HCCs (a maximum diameter of 3 cm or smaller), three or fewer lesions with no extrahepatic metastasis (Table 1), serum MtCK activity was additionally analyzed in more advanced HCC patients. Serum MtCK activity of those patients was 10.4 ± 9.2 U/L (n = 20), which was comparable to, not higher than, that in the HCC patients in the original cohort. Among them, nine patients responded to 5-fluorouracil and interferon-α [23] or transcatheter arterial chemoembolization. Among the responders, serum MtCK activity decreased to 31.7% of the value prior to the treatment in one patient, and overall, serum MtCK activity decreased significantly to 77.1% of the value prior to treatment (n = 9, $p = 0.003$).

High level of serum ubiquitous MtCK activity in HCC patients and of ubiquitous MtCK mRNA expression in HCC cell lines

Regarding MtCK, two tissue-specific isoenzymes are known, i.e., sarcomeric MtCK is found in striated muscles of vertebrates, while ubiquitous MtCK has been detected in most other tissues including brain, kidney, and sperm [24]. Thus, we examined which of the two isoenzymes was increased in the sera of HCC patients with high levels of MtCK activity. Specific antibodies to sarcomeric MtCK and ubiquitous MtCK were applied separately for the measurement of MtCK activity in 135 patients with HCC. Sarcomeric MtCK activity was under minimum detection limit of 1.9 U/L in 131 patients; in the remaining four patients, sarcomeric MtCK activity was 2.0, 2.2, 2.5, and 2.6 U/L, respectively. In the latter four patients, ubiquitous MtCK activity was 13.6, 5.2, 9.2, and 5.1 U/L, respectively. Thus, a small increase in sarcomeric MtCK activity was observed in only four out of 131 patients, which might be explained by a measurement error near the minimum detection limit. Collectively, the increase in serum MtCK activity in patients with HCC was mostly due to ubiquitous MtCK activity.

To examine other CK isoenzymes, the sera of HCC patients were analyzed using electrophoresis. As shown in Fig. 4A, octameric MtCK bands were found in the samples with high MtCK activities (>30 U/L; lanes 2–8), and dimeric MtCK bands were also found in these samples after incubation with anti-CK-M antibody because of close migration of the dimeric MtCK to the position of CK-MM [19]. Of note, no correlation was seen between serum MtCK activity and serum CK-MM activity or CK-MB activity. The sera of HCC patients were also examined using an immunoblot analysis. As demonstrated in Fig. 4B, serum CK-B did not correlate with serum ubiquitous MtCK, although CK-MB and CK-BB were not analyzed separately. Collectively, no correlation was observed between serum ubiquitous MtCK activity and other serum CK isoenzyme activities.

Finally, ubiquitous *MtCK* mRNA expressions in HCC cell lines, JHH7, Alex, HuH7, and HepG2 were determined using real-time PCR. The ratio of ubiquitous *MtCK* mRNA to *18s* rRNA was much higher in HCC cell lines than in the normal human liver, as depicted in Fig. 4C.

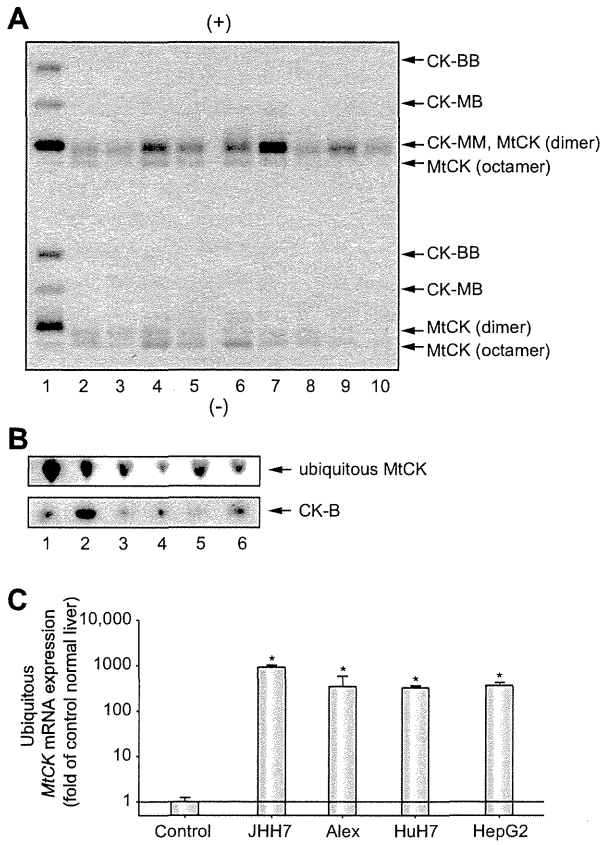

Fig. 4. CK and MtCK isoenzymes in the sera of HCC patients and in HCC cell lines. (A) MtCK and other CK isoenzymes in the sera of HCC patients. The sera of HCC patients with high MtCK activities (>30 U/L; lanes 2–8) and low MtCK activities (<8 U/L; lanes 9–10) were analyzed using electrophoresis with or without prior incubation with anti-CK-M antibody. Lane 1, CK isoenzyme controls. The octameric MtCK bands were found in the samples with high MtCK activities and the dimeric MtCK bands were also found in these samples after incubation with anti-CK-M antibody because of close migration of the dimeric MtCK to the position of CK-MM. (B) Ubiquitous MtCK and CK-B in the sera of HCC patients. The sera of HCC patients with high MtCK activities (>30 U/L; lanes 1–2), intermediate MtCK activities (8–9 U/L; lanes 3–4) and low MtCK activities (<3 U/L; lanes 5–6) were examined using an immunoblot analysis for ubiquitous MtCK and CK-B. (C) Ubiquitous *MtCK* mRNA expression in HCC cell lines and the control normal liver. Ubiquitous *MtCK* mRNA expression in human HCC cell lines, JHH7, Alex, HuH7, and HepG2, and the control normal liver was quantitated using real-time PCR, and the relative amount was normalized to the signal of *18s* rRNA. Columns and bars represent means ± SD of duplicate samples. The asterisk (*) indicates a significant difference from the control normal liver.

Discussion

Healthy liver tissue is one of the few tissues that, in general, do not express detectable amounts of MtCK or cytosolic CK isoforms [14]. Thus, their expression in the liver is assumed to be a sign of pathological development associated with, for example, ischemic-reperfusion injury [25] or tumor formation [15]. The enzyme described as "Macro CK" [26,27] in previous reports has in fact been identified as ubiquitous MtCK, and a correlation between serum ubiquitous MtCK level and the pathological condition of nephrotoxicity in HIV patients receiving tenofovir has been reported [28]. Although the role of CK expression in the pathological liver has not been fully elucidated, CK expression in the liver of transgenic mice reportedly provokes tolerance against tumor necrosis factor-α-induced apoptosis [29], protection against hypoxia or endotoxin perfusion [30–32], and inhibition of pro-apoptotic mechanisms [33], suggesting a beneficial role of CK expression in the liver.

In the current study, serum activity of MtCK was significantly higher in patients with cirrhosis and HCC caused by HBV or HCV virus than in subjects with no liver diseases. Among the patients with cirrhosis, serum MtCK activity was significantly higher in patients with HCC than in those without HCC. We also observed that serum MtCK activity decreased significantly after treatment with RFA, although the number of patients analyzed was small. Thus, our findings may raise a possibility that MtCK, measured by the novel immune-inhibition method, may be useful as a serum marker of HCC. The ROC curve comparing cirrhotic patients with or without HCC showed that MtCK was superior to AFP but inferior to DCP for the diagnosis of HCC. Serum MtCK activity above this cut-off was found in 52.9% and 63.2% of HCC patients with AFP levels below 20 ng/ml and DCP levels below 40 mAu/ml, respectively, suggesting the potential utility of MtCK for the diagnosis of HCC in patients with normal or mildly elevated AFP and/or DCP levels. Furthermore, serum MtCK activity was also useful for predicting a single HCC $\leqslant$2 cm in diameter, suggesting the potential usefulness of serum MtCK activity to detect early HCC.

