of resected hepatic tumors or ultrasonography-guided needle biopsy specimens. In cases in which resection was not indicated and it was necessary to avoid biopsy of the tumor because of the possibility of needle tract seeding of the cancer cells in association with biopsy, especially in patients with advanced tumors, the diagnosis of HCC was based on the imaging findings of selective hepatic angiography and computed tomography (CT). These included hypervascularity on angiographic images and a high-density mass on arterial-phase dynamic CT images, and a low-density mass on portal-phase dynamic CT images. When findings indicative of HCC were not obtained by means of dynamic CT or angiography, CT during hepatic arteriography and CT during arterial portography or T1- and T2-weighted imaging

associated with superparamagnetic iron oxide-enhanced magnetic resonance imaging (MRI) were performed after the 1990s.

Individual decisions regarding treatment were made primarily on the basis of the treatment guidelines for HCC in Japan. Patients were initially assessed for eligibility for hepatic resection. In hepatic resection, the tumor was resected with an ample margin as hepatectomy, and enucleation of the HCC tumor without margin was not performed as surgical treatment. Only patients who had class A liver function by Child–Pugh classification[6] (with some exceptions) and 15-min retention of indocyanin green test of ≤30%, and had no more than three HCC tumors, were considered for surgical treatment. When patients declined or were deemed ineligible for surgical treatment, they underwent non-surgical treatment. Patients were first considered to be offered locoregional ablative therapies (LAT). Patients who had no more than three HCC tumors with a maximal tumor size ≤3 cm were considered for LAT. Before the year 1995, percutaneous ethanol injection (PEIT) was performed for all patients as LAT, because other modalities for LAT were not available. Some patients underwent percutaneous microwave thermocoagulation (PMCT) during 1996–2000. After the year 2000 when radiofrequency ablation (RFA) became available for LAT, all patients underwent RFA with some exceptions. Patients who were ineligible for both surgery and LAT were offered transcatheter arterial chemoembolization (TACE). No patient underwent liver transplantation as a treatment, because it is extremely difficult to find a cadaveric donor for transplantation in Japan due to religious reasons. In addition, living-donor liver transplantation was not performed at our institution during the study period. No patients received molecular-targeted drugs during the study period.

The etiology of underlying liver disease, characteristics and the progression of HCC, liver function at the time of HCC diagnosis, and patient survival rates were analyzed on the basis of clinical records. The Child–Pugh classification was determined as an indicator of liver function. Tumor staging was performed according to the American Joint Committee on Cancer (AJCC) classification system.[7] In cases in which pathological evaluation was not available, vascular invasion was assessed by means of dynamic CT and angiography. The initial treatment for HCC was also investigated. Patients were stratified into seven periods by year of HCC diagnosis: 1981–1984, 1985–1988, 1989–1992, 1993–1996, 1997–2000, 2001–2004, and 2005–2008.

All patients were followed up from 0.1 months to 241.1 months (median follow-up period: 19.1 months) at our institution after diagnosis and treatment. Patients were followed up with ultrasonography, and CT or MRI was performed every 3–6 months. In addition, regular monitoring of serum tumor markers (α-fetoprotein [AFP] and des-gamma-carboxy prothrombin [DCP]) was performed every 3 months. When the elevation of tumor markers was observed, additional imaging examinations (usually by CT or MRI) were performed to check the presence of HCC. When the recurrence of HCC was confirmed, patients underwent treatment for recurrent HCC, as well as the treatment for initial HCC.

Statistical analysis

Values were expressed as mean ± standard deviation, unless otherwise indicated. Differences in percentages between groups were analyzed by the χ^2-test. Differences in mean quantitative values were analyzed by Mann–Whitney *U*-test. The date of HCC diagnosis was defined as time zero in the calculation of patient survival rates. Surviving patients and patients who died from a cause other than liver disease were censored. Patients who died from an HCC-related cause or liver failure were not censored. The Kaplan–Meier method[8] was used to calculate survival rates, and the log–rank test[9] was used to analyze differences in survival.

The Cox proportional hazards model[10] was used for the multivariate analysis of factors related to survival. The variables analyzed were the period of the diagnosis of HCC (1981–2008), patient age and sex, Child–Pugh class, tumor stage by AJCC, and initial treatment. Data analyses were performed with the JMP statistical software package (version 6.0, Macintosh version; SAS Institute, Cary, NC, USA). All *P*-values were derived from two-tailed tests, and $P < 0.05$ was accepted as statistically significant.

Results

Patient characteristics and HCC

The demographic characteristics of the 2013 patients included in this study are summarized in Table 1. The study patients included 1495 men and 518 women, with a mean age of 65.0 ± 9.6 (range: 21–93) years. Liver function at diagnosis of HCC was Child–Pugh class A in 1137 (56.5%) patients. HCC was stage I in 797 (39.6%) patients and stage II in 574 (28.5%) patients, according to the TNM stage classification of the AJCC.

With the exception of 356 (17.7%) patients who had not received treatment, all patients underwent treatment for HCC within 2 weeks after the diagnosis of HCC. Treatment included hepatectomy in 459 (22.8%) patients and LAT in 392 (19.5%) patients. Among patients receiving LAT, 190 patients were treated by PEIT and 189 patients were treated by RFA. HCC was treated by TACE in 618 (30.7%) patients. The diagnosis of HCC in 459 patients who underwent hepatectomy was based on a histological examination of tumor tissue taken from resected specimens. In patients treated by LAT, the diagnosis of HCC was made based on fine-needle biopsy of specimens from 162 of the 392 patients (41.3%). In the remaining 230 patients treated by LAT, the diagnosis was made based on the imaging findings. HCC was diagnosed by the imaging findings in all 618 patients who underwent TACE. A histological diagnosis was made in 21 of the 188 patients (11.2%) who underwent treatment other than surgery, LAT, or TACE, and 20 of the 356 patients (5.6%) who did not undergo treatment. In total, HCC was diagnosed histologically in 662 (32.9%) patients.

Characteristics and treatment for HCC by period

We analyzed the trends in the characteristics of patients with HCC by period. The numbers of patients who were diagnosed as having initial HCC (not a recurrence) were 141 patients during the period 1981–1984, 220 during 1985–1988, 292 during 1989–1992, 305 during 1993–1996, 334 during 1997–2000, 366 during 2001–2004, and 355 during 2005–2008. This number increased during the 1980s and 1990s and peaked during 2001–2004. Patient age at the diagnosis was increasing throughout the study period. The

Table 1 Clinical characteristics of study patients ($n = 2013$)

Age (mean ± SD, years) (range)	65.0 ± 9.6 (21–93)
Sex ratio (female/male)	518 (25.7%)/1495 (74.3%)
Etiology of underlying liver disease (HBV/HCV/HBV, HCV/non-HBV, non-HCV/non-HBV)	368 (18.3%)/1175 (58.4%)/23 (1.1%)/223 (11.1%)/224 (11.1%)
Child–Pugh class (A/B/C)†	1137 (56.5%)/650 (32.3%)/226 (11.2%)
Albumin (mean ± SD, g/dL)	3.50 ± 0.56
Total bilirubin (mean ± SD, mg/dL)	1.19 ± 1.28
Diagnostic modality (histology/other)	662 (32.9%)/1351 (67.1%)
AJCC tumor stage (I/II/III/IV)	797 (39.6%)/574 (28.5%)/554 (27.5%)/88 (4.4%)
Tumor size (mean ± SD, cm) (range)	5.70 ± 3.37 (0.5–29.4)
Tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm)	572 (28.4%)/677 (33.6%)/764 (38.0%)
Tumor number (single/multiple)	870 (43.2%)/1143 (56.8%)
Vascular invasion (absent/present)	1398 (69.4%)/615 (30.6%)
Initial treatment	
No treatment	356 (17.7%)
Hepatectomy	459 (22.8%)
LAT	392 (19.5%)
TACE	618 (30.7%)
Other	188 (9.3%)

†Category of Child–Pugh class A includes patients without cirrhosis. Other treatment included repeated arterial infusion chemotherapy ($n = 93$), one-shot arterial infusion of anticancer drug ($n = 61$), systemic chemotherapy ($n = 26$), and radiation ($n = 8$). AJCC, American Joint Committee on Cancer; HBV, hepatitis B virus; HCV, hepatitis C virus; LAT, locoregional ablative therapy, including percutaneous ethanol injection, percutaneous microwave thermocoagulation, and radiofrequency ablation; non-HBV, hepatitis B virus was negative (hepatitis C virus was not tested before 1990); non-HBV, non-HCV, both hepatitis B virus and hepatitis C virus were negative; SD, standard deviation; TACE, transcatheter arterial chemoembolization.