As described earlier, MtCK once attracted attention as a potential tumor-associated marker in the serum including HCC [15], however, the serum MtCK level was not judged to be a useful marker of HCC [18]. Previous investigations reported that an increase in serum MtCK level was detectable only in cases with advanced HCC [16] and that the sensitivity of serum MtCK level for the diagnosis of HCC was relatively low [18]. In contrast, a relatively higher sensitivity of serum MtCK activity for the detection of a single HCC $\leqslant$2 cm was observed in the current study. These differences can be explained by the methodology used to measure MtCK activity. In the previous studies, MtCK level was measured using electrophoresis and densitometry [16,18]. On the other hand, the enzymatic activity of MtCK was directly determined following the immuno-inhibition in the current study. The presently reported method may be superior to previous methods for quantifying MtCK activity. Furthermore, MtCK is known to exist in the serum as a dimer and an octamer [14]. After electrophoresis, dimeric MtCK is found close to the electrophoretic position of CK-MM, while the octameric MtCK is electrophoresed cathodic to CK-MM [34]. This close migration of the dimeric MtCK to the position of CK-MM in the zymogram could cause overlapping of the dimeric MtCK with the CK-MM band. In fact, the dimeric MtCK was missed in the evaluation of MtCK activity in a previous study [16] and the current study. In contrast, our current method is free from this problem, being capable of measuring both dimeric and octameric MtCK [19]. Collectively, the utility of MtCK as a serum marker for HCC has been clarified as a result of this improved methodology.

Another advantage of this novel method is its applicability for an automatic analyzer. Using this method, serum MtCK activity of a large number of serum samples can be quickly measured, reducing the turnaround time of routine laboratory tests and ultimately increasing its value when used in the clinical setting.

When considering serum MtCK activity as a potential marker for HCC, its limitation is that the correlation between serum MtCK activity and the stage of HCC was not observed in contrast to the previous reports [16,18]. Because CK including MtCK is not naturally secreted from the cells, it is speculated that the active release of MtCK from the tissue with the higher expression of MtCK may be necessary for its serum activity to be increased. Although a higher mRNA expression of ubiquitous MtCK in four HCC cell lines than in the normal liver tissue was determined in the current study, the releasing mechanism of MtCK into the blood stream in HCC remains to be clarified. If this releasing mechanism might not be correlated with the stage of HCC, it may explain the failed correlation between serum MtCK activity and the stage of HCC. This potential releasing mechanism may include mitochondrial dysfunction as the commitment step in hepatocyte cell death [35]. Because continuous hepatocyte cell death is a main feature of liver cirrhosis [36], mitochondrial dysfunction may be linked to the abundant appearance of MtCK in the blood of cirrhotic patients. It should be further elucidated whether this mitochondrial dysfunction may be involved in the release of MtCK also in the HCC tissue.

As another limitation of this study, it should be noted that the analyzed HCC patients were predominantly those with recurrence, because they were enrolled consecutively. Thus, the performance of MtCK to predict HCC at the first occurrence in cirrhotic patients, especially a less than 2 cm HCC detected at an ultrasound screening, should be further evaluated.

Although the correlation between serum MtCK activity and the stage of HCC was not observed, its increase in patients with early HCC should be noted. Unlike AFP or DCP, the performance of MtCK for the prediction of early HCC was not reduced compared to that of all HCCs. On the other hand, the increase of serum MtCK activity has not been observed in early stage of gastric cancer or colorectal cancer (data not shown). It is possible that the increase of serum MtCK activity in its early stage may be a specific phenomenon of HCC. In conclusion, serum MtCK activity merits consideration as a novel marker for HCC to be further tested as for its diagnostic and prognostic power.

Conflict of interest

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

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CME

Open

Radiofrequency Ablation for Hepatocellular Carcinoma: 10-Year Outcome and Prognostic Factors

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OBJECTIVES: **Radiofrequency ablation (RFA) is widely performed for hepatocellular carcinoma (HCC). However, there has been no report on 10-year outcome of RFA. The objective of this study was to report a 10-year consecutive case series at a tertiary referral center.**

METHODS: **We performed 2,982 RFA treatments on 1,170 primary HCC patients and analyzed a collected database.**

RESULTS: **Final computed tomography images showed complete tumor ablation in 2,964 (99.4%) of 2,982 treatments performed for the 1,170 primary HCC patients. With a median follow-up of 38.2 months, 5- and 10-year survival rates were 60.2% (95% confidence interval (CI): 56.7–63.9%) and 27.3% (95% CI: 21.5–34.7%), respectively. Multivariate analysis demonstrated that age, antibody to hepatitis C virus (anti-HCV), Child-Pugh class, tumor size, tumor number, serum des-γ-carboxy-prothrombin (DCP) level, and serum lectin-reactive α-fetoprotein level (AFP-L3) were significantly related to survival. Five- and 10-year local tumor progression rates were both 3.2% (95% CI: 2.1–4.3%). Serum DCP level alone was significantly related to local tumor progression. Five- and 10-year distant recurrence rates were 74.8% (95% CI: 71.8–77.8%) and 80.8% (95% CI: 77.4–84.3%), respectively. Anti-HCV, Child-Pugh class, platelet count, tumor size, tumor number, serum AFP level, and serum DCP level were significantly related to distant recurrence. There were 67 complications (2.2%) and 1 death (0.03%).**

CONCLUSIONS: **RFA could be locally curative for HCC, resulting in survival for as long as 10 years, and was a safe procedure. RFA might be a first-line treatment for selected patients with early-stage HCC.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignant neoplasm in the world (1). Only 20% of HCC patients are candidates for resection (2). Furthermore, recurrence is frequent even after apparently curative resection. Liver transplantation is restricted by organ donor shortage. Thus, various nonsurgical therapies have been introduced (3–5). Among these, image-guided percutaneous ablation is considered best for early-stage HCC.

Ethanol injection was formerly the standard procedure among the various percutaneous ablation techniques. Randomized controlled trials, however, have demonstrated that radiofrequency ablation (RFA) has a more reliable local antitumor effect, leading to a lower local tumor progression risk and higher survival rates (6–9). RFA has largely replaced ethanol injection (10).

Several reports on 5-year outcome of RFA exist (11–17); however, no study has covered 10-year outcome. We report on a 10-year consecutive case series at a tertiary referral center. We analyzed antitumor effect, patient survival, local tumor progression, and distant recurrence rates, variables relevant to these outcomes, and complications. To our knowledge, this study documents the largest number of RFA treatments performed at a single institution.

METHODS

RFA indications

RFA was the treatment of choice in HCC patients satisfying the following criteria: (i) ineligible for surgical resection/liver transplantation or patient refusal for surgery; (ii) no extrahepatic metastasis/vascular invasion; and (iii) no other malignancies that

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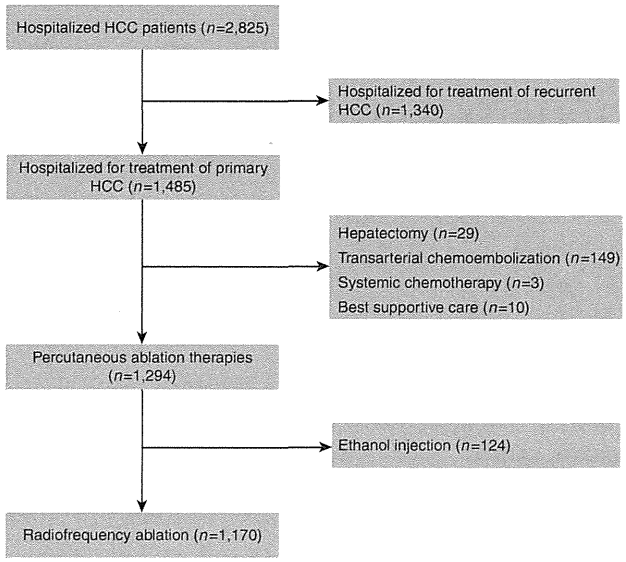

Figure 1. Flow of patients in this study. HCC, hepatocellular carcinoma.

may determine the patient's prognosis. Exclusion criteria were as follows: (i) tumor not visualized by ultrasonography/not accessible percutaneously; (ii) total bilirubin level ≥3.0 mg/dl; (iii) platelet count $< 50\times10^9$/l or prothrombin activity < 50%; (iv) refractory ascites; (v) enterobiliary reflux; and (vi) adhesion between the tumor and the gastrointestinal tract. In general, we performed RFA on Child-Pugh class A or B patients, a single tumor ≤5 cm in diameter, or three or fewer tumors ≤3 cm in diameter. In cases beyond these conditions, we performed RFA on patients who were likely to benefit from this procedure for possible cure or prolongation of life. No patients were excluded solely on account of tumor location (18). Informed consent was obtained from each patient. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the institutional review board (Registration ID: P98C05-11Y).

Patients

In this cohort study, we analyzed a prospectively collected computerized database. Between February 1999 and December 2009, 2,825 HCC patients were admitted once or more to the Department of Gastroenterology, the University of Tokyo (**Figure 1**). At initial hospitalization, 1,485 had primary HCC and the remaining 1,340 had recurrent HCC. In the recurrent HCC patients, primary HCC had previously been treated by therapies other than RFA.

Of the 1,485 primary HCC patients, 1,294 (87.1%) underwent percutaneous ablation as the initial treatment, including RFA. The remaining 191 patients underwent other therapies: hepatic resection, 29 patients with good liver function and who consented to an operation; transarterial chemoembolization, 149 with multinodular or large tumors that could not be treated by ablation therapies; systemic chemotherapy, three with extrahepatic metastasis; and only supportive care, 10 with decompensated cirrhosis or poor general condition.

Of the 1,294 patients treated by percutaneous ablation, 1,170 underwent RFA and the other 124 underwent ethanol injection. The choice of therapy was made as follows: between April 1999 and January 2001, 232 patients with three or fewer tumors, each ≤3 cm in diameter, and Child-Pugh class A or B liver function were entered into a randomized controlled trial to compare RFA with ethanol injection (6). Patients outside these inclusion criteria were mostly treated by RFA. After this trial, RFA was generally the treatment of choice, and ethanol injection was administered only to those considered unsuitable for RFA; ethanol injection was administered to those with either enterobiliary reflux or adhesion of the tumor to the gastrointestinal tract.

HCC was diagnosed based on typical imaging findings; that is, early-phase enhancement and late-phase contrast washout on dynamic computed tomography (CT) (19). HCC diagnosis was also confirmed by biopsy in 1,078 (92.1%) of the 1,170 patients with RFA-treated primary HCC. A total of 998 (85.3%) were diagnosed as having liver cirrhosis.

In general, transarterial chemoembolization was combined with RFA in patients with either ≥4 tumors or those with even one tumor >3.0 cm in diameter, although indication criteria of this combination had changed over time. The combination of transarterial chemoembolization with RFA was performed in 324 primary HCC patients.

Treatment methods

RFA was performed on an inpatient basis. Preoperative planning including evaluation of all imaging studies, and careful ultrasound examination was performed to identify the tumors and determine the access routes.

The procedure was performed according to an institutional protocol and in the presence of three physicians. One physician inserted the electrode under ultrasound guidance while another assisted the procedure; at least one had 8-year or longer experience of percutaneous ablation therapies. The remaining physician was responsible for the ultrasound machine and data recording. Video recording was performed occasionally to improve and standardize the procedure.

The precise techniques of RFA are described elsewhere (6). Briefly, all RFA procedures were performed percutaneously under ultrasound guidance (Power Vision 8000, Aplio XV or Aplio XG; Toshiba, Tokyo, Japan). We used artificial pleural effusion (20) or artificial ascites (21) for tumors, which were in the hepatic dome or adjacent to the gastrointestinal tract. After administration of sedatives and local anesthesia, a 17-gauge cooled-tip electrode (Cool-Tip; RF Ablation System, Covidien, Boulder, Colombia, CO) was inserted. Radiofrequency energy was delivered for 6–12 min for each application. For large tumors, the electrode was repeatedly inserted into different sites, such that the entire tumor could be enveloped by assumed necrotic volumes. Following the procedure, the patient remained in bed until the next morning.

A CT scan with a 5-mm section thickness was performed 1–3 days after RFA to evaluate technique effectiveness (22). Complete ablation was defined as hypoattenuation of the entire tumor. We intended to ablate not only the tumor but also some of the liver

parenchyma surrounding it. When we suspected that unablated tumor portions remained, the procedure was repeated. We did not predefine the procedure number in a treatment: treatment was generally continued until CT imaging demonstrated necrosis of the entire tumor.

Follow-up

To detect recurrence at an early stage, serum α-fetoprotein (AFP), lectin-reactive AFP (AFP-L3), and des-γ-carboxy-prothrombin (DCP) levels were measured monthly, and CT and ultrasonography were performed every 4 months. Local tumor progression was defined as the appearance of viable cancer tissue touching the initially treated tumor (22) and distant recurrence as the emergence of one or several tumor(s) separate from the primary site. Chest CT or bone scintigraphy was performed if extrahepatic recurrence was suspected. RFA was used for recurrence if the patient still met the indication criteria. If multiple recurrences were not treatable with RFA, transarterial chemoembolization was generally performed.

Statistical analysis

This is a report of a consecutive case series: all RFA treatments performed on primary HCC patients at the Department of Gastroenterology, University of Tokyo between February 1999 and December 2009 were included and none was excluded. Data are presented as mean±s.d. for quantitative variables, and as absolute frequencies for qualitative variables.

A "procedure" was defined as a single intervention episode comprising one or more ablation performed on one or more tumors and a "treatment" as the completed effort to ablate one or more tumors. A treatment comprised one or more procedures (22).

"Technique effectiveness" rate was defined as the percentage of successfully eradicated macroscopic tumors, as evidenced by CT scan 1–3 days after the last procedure (22).

Overall survival was calculated in the 1,170 primary HCC patients. Survival curves were generated by the Kaplan–Meier method. In addition to overall survival, some subgroup analyses were performed with clinical characteristics including tumor size, tumor number, and liver function. Recurrence was evaluated in 1,138 of the 1,170 primary HCC patients; the remaining 32 patients were excluded from the recurrence analysis because some small tumors had been left untreated by RFA on account of detection failure by ultrasonography. Recurrence rates were calculated by the Gaynor's method (23). All time estimates were made from the date of the first RFA. The follow-up was finalized at either death or the last visit to the outpatient clinic before 31 December 2009. Transplanted patients were censored from this study at the date of transplantation.

The prognostic relevance of 19 baseline variables (**Table 1**), the combination of transcatheter arterial chemoembolization (TACE) with RFA, HCC recurrence, and the number of RFA sessions to survival was analyzed by univariate and multivariate Cox proportional hazards regression models. The prognostic relevance of 19 baseline variables (**Table 1**), the combination of TACE with RFA, and the number of RFA sessions to local tumor progression and distant recurrence was also analyzed by univariate and multivariate models. All variables with a P value <0.05 by univariate comparison were subjected to multivariate analysis. Some continuous variables in which log-linearity could not be assumed were transformed into categorical variables. In multivariate analysis, we evaluated two models that contained either Child-Pugh class or its components to avoid multicollinearity. A stepwise variable selection was performed with Akaike Information Criteria in multivariate analysis. The results of multivariate analyses were presented as a hazard ratio with corresponding 95% confidence interval (CI), with P values from the Wald test. All significance tests were two-tailed, and differences with a P value <0.05 were considered statistically significant.

Complications were defined according to the guidelines of the Society of Interventional Radiology (24).

RESULTS

Antitumor effect

We performed a total of 2,982 RFA treatments for the 1,170 primary HCC patients, comprising 4,514 procedures. Thus, procedure number per treatment was 1.52±0.78. Many patients undergoing RFA for treatment of primary HCC received iterative RFA treatments for recurrence. A total of 485 patients underwent RFA treatment once, 247 twice, 177 thrice, 94 four times, 70 five times, 35 six times, 23 seven times, 14 eight times, 7 nine times, 7 ten times, 6 eleven times, 2 twelve times, 2 thirteen times, and 1 fourteen times.