Table 2 Clinical characteristics of study patients between periods 1992–2000 and 2001–2008

	Periods 1992–1996 and 1997–2000 ($n = 639$)	Periods 2001–2004 and 2005–2008 ($n = 721$)
Age (mean ± SD, years) (range)[1]	64.7 ± 8.8 (36–93)	68.2 ± 9.3 (21–91)
Sex ratio (female/male)	172 (26.9%)/467 (73.1%)	203 (28.2%)/518 (71.8%)
Etiology of underlying liver disease (HBV/HCV/HBV, HCV/non-HBV, non-HCV)	94 (14.7%)/463 (72.5%)/12 (1.9%)/70 (10.9%)	114 (15.8%)/503 (69.8%)/9 (1.2%)/95 (13.2%)
Child–Pugh class (A/B/C)[2]	380 (59.5%)/197 (30.8%)/62 (9.7%)	497 (68.9%)/169 (23.5%)/55 (7.6%)
Albumin (mean ± SD, g/dL)[3]	3.31 ± 0.62	3.59 ± 1.09
Total bilirubin (mean ± SD, mg/dL)	1.33 ± 1.76	1.20 ± 1.37
AJCC tumor stage (I/II/III/IV)[4]	266 (41.6%)/190 (29.7%)/157 (24.6%)/26 (4.1%)	369 (51.2%)/199 (27.6%)/124 (17.2%)/29 (4.0%)
Tumor size (mean ± SD, cm) (range)	4.28 ± 3.39 (0.5–19.0)	4.07 ± 3.25 (0.5–19.2)
Tumor size (≤2 cm/>2 cm and ≤5 cm/>5 cm)	221 (34.6%)/221 (34.6%)/197 (30.8%)	237 (32.9%)/300 (41.6%)/184 (25.5%)
Tumor number (single/multiple)[5]	282 (44.1%)/357 (55.9%)	392 (54.4%)/329 (45.6%)
Vascular invasion (absent/present)[6]	487 (76.2%)/152 (23.8%)	599 (83.1%)/122 (16.9%)
AFP (median, ng/mL) (range)[7]	38.0 (0.0–595 000)	24.7 (0.8–2 402 000)
DPC (median, mAU/mL) (range)[8]	62.0 (10.0–8 000)	38.2 (10.0–75 000)
Antiviral therapy for HBV infection[9]	8 (7.5%)	72 (58.5%)
Antiviral therapy for HCV infection[10]	36 (7.6%)	73 (14.3%)
Eradication of HCV by antiviral therapy	8 (1.7%)	17 (3.3%)

[1]$P < 0.0001$; [2]$P = 0.0013$; [3]$P < 0.0001$; [4]$P = 0.0011$; [5]$P = 0.0002$; [6]$P = 0.0020$; [7]$P = 0.0003$; [8]$P = 0.0027$; [9]$P < 0.0001$; [10]$P = 0.0012$. †Category of Child-Pugh class A includes patients without cirrhosis. AFP, α-fetoprotein; AJCC, American Joint Committee on Cancer; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; non-HBV, non-HCV, both hepatitis B virus and hepatitis C virus were negative; SD, standard deviation.

mean age was 60.6 ± 9.1 during the period 1981–1984, 61.4 ± 10.0 during the period 1985–1988, 62.3 ± 9.2 during the period 1989–1992, 63.8 ± 8.5 during the period 1993–1996, 65.5 ± 9.0 during the period 1997–2000, 68.0 ± 9.1 during the period 2001–2004, and 68.5 ± 9.5 during the period 2005–2008. The prevalence of patients with Child–Pugh class A liver function at diagnosis and the prevalence of patients with AJCC tumor stage I continued increasing after the period 1985–1988. In contrast, the prevalence of patients with maximal tumor size < 2 cm markedly increased between the period 1985–1988 and the period 1989–

1992, but reached a plateau after the period 1989–1992 (Fig. 1). The median serum AFP value at a diagnosis continued decreasing throughout the study period, and the median serum DCP value also continued decreasing after the period 1989–1992 (data not shown).

When the characteristics were compared between two periods (8 years) before the year 2000 (1992–1996 and 1997–2000, $n = 639$) and two periods after the year 2000 (2001–2004 and 2005–2008, $n = 721$) to elucidate the characteristics of patients with HCC after the year 2000, the age of the patients, as well as their serum albumin levels, were significantly higher after the year 2000 (64.7 ± 8.8 years *vs* 68.2 ± 9.3 years and 3.31 ± 0.62 g/dL *vs* 3.59 ± 1.09 g/dL, both $P < 0.0001$). After the year 2000, the percentage of patients with Child–Pugh class A liver function was significantly higher (59.5% *vs* 68.9%, $P = 0.0013$), and the percentage of patients with AJCC tumor stage I was significantly higher (41.9% *vs* 51.2%, $P = 0.0011$). Additionally, the percentage of patients with single HCC tumors (44.1% *vs* 54.4%, $P = 0.0002$) and the percentage of patients with HCC that lacked vascular invasion (76.2% *vs* 83.1%, $P = 0.0020$) were significantly higher after the year 2000. The serum AFP value at diagnosis was significantly lower after the year 2000 (38 ng/mL *vs* 24.7 ng/mL, $P = 0.0003$). Also, the serum DCP value at diagnosis was significantly lower after the year 2000 (62 mAU/mL *vs* 38.2 mAU/mL, $P = 0.0027$). In contrast, there was no decrease in maximal tumor size after the year 2000 in comparison to the periods before ($P = 0.4301$). The percentage of patients with hepatitis B virus (HBV) infection who were administered a nucleoside analog against HBV was significantly higher after the year 2000 (7.5% *vs* 58.5%, $P < 0.0001$). The percentage of patients with hepatitis C virus (HCV) infection who had undergone interferon-based antiviral therapy against HCV was significantly higher after the year 2000 (7.6% *vs* 14.3%, $P = 0.0012$), although the rate of patients in whom HCV was eradicated by antiviral therapy (i.e. sustained virological responders) was not significantly different.

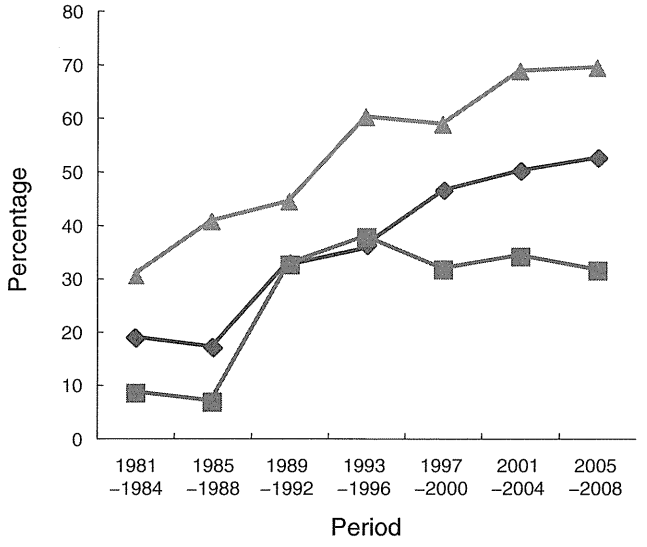

Figure 1 Changes in the percentage of patients with Child–Pugh class A or those without cirrhosis (green line), the percentage of patients with hepatocellular carcinoma (HCC) of maximal tumor size < 2 cm (red line), and the percentage of patients with HCC of American Joint Committee on Cancer (AJCC) tumor stage I (blue line) at the first diagnosis of HCC. Patients with Child–Pugh class A include patients with cirrhosis. ▲, Child–Pugh class A; ■, maximal tumor size < 2 cm; ◆, AJCC tumor stage I.