Technique effectiveness rate was 99.4% (2,964/2,982 treatments). It was similar between the initial RFA treatments and the other RFA treatments for recurrence ($P=0.98$). Complete ablation of the tumor was achieved in 1,163 (99.4%) of the 1,170 initial treatments and in 1,801 (99.4%) of the 1,812 other RFA treatments. However, technique effectiveness rate significantly differed with tumor size ($P=0.023$). No apparent viable portions remained in the treated tumor in 1,642 (99.6%) of 1,648 treatments for tumors ≤2.0 cm in diameter, in 923 (99.2%) of 930 treatments for tumors 2.1–3.0 cm, in 356 (98.9%) of 360 treatments for tumors 3.1–5.0 cm, and in 43 (97.7%) of 44 treatments for tumors >5.0 cm. Final CT imaging demonstrated residual cancer tissue in the remaining 18 treatments. We decided against performing additional procedures, because liver failure rather than HCC seemed to determine the prognosis in 10 treatments, and because additional RFA would have caused complications on account of poor visualization or inaccessibility in the other eight treatments.

Survival

The 19 baseline clinical characteristics of the 1,170 patients who underwent RFA for treatment of primary HCC are shown in **Table 1**. A total of 269 patients (23.0%) were >75 years old. In all, 422 patients had tumors ≤2.0 cm in diameter, 467 had tumors 2.1–3.0 cm, 246 had tumors 3.1–5.0 cm, and 35 had tumors >5.0 cm; 685 patients had 1 tumor, 395 had 2 or 3 tumors, and 90 had ≥4 tumors.

As of December 2009 (with a median follow-up of 38.2 months), 692 patients (59.1%) remained alive, 39 (3.3%) were lost to

Table 1. Baseline characteristics of the 1,170 patients undergoing radiofrequency ablation for primary hepatocellular carcinoma

Variable	
Age (years)	68.3±8.6
Males, *n* (%)	751 (64.1)
Viral infection	
HBs-Ag-positive, *n* (%)	127 (10.9)
Anti-HCV-positive, *n* (%)	870 (74.4)
Both positive, *n* (%)	13 (1.1)
Both negative, *n* (%)	159 (13.6)
Alcohol consumption >80 g/d	170 (14.5)
Ascites, *n* (%)	117 (10.0)
Encephalopathy, *n* (%)	24 (2.1)
Albumin (g/dl)	3.65±0.47
Total bilirubin (mg/dl)	0.95±0.49
Prothrombin time (%)	79.6±14.1
Platelet count ($\times 10^4$/mm^3)	11.9±5.6
AST (IU/l)	61.5±35.9
ALT (IU/l)	57.3±40.8
Child-Pugh class, n *(%)*	
A	868 (74.2)
B	291 (24.9)
C	11 (0.9)
Tumor size (cm)	2.54±1.04
Tumor number	1.8±1.2
Serum AFP (ng/dl), n *(%)*	
≤100	928 (793)
101–400	146 (12.5)
>400	96 (8.2)
Serum DCP (mAU/ml), n *(%)*[a]	
≤100	964 (83.1)
101–400	126 (10.9)
>400	70 (6.0)
Serum AFP-L3 (%), n *(%)*	
≤15	1,015 (86.8)
15.1–40	74 (6.3)
>40	81 (6.9)

AFP, α-fetoprotein; AFP-L3, lectin-reactive α-fetoprotein; ALT, alanine aminotransferase; AST, asparatate aminotransferase; DCP, des-γ-carboxy-prothrombin; HCV, hepatitis C virus.

Data are expressed as mean±s.d.

[a]Serum DCP level could not be measured in 10 patients because they were being administered warfarin.

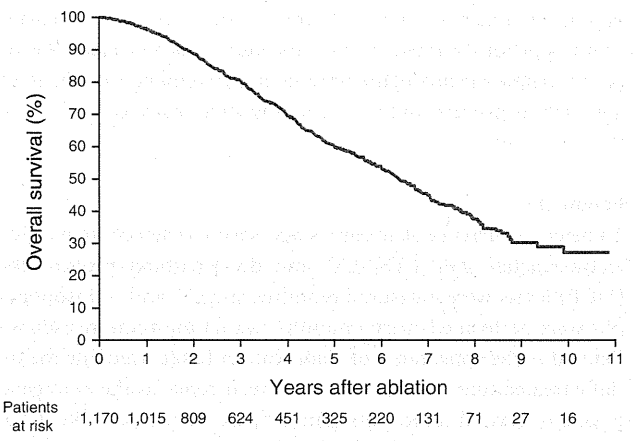

Figure 2. Overall survival in 1,170 primary hepatocellular carcinoma patients who underwent radiofrequency ablation.

follow-up, and 439 (37.5%) had died. Of the 1,170 patients, two were transplanted. The number of 5-, 7-, and 10-year survivors was 325, 131, and 16, respectively. The cause of death was HCC in 245 patients (55.8%), liver failure in 89 (20.3%), upper gastrointestinal bleeding in 11 (2.5%), complications related to the procedure in 3 (0.7%), liver-unrelated diseases in 81 (18.5%), and undetermined in 10 (2.3%).

The 1-, 3-, 5-, 7-, and 10-year survival rates of all 1,170 primary HCC patients were 96.6% (95% CI: 95.5–97.7%), 80.5% (95% CI: 78.0–83.1%), 60.2% (95% CI: 56.7–63.9%), 45.1% (95% CI: 40.9–49.6%), and 27.3% (95% CI: 21.5–34.7%), respectively (**Figure 2**; **Table 2**). Survival rates differed significantly with tumor size ($P<0.0001$), tumor number ($P=0.0003$), and Child-Pugh class ($P<0.0001$). In the Child-Pugh class A or B patients with a single tumor ≤5 cm in diameter, or three or fewer tumors ≤3 cm in diameter, the 5-year survival rate was 63.8% (95% CI: 59.9–67.9%), while in those outside these criteria, it was 46.4% (95% CI: 39.4–54.8%).

Univariate analysis showed 19 of the 22 variables relevant to survival. In multivariate analysis that contained Child-Pugh class but not its components, a model that contained age, antibody to hepatitis C virus (anti-HCV), Child-Pugh class, tumor size, tumor number, serum DCP level, and serum AFP-L3 level was selected (**Table 3**). The other model that contained the components of Child-Pugh class is shown in **Supplementary Table** online.

Recurrence

Recurrence developed in 741 patients. Local tumor progression alone was found in 25, local tumor progression with distant recurrence was found in 9, and distant recurrence alone was found in the other 707 patients. Of these 707 patients, 13 had the first recurrence in extrahepatic sites: 7 had lymph node metastasis, 3 had peritoneal seeding, 1 had lung metastasis, 1 had bone metastasis, and the remainder had both peritoneal seeding and lung metastasis. No recurrence developed in the remaining 397 patients.

Of the 741 patients, the first recurrence was treated by iterative RFA in 659 (88.9%), transarterial chemoembolization in 69 (9.3%), systemic chemotherapy in 4 (0.5%), surgical resection in 3 (0.4%), radiation therapy in 2 (0.3%), and supportive care in 4 (0.5%).

Table 2. Survival of patients undergoing radiofrequency ablation, based on tumor number, tumor size, and Child-Pugh class

Grading	*n*	Survival (%)					Median (years)	*P* value
		1-Year	3-Year	5-Year	7-Year	10-Year		
Overall survival	1,170	96.6	80.5	60.2	45.1	27.3	6.4	—
Tumor number								
Solitary	685	97.2	82.6	64.6	50.5	32.0	7.0	0.0003
2–3	395	95.7	77.9	54.4	39.4	19.9	5.6	
≥4	90	96.5	76.4	53.6	30.1	17.6	5.3	
Tumor size								
≤3 cm	889	97.2	83.8	65.1	47.3	30.7	6.7	<0.0001
>3 cm	281	94.8	71.0	46.5	38.0	18.6	4.6	
Child-Pugh class								
A	868	98.0	86.0	65.9	50.2	30.1	7.0	<0.0001
B	291	93.2	66.4	46.5	32.4	20.6	4.6	
C	11	81.8	58.4	23.4	23.4	—	3.1	
Combination of tumor number, tumor size, and Child-Pugh class								
Solitary, ≤3 cm	534	97.6	84.7	68.0	51.4	34.3	7.1	—
Solitary, ≤3 cm, Child-Pugh A	401	98.7	90.1	74.0	57.4	41.3	8.2	—
1–3 Tumors, ≤3 cm	822	97.1	83.7	65.2	48.8	32.5	6.9	—
Solitary, ≤5 cm, or 1–3 tumors, ≤3 cm	947	97.2	82.8	63.8	48.8	30.6	6.9	—
Child-Pugh A/B								
Satisfied the indication criteria of surgical resection proposed in the BCLC protocol[a]	237	98.6	90.5	75.9	61.1	38.1	8.7	—

BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.
[a]Child-Pugh class A with a normal level of bilirubin, no significant portal hypertension, and a single HCC.