An analysis of the initial treatment selected (Table 3) demonstrated that the percentage of patients who underwent surgery as an initial treatment increased from the period 1997–2000, and was approximately 35% in the periods after the year 2000 (1997–2000 *vs* 2001–2004, $P = 0.0002$). In contrast, patients who underwent TACE decreased from over 50% in the period 1985–1988, to approximately 20% in the periods after the year 2000 (1997–2000 *vs* 2001–2004, $P < 0.0001$). LAT treatment increased from the period 1989–1992 (1989–1992 *vs* 1993–1996, $P = 0.0004$), and was almost constant after this period, with some fluctuations. However, when the details of the treatment were analyzed in patients who underwent LAT, the percentage of patients who underwent RFA as LAT markedly increased in the period after the year 2000 (1997–2000 *vs* 2001–2004, $P < 0.0001$), and most patients underwent RFA in this period. In six patients who underwent PEIT as LAT after the year 2000, a HCC tumor was located just beside the bowel (2 patients), gallbladder (1 patient), and main trunk of the intrahepatic bile duct (3 patients), and therefore, RFA should have been avoided. We found no patient who underwent RFA before the period 1993–1996. The percentage of patients who underwent RFA among patients treated by LAT was 21.9% in the period 1997–2000. It was 95.1% and 98.7% in the periods 2001–2004 and 2005–2008, respectively.

Table 3 Initial treatment for hepatocellular carcinoma by periods

Periods	Surgery	Locoregional ablative therapies				TACE	Others	None
		Total	PEIT	PMCT	RFA			
1981–1984 ($n = 141$)	19 (13.5)	0	0	0	0	39 (27.7)	38 (26.9)	45 (31.9)
1985–1988 ($n = 220$)	25 (11.4)	6 (2.7)	6 (2.7)	0	0	115 (52.3)	31 (14.1)	43 (19.5)
1989–1992 ($n = 292$)	46 (15.8)	50 (17.1)	50 (17.1)	0	0	114 (39.0)	28 (9.6)	54 (18.5)
1993–1996 ($n = 305$)	42 (13.8)	91 (29.8)	90 (29.5)	1 (0.3)	0	108 (35.4)	23 (7.5)	41 (13.5)
1997–2000 ($n = 334$)	72 (21.5)	64 (19.2)	38 (11.4)	12 (3.6)	14 (4.2)	109 (32.6)	25 (7.5)	64 (19.2)
2001–2004 ($n = 366$)	127 (34.7)	103 (28.2)	5 (1.4)	0	98 (26.8)	59 (16.1)	22 (6.0)	55 (15.0)
2004–2008 ($n = 355$)	128 (36.1)	78 (22.0)	1 (0.3)	0	77 (21.7)	74 (20.8)	21 (5.9)	54 (15.2)

PEIT, percutaneous ethanol injection therapy; PMCT, percutaneous microwave thermocoagulation therapy; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

Survival rate for patients with HCC by period

The survival rates were compared according to the periods of initial HCC diagnosis (Fig. 2). The survival rates increased throughout the periods 1981–1984, 1985–1988, and 1989–1992 (1981–1984 *vs* 1985–1988, $P = 0.0003$; 1985–1988 *vs* 1989–1992, $P = 0.0009$; 1989–1992 *vs* 1993–1996, $P = 0.0383$). We found no difference in the survival rates between the periods 1993–1996 and 1997–2000 ($P = 0.5887$). In contrast, the survival rate markedly increased in the period 2001–2004 in comparison to the period 1997–2000 ($P < 0.0001$). Again, we found no difference in the survival rates between the periods 2001–2004 and 2005–2008 ($P = 0.5151$).

When we compared the survival rates according to treatment, it was highest in patients treated by surgery, followed by patients treated by LAT, by TACE, by other treatment, and patients without treatment in this order (data not shown). In patients who underwent LAT, the survival rate was higher in patients treated by RFA than those treated by PEIT ($P = 0.0073$, Fig. S1).

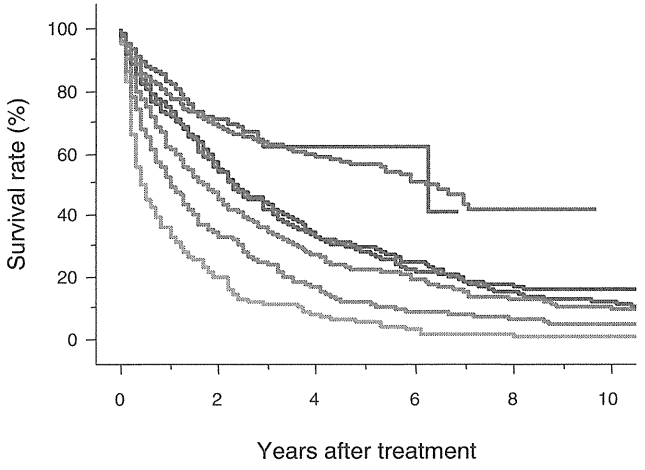

Figure 2 Survival rate of patients with hepatocellular carcinoma (HCC) according to the period of initial HCC diagnosis. ▬, 1981–1984 ($n = 141$); ▬, 1985–1988 ($n = 220$); ▬, 1989–1992 ($n = 292$); ▬, 1993–1996 ($n = 305$); ▬, 1997–2000 ($n = 334$); ▬, 2001–2004 ($n = 366$); ▬, 2004–2008 ($n = 355$).

Multivariate analyses for factors affecting survival rates

We conducted a multivariate analysis to determine factors that were associated with patient survival rate (Table 4). The analysis revealed that Child–Pugh class (B and C), AJCC tumor stage (II and III), treatment (surgery, LAT, TACE, and other treatments), and the period of diagnosis (periods 1997–2000, 2001–2004, and 2005–2008) were independently associated with patient survival rate.

Discussion

In the present study, we analyzed several trends in patients with HCC, including the periods after the year 2000. The incidence of HCC is reportedly increasing in the USA and other Western countries.[11,12] In contrast, a recent study demonstrated that the incidence

Table 4 Multivariate analysis of factors associated with patient survival

Factor		Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*P*-value
Age		0.0038	0.0033	1.36	1.0038 (0.9974–1.0102)	0.2428
Sex	Male				1	
	Female	−0.0566	0.0337	2.87	0.9449 (0.8845–1.0095)	0.0904
Child–Pugh class	A†				1	
	B	0.2379	0.0328	52.00	1.2686 (1.1895–1.3529)	< 0.0001
	C	0.4768	0.0481	89.41	1.6109 (1.4658–1.7703)	< 0.0001
AJCC tumor stage	Stage I				1	
	Stage II	0.2559	0.0380	45.36	1.2916 (1.1990–1.3915)	< 0.0001
	Stage III	0.6170	0.0431	206.14	1.8534 (1.7032–2.0168)	< 0.0001
	Stage IV	0.7973	0.0704	97.98	2.2196 (1.9334–2.5481)	< 0.0001
Treatment	No treatment				1	
	Surgery	−0.7011	0.0599	139.24	0.4960 (0.4411–0.5578)	< 0.0001
	LAT	−0.6365	0.0559	129.84	0.5291 (0.4742–0.5904)	< 0.0001
	TACE	−0.4039	0.0451	77.19	0.6677 (0.6113–0.7294)	< 0.0001
	Other	−0.1233	0.0530	5.49	0.8840 (0.7968–0.9808)	0.0192
Period	1981–1984				1	
	1985–1988	−0.0832	0.0569	2.12	0.9202 (0.8231–1.0287)	0.1456
	1989–1992	−0.0818	0.0543	2.23	0.9215 (0.8285–1.0249)	0.1350
	1993–1996	−0.0824	0.0557	2.16	0.9209 (0.8256–1.0272)	0.1419
	1997–2000	−0.1208	0.0553	4.67	0.8862 (0.7952–0.9877)	0.0306
	2001–2004	−0.2904	0.0621	21.52	0.7480 (0.6623–0.8448)	< 0.0001
	2005–2008	−0.2988	0.0707	18.33	0.7417 (0.6456–0.8520)	< 0.0001

†Category of Child–Pugh class A includes patients without cirrhosis. AJCC, American Joint Committee on Cancer; LAT, locoregional ablative therapies including percutaneous ethanol injection therapy, percutaneous microwave thermocoagulation therapy, and radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

of HCC in urban areas of Japan began to decrease by the year 2000.[13] However, we did not find a decrease in the number of patients with HCC during this period. This authors' institution is located in a county area, and the results indicated that the incidence of HCC has not started to decrease and remains constant even after the year 2000 for county regions of Japan.

In the comparisons based on the period of the HCC diagnosis in the present study, patient age continued to increase throughout the study period. One of the most important risk factors for the development of HCC worldwide[14,15] is chronic viral hepatitis, and the majority of patients with HCC in Japan have chronic HCV infection.[16] The age of Japanese individuals with HCV infection is increasing, thus contributing to the higher patient age found in this study.

When we analyzed the characteristics of patients with HCC who were diagnosed after the year 2000, in comparison to those diagnosed in the 1990s, the prevalence of patients with liver function of Child–Pugh class A significantly increased, along with the serum albumin level and patient age. This indicates the trend that patients had better liver function at first diagnosis of HCC after the year 2000. At the same time, it indicates that HCC develops more frequently in livers with less progressed fibrosis, and even in livers without cirrhosis, after the year 2000. The reason for this better liver function at diagnosis is unknown. It could be due to the increase in patient age at diagnosis in Japan, as it has been reported that HCC develops in the liver with less progressed fibrosis in cases of high-aged patients.[17] It could also be due to the increase in the number of patients with a history of several antiviral therapies, including nucleoside analogs for patients with HBV infection and interferon-based therapy for those with HCV infection. Indeed, the percentage of patients who underwent antiviral therapy markedly increased after the year 2000 in both patients with HBV and HCV infection. Further studies will be needed to clarify the reason for the increase of HCC patients with better liver function in the period after the year 2000.

With respect to HCC tumor progression at diagnosis, we did not find an improvement in the size of HCC tumor at diagnosis in the period after the year 2000. The size of maximal HCC tumor at diagnosis reached a plateau after the period 1993–1996. Tremendous effort has been made to increase the detection of small-size HCC tumors, especially during the 1990s. This has led to the development of various scanning techniques and imaging apparatuses,[18,19] identification of highly-sensitive and specific tumor markers,[20,21] and the establishment of surveillance for patients at high-risk of developing HCC.[5,22] It appears that this effort reached a limit during the 1990s, as it currently is difficult to detect smaller HCC tumors. In contrast to the size of maximal HCC tumors, the prevalence of single HCC tumors or HCC without vascular invasion significantly increased after the year 2000. This resulted in an increase in patients with an earlier tumor stage of HCC. Also, serum AFP and DCP values at diagnosis, which are reported to be a biomarker of biological malignant features of HCC, continued decreasing after the year 2000. Advances in techniques for the early detection of HCC continue to effect the improvement not of the size of HCC tumors but of these factors after the year 2000; less advanced HCC continues increasing, even in the same size HCC tumors.

In regards to patient prognosis, the survival rates were almost constant during the period 1993–2000. This is associated with the fact that the detection of small-sized HCC tumors reached a plateau during this period. In contrast, the survival rates significantly increased after the year 2000. The increase in patients with better liver function at diagnosis after the year 2000 resulted in an increase of the percentage of patients who underwent hepatectomy as an initial treatment in this period. This increase in the percentage of patients who underwent hepatectomy for the treatment for HCC might have contributed to the improvement in the survival rate of patients with HCC diagnosed after the year 2000. In addition, the percentage of patients who underwent RFA markedly increased after the year 2000. Considering the higher survival rate in patients who underwent RFA as LAT than that in patients treated by PEIT, the emergence of RFA as a treatment modality for HCC might also have played a role in the increasing survival rate after the year 2000.

In conclusion, the survival rate of patients with HCC continues to increase after the year 2000 in Japan. This is not due to improvements in the detection of small HCC tumors, as observed in 1990s, but is a consequence of the increase in the percentage of patients with better liver function and patients with single HCC tumors or HCC without vascular invasion with low serum tumor marker levels at first diagnosis. These changes resulted in an increase in the number of patients who underwent radical curative treatment and contributed to the continuing improvement of patient prognosis. Further studies will be necessary to elucidate the reasons for these changes in the characteristics of patients with HCC after the year 2000.

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Supporting information

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

Figure S1. Survival rate of patients with hepatocellular carcinoma treated by percutaneous ethanol injection therapy and radiofrequency ablation as a locoregional ablative therapy.

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BMC Research Notes

RESEARCH ARTICLE Open Access

Markedly lower follow-up rate after liver biopsy in patients with non-alcoholic fatty liver diseases than those with viral hepatitis in Japan

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

Abstract

Background: Patients with non-alcoholic fatty liver diseases (NAFLD) are recommended to have periodic follow-up exams because these patients are at increased risk of the presence of non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis or hepatocellular carcinoma. We investigated the follow-up status of NAFLD patients after a liver biopsy examination.

Methods: We compared the follow-up rates of NAFLD patients who had received an ultrasonography-guided liver biopsy and patients who had received a liver biopsy for chronic viral hepatitis (hepatitis B or C).

Results: The 1- and 3-year follow-up rates after the liver biopsy were 92.7% and 88.3% for patients with chronic HBV infection, and 93.4% and 88.2% for patients with chronic HCV infection, respectively. In contrast, the follow-up rates for NAFLD patients were 77.6% and 49.9%, respectively, which were significantly lower than those of patients with chronic viral hepatitis ($p < 0.0001$). Among NAFLD patients, the respective 1- and 3-year follow-up rates were 73.0% and 44.6% for patients with simple steatosis and 80.0% and 52.4% for patients with NASH based on a pathologic diagnosis, without significant difference between these two subgroups ($p = 0.5202$).

Conclusions: The outpatient-based follow-up rate after a liver biopsy was significantly lower in NAFLD patients compared to patients with chronic viral hepatitis, regardless of the presence of NASH. It is important to determine how to maintain regular hospital visits for NAFLD patients, preventing patient attrition.

Keywords: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, simple steatosis, follow-up, compliance

Background

Nonalcoholic fatty liver disease (NAFLD) encompasses a histological spectrum that ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and end-stage liver disease. NAFLD is one of the most common liver diseases in both Western and Asian countries [1-5], affecting 30% of the general Western adult population [6].

Because NAFLD includes patients with NASH that can progress to cirrhosis [1,7,8] and because it is still controversial whether simple steatosis converts to NASH, it is important to carefully monitor NAFLD patients at regular follow-up exams. Furthermore, in the absence of treatment modalities with proven efficacy, outpatient-based weight management is currently an important treatment for NAFLD patients. However, it is unclear whether NAFLD patients have a sufficient understanding of the importance of periodic follow-up visits compared to patients with viral hepatitis or other liver diseases. Whereas the follow-up rate of patients with chronic viral hepatitis (chronic hepatitis B or C) is high in Japan, the follow-up rate of patients with NAFLD, in comparison to patients with viral hepatitis, is not clarified.