The 1-, 3-, 5-, 7-, and 10-year rates of local tumor progression with or without distant recurrence were 1.4% (95% CI: 0.7–2.1%), 3.2% (95% CI: 2.1–4.3%), 3.2% (95% CI: 2.1–4.3%), 3.2% (95% CI: 2.1–4.3%), and 3.2% (95% CI: 2.1–4.3%), respectively (**Figure 3**). Univariate analysis demonstrated that prothrombin time and serum AFP, DCP, and AFP-L3 levels were correlated to local tumor progression, whereas multivariate analysis showed that serum DCP level alone was significantly correlated. Tumor size was not correlated to local tumor progression.

The 1-, 3-, 5-, 7-, and 10-year rates of distant recurrence without local tumor progression were 25.6% (95% CI: 23.0–28.2%), 63.3% (95% CI: 60.2–66.4%), 74.8% (95% CI: 71.8–77.8%), 78.1% (95% CI: 75.1–81.2%), and 80.8% (95% CI: 77.4–84.3%), respectively. Univariate analysis demonstrated 14 variables relevant to distant recurrence, whereas multivariate analysis showed that anti-HCV, Child-Pugh class, platelet count, tumor size, tumor number, serum AFP level, and serum DCP level were significantly related to distant recurrence (**Table 3**).

Complications

A total of 67 complications were encountered (**Table 4**). The incidence rates of complications per treatment and per procedure were 2.2% (67/2,982) and 1.5% (67/4,514), respectively. One patient died of hepatic failure on account of massive hepatic infarction 7 days after the last RFA procedure. He had undergone 12 RFA treatments in 8 years. The treatment mortality rate was 0.03%.

DISCUSSION

This study describes our 10-year clinical experience with RFA at a high-volume center. We performed the 2,982 RFA treatments on a total of the 1,170 primary HCC patients, showing that RFA has a high antitumor effect. Tumors were judged to have been completely ablated by final CT imaging in 99.4% of the treatments. Complete response was achieved not only in the first RFA but also in iterative RFA for recurrence. Although complete response rate differed with tumor size, there was not a sharp drop-off in effectiveness. The complete response rate may be higher in this study than others probably because we generally repeated the procedure until CT imaging demonstrated complete tumor necrosis, whereas many other studies limited the procedure number of RFA to 2–3 (11,13,15). Complete ablation of tumors has been reported to be related to improved survival (25). There were the 18 treatments in which we did not perform additional RFA for residual cancer tissue. In those treatments, usefulness of RFA had been unclear at the initial session because of liver dysfunction or tumor burden.

Table 3. Multivariate analysis of variables relevant to survival, local tumor progression, and distant recurrence

Variable	Multivariate analysis Hazard ratio (95% CI)	*P* value
Survival		
Age (per year)	1.03 (1.02–1.04)	<0.0001
Anti-HCV-positive	1.34 (1.03–1.76)	0.03
Child-Pugh class		
A	1	
B or C	2.08 (1.69–2.56)	<0.0001
Tumor size (cm)		
≤2.0	1	
2.1–3.0	1.40 (1.10–1.80)	0.007
3.1–5.0	1.80 (1.37–2.38)	<0.0001
>5.0	1.50 (0.90–2.49)	0.12
Tumor number		
Solitary	1	
2–3	1.28 (1.04–1.59)	0.02
≥4	1.58 (1.13–2.21)	0.008
Serum DCP (mAU/ml)		
≤100	1	
101–400	1.22 (0.88–1.69)	0.24
>400	1.66 (1.14–2.42)	0.008
Serum AFP-L3 (%)		
≤15	1	
>15	1.45 (1.11–1.91)	0.008
Local tumor progression		
Serum DCP (mAU/ml)		
≤100	1	
101–400	2.51 (1.02–6.20)	0.05
>400	6.52 (2.63–16.1)	<0.0001
Distant recurrence		
Anti-HCV-positive	1.44 (1.19–1.75)	0.0002
Child-Pugh class		
A	1	
B or C	1.23 (1.03–1.45)	0.02
Platelet count (/l)		
$>10^{11}$	1	
$\leq 10^{11}$	1.36 (1.12–1.64)	0.002
Tumor size (cm)		
≤2.0	1	
2.1–3.0	1.30 (1.10–1.55)	0.003
3.1–5.0	1.29 (1.05–1.60)	0.02
>5.0	1.25 (0.75–2.08)	0.4
Tumor number		
Solitary	1	
2–3	1.36 (1.16–1.59)	0.0002
≥4	2.02 (1.53–2.66)	<0.0001
Serum AFP (ng/dl)		
≤100	1	
101–400	1.15 (0.92–1.44)	0.22
>400	1.36 (1.03–1.81)	0.03
Serum DCP (mAU/ml)		
≤100	1	
101–400	1.19 (0.92–1.54)	0.19
>400	1.72 (1.22–2.42)	0.002

AFP, α-fetoprotein; CI, confidence interval; DCP, des-γ-carboxy-prothrombin; HCV, hepatitis C virus.

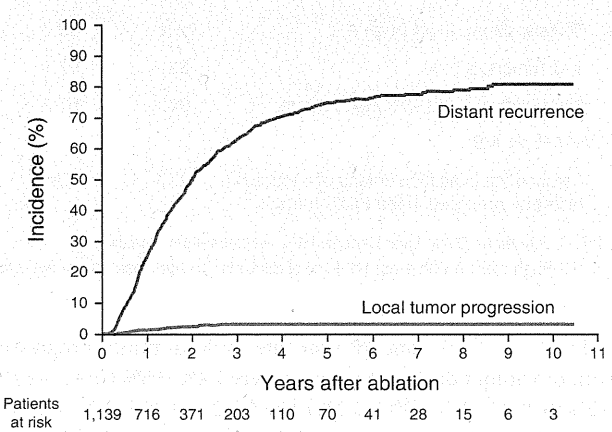

Figure 3. Local tumor progression and distant recurrence in patients who underwent radiofrequency ablation.

This study shows that RFA could achieve long-term survival for as long as 10 years. Sixteen patients treated by RFA survived for >10 years. The variables relevant to survival were similar to those found in previous studies on ethanol injection (26,27), RFA, hepatic resection (28), and transarterial chemoembolization (29). Both liver function and tumor-related factors were associated with survival. In addition, age and anti-HCV were relevant to survival in this study. Age was among the prognostic factors, probably because 23.0% of the patients were >75 years old, which resulted in a higher percentage (18.5%) of liver-unrelated deaths in this study compared with others. Anti-HCV was among the prognostic factors, probably because anti-HCV-positive patients developed distant recurrence more frequently.

HCC frequently recurred after RFA; most recurrences were, however, not local tumor progression but distant recurrence. Frequent recurrence is not specific to RFA. After hepatic resection, the

Table 4. **Complications in 2,982 treatments of radiofrequency ablation for hepatocellular carcinoma**

Complication	No. of complications
Neoplastic seeding	24
Liver abscess	6
Hemoperitoneum	12
Hemothorax	5
Symptomatic pleural effusion	1
Massive hepatic infarction	6
Gastrointestinal perforation or penetration	5
Hemobilia	2
Skin burn	1
Pneumothorax	3
Gallbladder injury	1
Cerebral infarction	1

tumor recurrence rate exceeds 70% at 5 years (30,31). In this study, periodic follow-up detected most recurrences at limited stage. RFA was performed again for first recurrence in almost 90% of cases, although multimodal treatments were used in a long-term follow-up. On the other hand, repeat resection rate for first recurrence has been reported to range from 10.4 to 30.6% (31,32). Because RFA is less invasive than hepatic resection, iterative RFA can be performed for recurrence more easily.