In the present study, we investigated the follow-up status of NAFLD patients, including patients with both simple steatosis and NASH, after the disease diagnosis was histologically confirmed by a liver biopsy.

* Correspondence: hmtoyoda@spice.ocn.ne.jp
Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

Methods

Patients and Follow-up Visits

A total of 610 patients had undergone an ultrasonography-guided liver biopsy examination at our institution to confirm a diagnosis of liver diseases during a 5-year period between April 2003 and March 2008. These patients received a liver biopsy to evaluate of the grade of liver damage (activity of hepatitis), examine the degree of liver fibrosis, and investigate or confirm the diagnosis. The patients included 305 males and 305 females with a mean age of 53.9 ± 12.7 years. All patients underwent an ultrasonography-guided fine needle liver biopsy using a 17 G biopsy needle during their 3 to 4 days of hospitalization. Among these patients, 69 patients had chronic hepatitis B virus (HBV) infection, 354 patients had chronic hepatitis C virus (HCV) infection, and 135 patients had NAFLD by clinical evaluation. We analyzed the follow-up rates of these patient groups after liver biopsy.

The patients were informed of the pathologic results of the biopsy when they visited the outpatient clinic 2 or 3 weeks after the biopsy. Patients with chronic viral hepatitis (hepatitis B or C) were advised to receive regular periodic follow-up exams every 3 to 6 months to monitor liver damage, fibrosis, and the development of HCC. Patients who were diagnosed with NASH were informed that they could potentially progress to cirrhosis and were at increased risk of developing hepatocellular carcinoma (HCC). They were also advised to have regular follow-up visits at our institution every 3 to 6 months. Patients who were diagnosed with simple steatosis were informed that it has not been fully determined if simple steatosis is converts to NASH and were advised to have regular follow-up exams at our institution every 6 months.

Our institution provides appointment-based outpatient care, and most patients who visit the clinic reserve the next available appointment. If a patient did not visit the clinic despite a scheduled appointment, the patient was telephoned, advised to maintain their regular visits, and given a new appointment. This telephone contact was repeated at least three times. Patients were routinely monitored from the biopsy date until the end of December 2010.

The study protocol was in compliance with the Helsinki Declaration and was approved by the institutional review board of the Ogaki Municipal Hospital for the review of patient clinical records. Written informed consent was obtained from patients who continued to be followed up.

Clinical and Pathologic Diagnosis of Non-alcoholic Fatty Liver Diseases, Simple Steatosis, and Non-alcoholic Steatohepatitis

A patient was clinically diagnosed with a fatty liver before receiving a biopsy if they met made when the following criteria: 1) persistently abnormal liver function tests for more than 3 months, 2) ultrasonographic images showing steatosis, 3) no evidence of alcohol abuse, and 4) exclusion of other liver diseases and other known causes of steatosis based on the results of specific clinical, biochemical, or imaging studies. The ultrasonographic findings of steatosis were based on known criteria that are used to diagnose a fatty liver by ultrasonography (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring). The first two factors were used as definitive criteria, while the last two factors were taken into account as needed. NAFLD was pathologically diagnosed based on pathologic findings in the biopsied liver specimens. The liver biopsy specimens were stained with hematoxylin/eosin, Masson's trichrome, and PAS and then examined by experienced pathologists. A liver with steatosis involving at least 10% of the hepatocytes was considered to have NAFLD. The presence of NASH was defined according to the NAFLD activity score (NAS) as proposed by the Center for Neuroscience Research and National Institutes of Health [9].

Statistical Analyses

Quantitative values are reported as the mean ± SD. Between-group differences were analyzed by the chi-square test. Differences in quantitative values between two groups were analyzed by the Mann-Whitney *U* test. Follow-up rates were analyzed using the Kaplan-Meier method, and the log-rank test was used to compare the follow-up rates. The date of liver biopsy was defined as time zero when calculating the patient follow-up rates. Patients who continued their follow-up schedule were censored. Patients who missed a follow-up visit were not censored. All p-values were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Patient Characteristics

Table 1 shows the clinical characteristics of the patients who underwent a liver biopsy. Patients with chronic HCV infection were significantly older than patients with chronic HBV infection or NAFLD patients (both, $p < 0.0001$). The prevalence of females was significantly higher among patients with chronic HCV infection compared to NAFLD patients ($p = 0.0094$). The serum ALT activity was significantly higher in NAFLD patients than in patients chronically infected with HCV ($p < 0.0001$). In contrast, there were no differences in the serum ALT activity between patients with NAFLD and those with chronic HBV infection ($p = 0.4773$).

Follow-up Status after the Liver Biopsy

The follow-up rates of patients after the liver biopsy are shown in Figure 1 according to the underlying liver

Table 1 Clinical characteristics of patients who received a liver biopsy.

	NAFLD (n = 135)	Chronic HBV infection (n = 69)	Chronic HCV infection (n = 354)
Age (years: mean ± S. D.)	48.8 ± 14.7	47.1 ± 12.3	56.5 ± 11.0
Sex (male/female) (%)	84(62.2)/51 (37.8)	40(58.0)/29 (42.0)	172(48.6)/182 (51.4)
AST (IU/L: mean ± S.D.)	58.0 ± 42.6	78.2 ± 67.4	55.9 ± 45.4
ALT (IU/L: mean ± S.D.)	94.7 ± 67.8	126.3 ± 119.6	70.9 ± 74.1

NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase

diseases. The 1- and 3-year follow-up rates after liver biopsy were 92.7% and 88.3% for patients with chronic HBV infection, 93.4% and 88.2% for patients with chronic HCV infection, and 77.6% and 49.9% for NAFLD patients, respectively. There were no differences in the follow-up rates between patients with chronic HBV infection and those with chronic HCV infection (p = 0.7299). By contrast, the post-liver biopsy follow-up rate was significantly lower in NAFLD patients compared to those with HBV infection or HCV infection (both, $p < 0.0001$); more than 50% of patients discontinued their follow-up exams within 3 years after the liver biopsy. When we compared the post-liver biopsy follow-up rates between patients with chronic HCV infection and those with NAFLD focusing on patients who had not been administered medication, the follow-up rate of patients with NAFLD was significantly lower than that of patients with HCV ($p < 0.0001$, Figure 2). In addition, when we compared the follow-up rates between NAFLD patients and patients who had been chronically infected with HCV but were successfully treated with an antiviral therapy with interferon/peginterferon with or without ribavirin (sustained virologic responders), NAFLD patients had a significantly lower follow-up rate ($p < 0.0001$, Figure 3).

Comparison of Characteristics Between Non-alcoholic Fatty Liver Disease Patients Who Continued and Discontinued Their Follow-up Exams

When the background characteristics were compared between NAFLD patients who continued and discontinued their periodic follow-up visit (Table 2), the patients who discontinued their follow-up visit were significantly younger than those who continued their regular follow-up exams. The prevalence of NASH and simple steatosis by pathologic evaluation did not differ between patients who had continued and discontinued their follow-up exams. Also, the percentage of patients who had been administered medication did not differ between patients who continued and discontinued their follow-up visit. Figure 4 compares the follow-up rates after the liver biopsy between patients with NASH and those with simple steatosis. The 1- and 3-year follow-up rates were

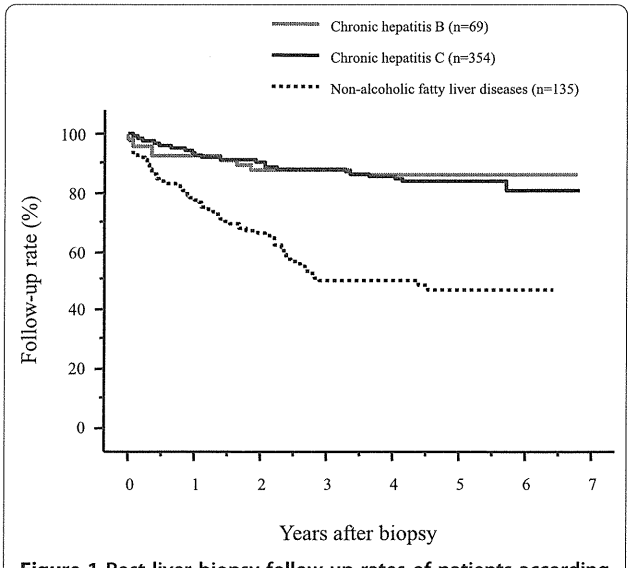

Figure 1 Post-liver biopsy follow-up rates of patients according to the underlying liver disease. The follow-up rate was significantly lower in patients with nonalcoholic fatty liver disease than in patients with chronic hepatitis B or C virus infection (both, $p < 0.0001$). There was no difference in the follow-up rate between patients with chronic HBV infection and those with chronic HCV infection ($p = 0.7299$).

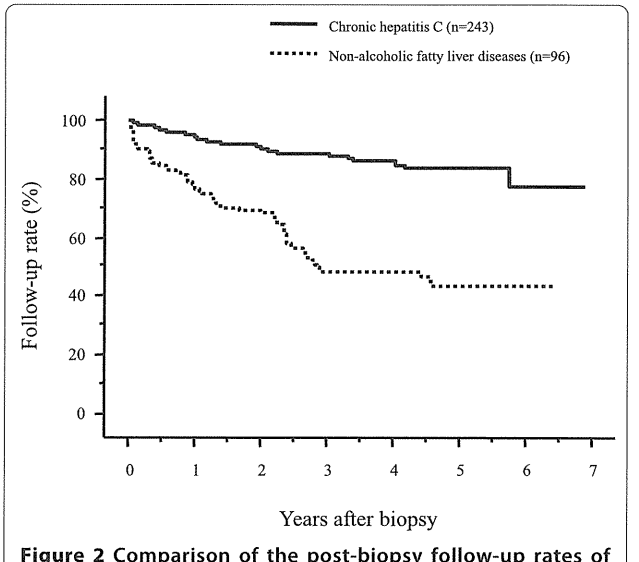

Figure 2 Comparison of the post-biopsy follow-up rates of patients with chronic hepatitis C virus (HCV) infection and those with nonalcoholic fatty liver disease (NAFLD), focusing on patients who had not been administered medication. The follow-up rate remained significantly lower in patients with NAFLD than in patients with chronic HCV infection ($p < 0.0001$).

Figure 3 Comparison of the post-liver biopsy follow-up rates in patients with nonalcoholic fatty liver disease (NAFLD) and patients who had been successfully treated for hepatitis C virus (HCV) with antiviral therapy (sustained virologic responders). The follow-up rate was significantly lower in NAFLD patients than in sustained virologic responders for HCV ($p < 0.0001$).

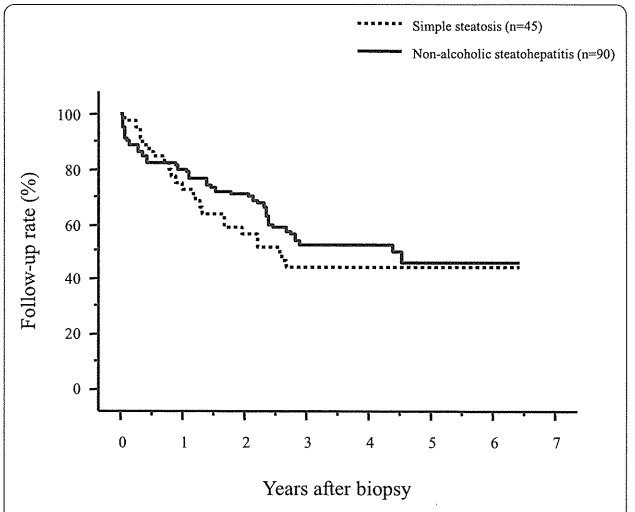

Figure 4 Comparison of the post-biopsy follow-up rates of patients with nonalcoholic fatty liver disease between patients with nonalcoholic steatohepatitis and those with simple steatosis. There were no differences in the follow-up rates (p = 0.5202).

73.0% and 44.6% for patients with simple steatosis and 80.0% and 52.4% for patients with NASH, respectively, without significant differences between these two groups (p = 0.5202). The presence of NASH, as determined by a pathologic diagnosis, did not influence the follow-up rate after the liver biopsy.

Among 65 patients who discontinued their follow-up visit for NAFLD, 13 patients (20.0%) had received periodic follow-up examinations for diabetes at the diabetes department of our institution, and 15 patients (23.1%) had received follow-up exams for diabetes by their family physician. However, in both cases, the patients were not examined for liver disease.

Discussion

Regular outpatient follow-up visits are important for patients with chronic liver diseases. In particular, previous studies have shown that periodic HCC surveillance in patients with liver diseases improves the patient survival due to the detection of early-stage HCC [10,11]. In addition, it is important for NAFLD patients to receive regular advice and be monitored for weight loss, exercise, and specific diets at outpatient clinics to prevent the conversion from simple steatosis to NASH and the progression of NASH. However, there is no study that investigated the outpatient follow-up of patients with NAFLD.

Table 2 Comparison of the characteristics of non-alcoholic fatty liver disease patients who continued and discontinued their follow-up exams after a liver biopsy.

	Patients who maintained their follow-up exams (n = 68)	Patients who discontinued their follow-up exams (n = 67)	*p*-value
Age (years: mean ± S.D.)	52.6 ± 13.4	44.9 ± 15.2	0.0035
Sex (male/female) (%)	42 (61.8)/26 (38.2)	42 (62.7)/25 (37.3)	0.9120
AST (IU/L: mean ± S.D.)	59.6 ± 35.0	56.4 ± 49.4	0.1208
ALT (IU/L: mean ± S.D.)	93.6 ± 70.5	95.9 ± 65.4	0.5928
Medication (yes/no) (%)	21 (30.9)/47 (69.4)	18 (26.9)/49 (73.1)	0.7442
Pathologic diagnosis (NASH/simple steatosis) (%)	47 (69.1)/21 (30.9)	43 (64.2)/24 (35.8)	0.6691

AST, aspartate aminotransferase; ALT, alanine aminotransferase; NASH: non-alcohol steatohepatitis.

The results of the present study revealed that NAFLD patients have a markedly reduced follow-up rate after they receive their initial diagnostic liver biopsy, in comparison to those with viral hepatitis. The follow-up rate of NAFLD patients was significantly lower than that of patients with chronic HCV infection, even when focusing on patients who had not been administered medication. In addition, the post-biopsy follow-up rate of NAFLD patients was lower in comparison to patients whose chronic HCV infection in was eradicated with antiviral therapy, in whom the follow-up rate was lower compared to patients with ongoing HCV infection [12]. In a previous study on patients with alcoholic liver disease, Trabut et al. reported that a liver biopsy did not further motivate patients to abstain from alcohol [13]. Similarly, in these findings, a liver biopsy did not motivate patients to attend follow-up visits that were designed to control and monitor NAFLD.

When we compared NAFLD patients who continued and discontinued their follow-up visits, there was a significant difference in age between these two subgroups, and younger patients were more likely to miss subsequent follow-up exams. This could be partially due to the difficulty in visiting the hospital due to the limited appointment times, and partly because younger patients tend to be less interested in their health than older patients. Interestingly, there were no differences in the follow-up rates between patients with and without NASH. The presence of NASH did not influence the follow-up rate of NAFLD patients, despite the fact that these patients were told that they were at increased risk of developing cirrhosis or HCC. This could be partly because NAFLD patients often do not have accurate and detailed knowledge on the natural course of NASH and the potential occurrence of HCC. The increased awareness of the risk of HCC in patients with chronic viral hepatitis has been established in Japan and could have increased the number of patients under surveillance. However, the present study indicates that patients in Japan are insufficiently aware that they are at increased risk of NASH progressing to cirrhosis or developing HCC. Also, we observed the similar percentage of patients who had been administered medication between patients who continued and discontinued regular follow-up visit. Therefore, the administration of medication did not prevent the drop out from regular visit in NAFLD patients. The medication that had been administered for NAFLD patients included ursodeoxycolic acid, or drugs for diabetes, hypertension, or hyperlipidemia, and was not drugs for NAFLD in the absence of definitive drugs for NAFLD. The emergence of drugs for NASH or NAFLD may increase the follow-up rate of patients with NAFLD in the future.