Local tumor progression was found less frequently in this study than in other studies, having been reported to be around 10% at 3 years following RFA (13,14). Furthermore, different from the findings in previous reports (33,34), tumor size was not related to local tumor progression in this study. These differences are probably because we repeated RFA until we considered we had ablated not only the tumor itself but also some of the liver tissue surrounding it. Furthermore, to avoid local tumor progression, we were more cautious in the treatment of larger tumors when deciding whether sufficient ablation had been performed. Only serum DCP level was significantly related to local tumor progression in this study. Elevated serum DCP level may be related to the malignant potential of HCC such as the development of portal venous invasion (35).

The frequency of distant recurrence in this study was similar to that reported in other studies (13). Among the variables significantly related to distant recurrence, tumor size, tumor number, serum AFP level, and serum DCP level were probably related to micrometastasis, which had not been detected by imaging modalities before the treatment, while anti-HCV, Child-Pugh class, and platelet count were related to metachronous multicentric carcinogenesis, which developed based on underlying chronic liver disease.

From the viewpoint of survival and distant recurrence, patients with 2.1–5.0 cm tumors had significantly worse outcomes than those with ≤2.0 cm tumors while those with tumors >5.0 cm did not have worse rates than those with tumors ≤2 cm. This is probably because the number of patients with tumors >5.0 cm ($n=35$) were not large enough for the difference to be statistically significant. Another possibility is selection bias. It is possible that patient with tumors >5.0 cm who underwent RFA had more favorable conditions for survival and distant recurrence except tumor size than those with 2.1–5.0 cm tumors.

In this study, 324 of the 1,170 patients were treated with combination of TACE and RFA at the initial treatment. Thus, we evaluated the combination as a possible variable that influences survival or recurrence. Univariate analysis demonstrated that the combined therapy was significantly correlated to overall survival, whereas multivariate analysis did not show the relationship. TACE was generally combined with RFA in patients with either ≥4 tumors or those with even one tumor >3.0 cm in diameter. This is why the correlation was significant in univariate analysis, while it was not in multivariable model in which the effect of other risk factors, such as tumor number and tumor size were adjusted. The combination of TACE and RFA was not significantly related to either local tumor progression or distant recurrence.

RFA was a safe procedure. Although many patients treated by RFA in this study were at high risk for surgical treatment because of advanced cirrhosis or other comorbidities, complications occurred in only 2.2% of the treatments. Other investigators have also reported low complication rates of 0–6.1% (11,13–16). For hepatic resection, morbidity rates of 38–47% have been reported even in recent studies (36–38).

To date, percutaneous ethanol injection has been considered the standard in ablation (5). However, randomized controlled trials have demonstrated the superiority of RFA (6–9), with RFA now largely replacing ethanol injection. We have also shifted from ethanol injection to RFA (10). At our department, RFA is currently the first option and ethanol injection is performed only on patients on whom RFA cannot be performed safely because of either enterobiliary reflux, adhesion between the tumor and the gastrointestinal tract, or other reasons.

Surgical resection has been considered the treatment of choice for HCC. Our first option for resectable HCC was also surgery. However, most patients who came to our department visited us because they did not want surgical resection. Thus, many patients in this study underwent RFA not because of unresectable tumor but because of refusal of surgery. Those who preferred surgery would have directly gone to the surgical department that has extensive experience in hepatic resection (38).

It is not easy to compare outcomes between RFA and surgical resection; the indications are different between the two treatments. Furthermore, indications for each treatment are different from institution to institution. Thus, a case adjudged to be treatable by RFA or surgical resection at an institution may not be given the same treatment at another. The best known indication criteria for surgical resection may be those proposed in the Barcelona Clinic Liver Cancer (BCLC) protocol (5), which states that surgical resection should be restricted to patients with performance status 0, Child-Pugh class A, single HCC, normal portal pressure, and normal serum bilirubin level. In patients satisfying those criteria, the 5-year survival rate is expected to be >70% (30). In this study, 237

(20.3%) of 1,170 patients satisfied those criteria and were thus considered good candidates for surgical resection; their 5-year survival rate was 75.9%, which appears satisfactory when compared with outcomes following surgical resection. Furthermore, in all 1,170 primary HCC patients treated by RFA, 5- and 10-year survival rates were 60.2% and 27.3%, respectively. In patients treated by surgical resection, 5- and 10-year survival rates were 34.4–70.0% and 10.5–52.0%, respectively (32,39–45). Although this is an observational study with no control, survivals following RFA appear comparable to those reported following surgical resection.

Two recent randomized controlled trials showed no significant difference in survival between RFA and surgical resection (46,47). Several nonrandomized controlled trials reported that RFA had similar overall survival rates to resection (48–50), while others found resection to be associated with higher survival rates (51–53). Further studies are necessary to resolve comparison of RFA with resection.

We have made strenuous efforts to standardize the RFA procedure. Although many physicians have participated in RFA at our institution, the procedure was invariably performed according to the institutional protocol and in the presence of experienced physicians. Video recording was also used to monitor the procedure. Additionally, preoperative planning and postoperative evaluation of technique effectiveness were also carried out by at least three physicians. We also believe that not only proficient practice of RFA but also detailed preoperative planning, cautious postoperative evaluation of therapeutic effect, and careful follow-up are vital to achieve satisfactory outcomes.

Source population in this study may represent selection bias, as we performed RFA on most patients who were hospitalized at our department; however, many patients with unfavorable tumor conditions for RFA might not have been referred to us. Therefore, caution is required when extrapolating our findings to the general population of HCC patients.

A second limitation is that study population cannot be clearly defined. This study was based on daily clinical practice over a 10-year period. Indication criteria of RFA have changed over time, mainly because another percutaneous ablation, that is, ethanol injection has also been performed. Furthermore, various treatments besides percutaneous ablation were available for HCC, such as surgical resection and transarterial chemoembolization, with frequently overlapping indications.

One further limitation is the fact that this was a single-center study; these results might not be reproducible consistently in other settings. To extrapolate the findings in this study to patients at other institutions, careful consideration should be given to differences in the indications, methods, expertise, performance of available ultrasound and CT equipment, and others. Treatment outcome may be influenced by the physicians' expertise and the institution's volume of care. We started ethanol injection in 1985 and microwave ablation in 1995, that is, before the introduction of RFA. Recently, we have performed over 900 RFA treatments per year, which may represent a far greater number of treatments than those in most other institutions. We would not recommend any change in daily clinical practice solely on the strength of our study findings.

In conclusion, our 10-year clinical experience shows that RFA could be locally curative, resulting in survival for as long as 10 years, and was a safe procedure. RFA might be a first-line treatment for selected patients with early-stage HCC.

CONFLICT OF INTEREST

Guarantor of the article: Shuichiro Shiina, MD, PhD.
Specific author contributions: Study concept and design, analysis and interpretation of data, and drafting of the manuscript: Shuichiro Shiina; analysis and interpretation of data and statistical analysis: Ryosuke Tateishi; study execution and data acquisition: Toru Arano, Koji Uchino, Kenichiro Enooku, Hayato Nakagawa, Yoshinari Asaoka, Takahisa Sato, Ryota Masuzaki, Yuji Kondo, and Tadashi Goto; revised the article critically for important intellectual content: Haruhiko Yoshida; Masao Omata, and Kazuhiko Koike. All authors have read and approved the submitted manuscript.
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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Radiofrequency ablation (RFA) has been widely performed for hepatocellular carcinoma (HCC).
- ✓ RFA has a more reliable local antitumor effect and higher survival than ethanol injection.
- ✓ There has been no report on 10-year outcome of RFA.

WHAT IS NEW HERE

- ✓ Five- and 10-year survival rates in 1,170 patients with primary hepatocellular carcinoma (HCC) were 60.2 and 27.3%, respectively.
- ✓ Age, antibody to hepatitis C virus, Child-Pugh class, tumor size, tumor number, serum des-γ-carboxy-prothrombin level, and serum lectin-reactive α-fetoprotein level were significantly related to survival.
- ✓ Five- and 10-year local tumor progression rates were both 3.2%. Five- and 10-year distant recurrence rates were 74.8 and 80.8%, respectively.