There are several limitations on this study. This is a retrospective study on the basis of the reviews of medical record. In addition, this is a study conducted in one region of Japan. All people in Japan are usually covered by a national medical insurance system, and the follow-up rates of patients with chronic hepatitis virus infection are very high. Therefore, the results observed in this study will not be applicable in other countries with a different medical insurance system or with different prevalence of patients with NAFLD and those with viral hepatitis.

Conclusions

NAFLD patients had a low post-liver biopsy follow-up rate. Thus, it is important to continue the current efforts to inform individuals in Japan of the risks of this disease. Furthermore, given the large number of NAFLD patients, it will be important to further examine methods to identify patients with NAFLD who are at higher risk for disease progression or are at risk for developing HCC [14,15]. For patients chronically infected with HBV or HCV, chronic hepatitis virus infection is a risk factor for progression to cirrhosis or development of HCC. In contrast, it is not easy to identify NAFLD patients who are at high risk for progressing to cirrhosis or developing HCC. It will be necessary to further identify patients who are at increased risk for progressing to cirrhosis or developing HCC, even among patients who are pathologically confirmed to have NASH, in order to further clarify and better monitor patients with NASH who are at higher risk for disease progression and HCC, identifying patients who we should be strongly advised to have regular follow-up exams.

List of abbreviations used

HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; NAFLD: non-alcoholic fatty liver diseases; NASH: non-alcoholic steatohepatitis.

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Authors' contributions

All authors (HT, TK, SK, MT, YH, AK, and TT) are managed patients with viral hepatitis and those with NAFLD at an outpatient clinic. HT and TT performed liver biopsy. HT carried out the acquisition of patient data on follow-up status and performed statistical analyses. HT and TK participated the design of the study. HT drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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CLINICAL STUDY

Transarterial Chemoembolization for Hepatitis B Virus–Associated Hepatocellular Carcinoma: Improved Survival after Concomitant Treatment with Nucleoside Analogues

Hidenori Toyoda, MD, Takashi Kumada, MD, Toshifumi Tada, MD, Yasuhiro Sone, MD, and Masashi Fujimori, MD

ABSTRACT

Purpose: To determine whether nucleoside analogue therapy is associated with improved survival in patients with hepatitis B virus (HBV)–associated hepatocellular carcinoma (HCC) who are treated solely with transarterial chemoembolization.

Materials and Methods: A retrospective chart review of patients diagnosed with HBV-associated HCC was performed to identify patients treated solely with chemoembolization. Relevant demographic and clinical data were extracted and recorded. The influence of therapy with nucleoside analogues (lamivudine, adefovir dipivoxil, or entecavir) was determined by estimating the survival function using the Kaplan-Meier product-limit method.

Results: The inclusion criteria for chemoembolization were met by 81 patients (67 men and 14 women, mean age 60.6 years ± 9.2); 21 (25.9%) of these patients had been treated with nucleoside analogues. The number of chemoembolization treatments was significantly greater in the patients who were treated with nucleoside analogues (3.43 ± 2.32) than in the patients who did not receive nucleoside analogues (1.82 ± 0.95; $P = .0022$). The 1-year, 3-year, and 5-year survival rates were 89.5%, 66.8%, and 40.5% in the patients treated with nucleoside analogues and 72.6%, 27.5%, and 14.3% in the patients not treated with nucleoside analogues. The survival rate was significantly higher in the patients who received nucleoside analogues ($P = .0051$). Nucleoside analogue intake was an independent factor that was associated with increased survival ($P = .0063$).

Conclusions: Administration of nucleoside analogues was associated with longer survival in patients with HBV-associated HCC who were treated with transarterial chemoembolization.

ABBREVIATIONS

AFP = alpha-fetoprotein, HBV = hepatitis B virus, HCC = hepatocellular carcinoma

Transarterial embolization was initially used to treat hepatocellular carcinoma (HCC) by Doyon et al (1) in 1974, and chemoembolization with gelatin sponge particles and anticancer agents was subsequently developed in Japan to treat inoperable HCC (2). Despite the increase in the number of patients who undergo complete curative treatments such as hepatectomy or radiofrequency ablation (3), transarterial chemoembolization continues to have an important role, both as an initial treatment and as a therapeutic alternative for recurrent disease (4) because of the advanced nature of HCC at diagnosis and the high rate of recurrent disease (5). The benefits resulting from chemoembolization have long been a subject of debate (6–10), but two randomized trials found that chemoembolization was associated with higher survival compared with symptomatic treatment (4,11,12).

Because of poor liver function, patients with HCC do not always receive chemoembolization. Repeated chemoembolization treatments for HCC may cause liver function to deteriorate despite the fact that the deterioration of liver function by each chemoembolization treatment would be mild (13). If repeated chemoembolization treatments are to be used in cases of HCC recurrence, it is important to

From the Departments of Gastroenterology (H.T., T.K., T.T.) and Radiology (Y.S., M.F.), Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu, 503-8502, Japan. Received May 31, 2011; final revision received November 12, 2011; accepted November 14, 2011. Address correspondence to H.T.; E-mail: tkumada@he.mirai.ne.jp

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Tables E1 and E2 are available online at *www.jvir.org*.

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prevent the worsening of liver function in the intervals between the treatments for longer survival (14).

Nucleoside analogues against hepatitis B virus (HBV) have been used since the late 1990s to suppress the replication of HBV and to normalize transaminase levels. Therapy with nucleoside analogues against HBV is known to arrest the progression of hepatic dysfunction in patients with chronic hepatitis B. More recent studies have shown that these drugs prevent the development of liver failure, even in the patients with advanced liver fibrosis (15–19). However, it is unknown whether this beneficial effect of antiviral therapy translates into longer survival for patients with concomitant HCC who undergo chemoembolization. We conducted a retrospective review of our experiences using chemoembolization to treat HCC in patients with chronic HBV infection.

MATERIALS AND METHODS

Patients

The complete study protocol was approved by the institutional review board of our hospital and was performed in compliance with the Helsinki Declaration. Between July 1997 and December 2010, 1,359 patients were diagnosed with primary HCC at our institution. Chronic HBV infection was confirmed in 260 of these patients, and 95 of these 260 patients were treated with chemoembolization. Of these 95 patients, 14 underwent treatments other than chemoembolization for recurrent HCC (4 underwent hepatectomy and 10 underwent radiofrequency ablation), and the remaining 81 patients had been treated with chemoembolization alone for recurrent HCC tumors. Our study retrospectively examined these 81 patients.

HCC was diagnosed based on clinical criteria (20) in all 81 patients. Specifically, the patients had a pertinent clinical background (chronic HBV infection) and typical imaging results. The tumor usually was detected by B-mode ultrasonography with typical HCC imaging features, including a hypoechoic tumor or a tumor with a mosaic pattern with a halo. HCC was diagnosed when a high-density mass was detected on arterial phase dynamic computed tomography (CT) images combined with a low-density mass on portal phase dynamic CT images obtained with a single or multidetector helical CT scanner. All of the patients with possible HCC tumors underwent angiography using a unified CT-angiography system (Interventional-CT; Toshiba, Tokyo, Japan) (21,22). CT during arterial portography and CT during hepatic arteriography were also performed to evaluate the progression of HCC (23).

The patients included 67 men (82.7%) and 14 women (17.3%), with a mean age of 60.6 years ± 9.2. The liver function at diagnosis was Child-Pugh class A in 49 patients (60.5%). At the time of diagnosis, 52 patients (64.2%) had multiple initial HCC tumors. HCC was accompanied by branch portal vein invasion in 18 patients (22.2%), but no patients had HCC invasion of the main portal vein trunks or the left or right main portal vein (**Table E1**).

Chemoembolization for Hepatocellular Carcinoma and Follow-Up after Treatment

The treatment decisions were based principally on the Japanese HCC treatment guidelines (24). The patients were initially assessed for their eligibility for hepatic resection and subsequent local ablative therapies, including percutaneous ethanol injection, percutaneous microwave thermocoagulation, and radiofrequency ablation. The patients who were not eligible for curative treatment with surgery, local ablative therapies, or a combination of both were offered chemoembolization. The patients with Child-Pugh class C (25) liver function and the patients with HCC invasion of the main portal vein trunks and left or right main portal vein were not offered chemoembolization. Chemoembolization was performed by injecting an emulsion of 50 mg of farmorubicin hydrochloride (Epirubicin; Adria Laboratories, Columbus, Ohio) or 100 mg of cisplatin (IA-Call; Nihon-Kayaku, Tokyo, Japan) dissolved in 5 mL of iopamidol (Iopamiron, 370 mg I/mL; Schering, Tokyo, Japan) and mixed with 5 mL of iodized oil (Lipiodol Ultra Fluid; Guerbet, Paris, France). This procedure was followed by an injection of gelatin sponge particles (Gelfoam; Upjohn, Kalamazoo, Michigan). The total dose of the injected emulsion was determined by the volume of the liver that would be embolized. An unenhanced CT scan was obtained to confirm complete deposition of the iodized oil in the lesion and to complete the treatment.

After the first chemoembolization treatment, the patients were followed for 2.39–118.6 months (median follow-up period 19.3 months) at our institution with ultrasonography and CT or magnetic resonance imaging performed every 3–6 months. Serum tumor markers (alpha-fetoprotein [AFP], *Lens culinaris* agglutinin-reactive AFP, and des-gamma-carboxy prothrombin) were monitored every 3 months. When elevated tumor markers were detected, an additional imaging examination (usually CT or magnetic resonance imaging) was performed to check for recurrence or progression of HCC. If recurrence or progression was confirmed, retreatment was considered. Retreatment decisions were also based on the Japanese HCC treatment guidelines. Repeat chemoembolization was considered as a retreatment option in patients who had HCC recurrence or progression.

Statistical Analyses

The intergroup differences were analyzed using χ^2 and Mann-Whitney *U* tests for categorical and quantitative data. The date of the initial HCC treatment (chemoembolization) was defined as time zero when calculating the patient survival rates. Surviving patients and patients who died from causes other than liver disease were censored in the survival analysis. Patients whose death was caused by HCC

Table 1. Clinical Characteristics of Patients Who Did and Did Not Receive Nucleoside Analogues

	Nucleoside Analogues (+) (n = 21)	Nucleoside Analogues (−) (n = 60)	*P* Value
Age (mean ± SD, y) (range)	60.3 ± 8.9 (46–81)	60.6 ± 9.3 (37–78)	.7957
Sex ratio (female/male)	3 (14.3%)/18 (85.7%)	11 (23.3%)/49 (76.7%)	.9274
Child-Pugh class (A/B)	14 (66.7%)/7 (33.3%)	35 (58.3%)/25 (41.7%)	.6773
Albumin (mean ± SD, g/dL)	3.65 ± 0.45	3.33 ± 0.79	.0372
Total bilirubin (mean ± SD, mg/dL)	1.22 ± 0.72	0.98 ± 0.85	.0844
15-minute retention rate of ICG (%)*	24.8 ± 12.3	19.6 ± 13.8	.0691
Prothrombin (%)	81.1 ± 19.5	79.7 ± 20.4	.8209
Platelet count (× 1,000/mL)	112 ± 52	143 ± 82	.1867
Tumor size (mean ± SD, cm) (range)	4.30 ± 2.94 (1.2–11.5)	4.40 ± 3.24 (1.0–16.0)	.8083
Tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm)	4 (19.0%)/11 (52.4%)/6 (28.6%)	17 (28.3%)/25 (41.7%)/18 (30.0%)	.6282
Tumor number (single/multiple)	9 (42.9%)/12 (57.1%)	20 (33.3%)/40 (66.7%)	.4333
Portal vein invasion (absent/present)	18 (85.7%)/3 (14.3%)	45 (75.0%)/15 (25.0%)	.4744
AFP (median, ng/mL) (range)	56.7 (0.9–3,132)	61.4 (0.8–1,304,200)	.7836
AFP (≥ 20 ng/mL/< 20 ng/mL)	13 (61.9%)/8 (38.1%)	35 (58.3%)/25 (41.7%)	.9746
AFP-L3 (median, %) (range)	0.5 (0–64.0)	6.2 (0–60.7)	.3658
AFP-L3 (≥ 10%/< 10%)	7 (33.3%)/14 (66.7%)	24 (40.0%)/36 (60.0%)	.7769
DCP (median, mAU/mL) (range)	94.0 (16–8,000)	62.0 (10–75,000)	.7997
DCP (≥ 40 mAU/mL/< 40 mAU/mL)	13 (61.9%)/8 (38.0%)	41 (68.3%)/19 (31.7%)	.7854

AFP = alpha-fetoprotein; AFP-L3 = *Lens culinaris* agglutinin-reactive AFP; DCP = des-gamma-carboxy prothrombin; ICG = indocyanine green test.
* ICG test was not performed in 14 patients.

or liver failure were not censored. The survival function was estimated using the Kaplan-Meier product-limit method (26), and the log-rank test (27) was used to analyze the differences in survival.

The Cox proportional hazards model (28) was used to perform a multivariate analysis of the factors related to survival. The following variables were analyzed: patient age and sex, Child-Pugh class (A/B), tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm), number of tumors (single/multiple), portal vein invasion (absent/present), and treatment with nucleoside analogues against HBV. The data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, North Carolina). All *P* values were derived from two-tailed tests; $P < .05$ was considered statistically significant.

RESULTS

Comparison of Patient Characteristics According to Nucleoside Analogue Intake

The anti-HBV nucleoside analogues had been administered to 21 of the 81 patients (25.9%). Among the 21 patients who had received nucleoside analogues, 7 patients had already been taking nucleoside analogues at the initial HCC diagnosis, and the remaining 14 patients started nucleoside analogues after diagnosis of HCC. Seven patients were taking 100 mg of lamivudine (Zefix; GlaxoSmithKline, Tokyo, Japan), eight patients were taking 0.5 mg of entecavir (Baraclude; Bristol-Myers Squibb, Tokyo, Japan), and six patients were taking lamivudine and 10 mg of adefovir dipivoxil (Hepsera, GlaxoSmithKline) because of the emergence of lamivudine-resistant HBV. **Table 1** compares the background characteristics of the patients who had and had not been treated with nucleoside analogues. There were no significant differences between these two groups in patient age and sex, liver function, and tumor progression, although the serum albumin levels were higher in the patients who received nucleoside analogues.

Influence of Nucleoside Analogue Treatment on Survival and Progression-Free Survival

Table 2 shows the number of chemoembolization treatments that were performed for initial and recurrent HCC with respect to the nucleoside analogue intake. Chemoembolization could not be performed more than four times in the patients who had not received nucleoside analogues; however, it was performed more than four times in one-third of patients who did receive them. The number of chemoembolization treatments was significantly higher in the patients who had received nucleoside analogues than in the patients who were not treated with nucleoside analogues ($P = .0022$). In the patients who underwent chemoembolization treatments repeatedly, the interval between two chemoembolization treatment sessions did not differ significantly between the patients who were and were not treated with nucleoside analogues (6.27 months ± 2.66 in patients without nucleoside analogues vs 6.71 months ± 2.71 in