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ORIGINAL ARTICLE

Chronic hepatitis B in patients coinfected with human immunodeficiency virus in Japan: a retrospective multicenter analysis

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Abstract A nationwide survey in Japan revealed that about 6 % of human immunodeficiency virus (HIV)-positive patients are coinfected with hepatitis B virus (HBV). To further analyze the features of liver disease in HIV/HBV-coinfected patients, we analyzed 252 patients from six hospitals in the HIV/AIDS (acquired immunodeficiency syndrome) Network of Japan. The mean age was 39.5 years, and the proportion of male patients was very high (243 of 252; 96 %). The main transmission route was male homosexual contact (186 of 252; 74 %), followed by heterosexual contact. The HBV genotype was determined in 77 patients. Among them, genotype A HBV was the most frequent (58 of 77; 75 %) and was detected almost exclusively in homosexual patients. Acute hepatitis B was documented in 21 patients (8 %). Three of the 252 HIV/HBV-coinfected patients developed advanced liver disease with the complication of ascites, hepatic encephalopathy, or hepatocellular carcinoma. A comparison between patients not treated and those treated with antiretroviral drugs including anti-HBV drugs revealed that the baseline liver function was worse in treated patients. However, the serum albumin levels and platelet counts in both groups increased after treatment and were similar. Liver disease-associated death was not observed. Here, we characterize the clinical features of liver disease in HIV/HBV-coinfected patients in Japan for the first time. The findings suggest that antiretroviral therapy with anti-HBV drugs may retard the progression of a liver disease and prevent liver disease-associated death in such patients.

Keywords Acquired immunodeficiency syndrome · Chronic liver disease · HBV DNA · Genotype

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Introduction

The number of human immunodeficiency virus (HIV)-positive patients is growing in Japan [1]. Although combination therapy with antiretroviral agents has made HIV infection itself somewhat controllable in many cases since its introduction in 1996, and mortality from opportunistic infection has decreased, existing comorbidities are the focus of current patient care. In fact, more than 50 % of deaths in HIV-1-infected patients are not related to acquired immunodeficiency syndrome (AIDS); the mortality from liver disease is second only to AIDS-related mortality [2]. Risk factors related to significant liver

diseases among HIV-positive patients include a diagnosis of viral hepatitis [3], nonalcoholic fatty liver disease [4], and excessive alcohol consumption [5]. Among these factors, hepatitis B and hepatitis C are of particular importance because they can often lead to life-threatening diseases such as cirrhosis and hepatocellular carcinoma by themselves.

The estimated prevalence of chronic hepatitis B virus (HBV) infection in Japan is less than 1 %, or 0.9 million carriers [6]. However, about 6 % of HIV-positive patients are coinfected with HBV [7]; this coinfection rate is more than six times higher than that in the non-HIV population. In the United States, the HIV/HBV coinfection rate is reported to be in the range of 6–14 % [8–10].

Several issues make the management of HIV/HBV coinfection complicated. HBV infection tends to be persistent in HIV-positive patients [9, 11, 12]. Chronic HBV infection may lead to hepatitis, cirrhosis, or hepatocellular carcinoma. The progression of a liver disease associated with chronic HBV infection is more rapid in HIV/HBV-coinfected patients than in HBV-monoinfected patients [13].

Combination regimens of antiretroviral therapy (ART) for coinfected patients should be carefully determined. Initial combination regimens of ART for HIV/hepatitis C virus (HCV)-coinfected patients are basically the same as those for HIV patients without HCV infection. However, because some nucleoside reverse transcriptase inhibitors (NRTIs) used in HIV treatment have activity against HBV, and some NRTIs mainly used in HBV treatment have partial activity against HIV [14], careful choice of treatment agents is necessary in HIV/HBV coinfection. Abrupt discontinuation of NRTIs that are active against HBV may aggravate viral hepatitis. Administration of entecavir, which has a weak activity against HIV, to HIV/HBV-coinfected patients without simultaneous effective HIV treatment may cause the accumulation of drug-resistant HIV strains [15–17]. In such cases, drug resistance of HBV may occur as well [18].

Drug-induced liver injury following ART is another concern. HIV/HBV-coinfected patients show an increase in transaminase level at a higher rate [19, 20]. However, it is often unclear whether this increase is caused by drug hepatotoxicity because the treatment of HIV infection causes immune reconstruction in patients, which alone could contribute to the transaminase level increase in viral hepatitis.

The objective of this study is to clarify the clinical features of HIV/HBV coinfection in Japan and to clarify the impact of ART on liver function among HIV/HBV-coinfected patients. The estimated prevalence of chronic HBV infection among the general population in Japan is decreasing yearly, but it remains much higher than that in the United States [21], where universal hepatitis B vaccination is introduced. Thus, the detailed analysis of HIV/HBV coinfection in Japan is of particular importance.

Patients and methods

We have conducted a multicenter retrospective study based on the data from a nationwide survey in 2006 conducted by sending questionnaires to 372 member hospitals of the HIV/AIDS network of Japan as of January 2006, and part of the results was reported earlier [7]. Following the survey, 6 of the 207 hospitals that responded to the survey—Hokkaido University Hospital (Hokkaido, Japan), University of Tokyo Hospital (Tokyo, Japan), Nagoya University Hospital (Aichi, Japan), International Medical Center of Japan (currently, National Center for Global Health and Medicine, Tokyo, Japan), Osaka National Hospital (Osaka, Japan), and Hiroshima University Hospital (Hiroshima, Japan)—were chosen for further studies because more than two-thirds of the HIV/HBV-coinfected patients identified in the survey went to these hospitals, and because both HIV experts and hepatologists were following up those patients there.

The questionnaire sent to the hospitals included items regarding the number of patients who visited the hospitals at least once between January and December in 2006 as follows: (1) the number of HIV-positive patients; (2) the number of hepatitis B surface antigen (HBsAg)-positive patients among (1); (3) the number of patients among (2) who were determined at least once to have a serum alanine aminotransferase (ALT) level higher than 100 IU/l; (4) the number of HIV-positive patients who contracted HIV from blood products; (5) the number of HBsAg-positive patients among (4); (6) the number of patients among (5) who were determined at least once to have a serum ALT level higher than 100 IU/l; (7) the number of HIV-positive patients whose presumed transmission route is through homosexual contact; (8) the number of HBsAg-positive patients among (7); (9) the number of patients among (8) who were determined at least once to have a serum ALT level higher than 100 IU/l; (10) the number of HIV-positive patients who presumably contracted HIV through injection drug use; (11) the number of HBsAg-positive patients among (10); (12) the number of patients among (11) who were determined at least once to have a serum ALT level higher than 100 IU/l; (13) the number of HIV-positive patients whose transmission routes were classified as "others"; (14) the number of HBsAg-positive patients among (13); and (15) the number of patients among (15) who were determined at least once to have a serum ALT level higher than 100 IU/l.

We defined confirmed HIV infection with positivity for serum HBsAg as the criterion for HIV/HBV coinfection.

After identifying HIV/HBV-coinfected patients, medical records including laboratory data of these patients were reviewed between the date of the oldest available record for these patients and the final date of the record acquired by the end of the study. The laboratory data at the diagnosis or first recognition of HBV infection and the latest data in the study period were compared for analysis unless otherwise noted. HBV genotypes (A through D) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan) on the basis of the pattern of detection using monoclonal antibodies of a combination of epitopes on preS2-region products, each of which was specific for each genotype [22, 23].

Ethical issues

The respective ethics committees of the six hospitals approved the study. Informed consent was obtained from each study participant.

Statistical analyses

For the comparison of means of collected data, Student's *t* test (paired *t* test) was performed unless otherwise specified. The chi-square test was performed to determine the independence of clinical parameters.

Results

Two hundred and fifty-two patients were identified to have HIV/HBV coinfection. The mean age was 39.5 years, and the proportion of male patients was very high (243 of 252; 96.4 %). The main presumed transmission route of HIV was male homosexual contact (186 of 252; 73.8 %), followed by heterosexual contact. Among those HIV/HBV-coinfected patients, 21 of the 252 (8.3 %) acquired acute hepatitis during the study period (Table 1).

Table 1 Clinical background of HIV/HBV-coinfected patients

Number (male:female)	243:9
Age (year)	39.5 ± 9.6[a]
Presumed Transmission Route	
Transfusion	14
Homosexual contact	186
Heterosexual contact	24
Injection drug use	2
Others	4
Onset as acute hepatitis	21

[a] Mean ± standard deviation

The HBV genotype was determined in 77 patients. Among them, genotype A HBV was the most frequent (58 of 77; 75.3 %), followed far behind by genotype C (7 of 77; 9.1 %), which is the predominant genotype in the entire chronic hepatitis B population in Japan. Genotype B, which is also common in Japan, was found only in three patients (3.9 %). Genotype A was detected almost exclusively in homosexual patients (57 of 58; 98.3 %) (Fig. 1).

At the end of the study period, 113 patients (44.8 %) received some type of anti-HBV drug such as interferon, lamivudine, adefovir, or entecavir, not as part of anti-HIV treatment. Ninety-seven (38.5 %) patients were still taking anti-HBV drugs by the end of the study period. The median ALT level was 30.0 IU/l (5th percentile, 11.1; 95th percentile, 128.9), suggesting the existence of some liver injury. Liver function was normal in most HIV/HBV-coinfected patients. The mean serum albumin level was 4.1 ± 0.6 g/dl, and the median serum total bilirubin level was 0.8 mg/dl (5th percentile, 0.3; 95th percentile, 3.8). The mean platelet count was 21.0 ± 6.1 × 10^4/ml. The hepatitis B e antigen (HBeAg) was detected in 84 patients, and the HBV DNA level was high (higher than 100,000 IU/l) in 55 patients (Table 2). Three of the 252 (1.1 %) HIV/HBV-coinfected patients developed advanced chronic liver diseases, such as cirrhosis with the complication of ascites and/or hepatic encephalopathy, or hepatocellular carcinoma. Although we tried to retrieve information on alcohol consumption of the patients, it was available for only a limited number of patients (26 of 252); among the 26, only 2 patients had a habit of taking more than 60 g alcohol per day. The remaining 24 patients took alcohol only on social occasions. The antiretroviral agents used for these study patients are listed in detail in Table 3. Among those who had a known history of ART, 158 of 252 (62.7 %) received regimens that include anti-HBV drugs at least once previously, whereas 42 (16.7 %) did not, and no information is available for the remaining 52. The most common drug combination for HIV/HBV-coinfected patients was ATV/r + FTC/TDF (22 of 172; 12.8 %) (Table 4). FTC/TDF, composed of two drugs active against HBV, is recommended for HIV/HBV-coinfected patients

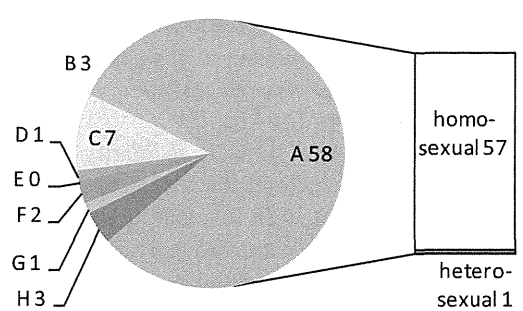

Fig. 1 Hepatitis B virus (HBV) genotype

Table 2 Liver function and related parameters of HIV/HBV-coinfected patients

Albumin (g/dl)	4.1 ± 0.6
Bilirubin[a] (mg/dl)	0.8 (5th percentile, 0.3; 95th percentile, 3.8)
ALT[a] (IU/l)	30.0 (5th percentile, 11.1; 95th percentile, 128.9)
WBC ($\times 10^3/\mu l$)	5.2 ± 1.6
Platelet ($\times 10^4/\mu l$)	21.0 ± 6.1
HBeAg (positive:negative)	84:68
HBV DNA (high:low)[b]	55:127

[a] Median and percentiles are provided instead of mean and standard deviation because of the nonnormality of the distribution

[b] HBV DNA level of 100,000 IU/l or higher is categorized as "high"

as one of the preferred NRTI backbones of the ART regimen [24].

We compared the clinical characteristics between patients who received the full ART and those who did not. Regarding the baseline statistical data, the observation period was longer for patients on ART, and there were more patients with AIDS in the ART group (10 of 64 vs. 52 of 162) (Table 5a). No significant difference was observed between the non-ART and ART groups in male/female ratio, age, transmission route, HBV markers, or advanced liver disease. Liver-related death was not observed, but hepatic failure with ascites and/or hepatic encephalopathy developed in 2 patients on ART and hepatocellular carcinoma developed in another patient.

Comparison between the ART group and the non-ART group revealed that the baseline liver function was worse in the ART group. At the beginning of the study period, the ART group showed a significantly lower CD4+ T-cell count than the non-ART group. The total white blood cell count and platelet count were also lower in the ART group. Although it is not statistically significant, the serum albumin level and prothrombin time (PT) index were lower in the ART group. However, at the end of the observation period, these parameters improved significantly in the ART group. The difference in CD4+ T-cell count between the ART and non-ART groups became marginal and became statistically insignificant (Table 5b).

Changes in the liver function of HIV/HBV-coinfected patients may not be fully explained by the changes in HBV activity because some parameters relevant to the estimation of liver function showed paradoxical changes. To clarify this observation, we compared the changes in liver function among HIV/HBV-coinfected patients on ART with respect to protease inhibitor (PI) use.

The mean serum total bilirubin level in patients on ART with PI use (PI group) at the beginning of the observation period was 1.1 mg/dl, whereas that in patients without PI use (non-PI group) was 0.8 mg/dl. The means at the end of the study period were 1.6 mg/dl in the PI group and 0.7 mg/dl in the non-PI group. Because the sample distribution of serum total bilirubin level did not follow the normal distribution by logarithmic transformation, we compared the means statistically. At the beginning, the difference in the mean between the PI group and the non-PI group was not significant ($p = 0.257$). At the end of the observation period, a statistically significant difference ($p = 0.001$) was observed. We then calculated the

Table 3 Antiretroviral treatment of HIV/HBV-coinfected patients

Antiretroviral drugs	Number of patients
NRTIs	
Zidovudine (AZT)	34
Didanosine (ddI)	9
Ddl / enteric coated	7
Zalcitabine (ddC)	1
Stavudine (d4T)	4
Lamivudine[a] (3TC)	84
Abacavir[3] (ABC)	38
Tenofovir[3] (TDF)	27
Emtricitabine (FTC) / TDF[a]	57
NNRTIs	
Nevirapine (NVP)	10
Efavirenz (EFV)	34
Delavirdine (DLV)	1
PIs	
Indinavir (IDV)	4
Ritonavir (RTV)	50
Nelfinavir (NFV)	8
Lopinavir (LPV)	3
Ritonavir-boosted LPV (LPV/r)	40
Atazanavir (ATV)	39
ATV/r	6
Fosamprenavir (FPV)	13

NRTI nucleoside reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor

[a] Agents with anti-HBV activity

Table 4 Antiretroviral regimens used for HIV/HBV-coinfected patients

Antiretroviral regimen	Number of patients
ATV/r + FTC/TDF	22
LPV/r + 3TC + TDF	8
LPV/r + FTC/TDF	7
EFV + FTC/TDF	6
ATV/r + 3TC + TDF	5

Table 5 Comparison of changes in clinical parameters of HIV/HBV-coinfected patients with or without antiretroviral therapy (ART)

a. Baseline statistical data	Natural course[a] (without ART)	With ART	*p* value (with vs. without ART)
Number (male:female)	84:6	159:3	0.105†
Age (year)	37.0 ± 10.3	39.0 ± 9.1	0.362
Observation period (month)	34.5 ± 55.5	50.9 ± 43.9	0.022*
Presumed transmission route	Blood products:homosexual contact:heterosexual contact:injection drug use:other		
	5:60:12:2:3	9:126:12:0:1	0.052†
Recognized acute hepatitis	10	11	0.243†
HBeAg (positive:negative)	42:18	100:40	0.394†
HBV DNA (high:low)	29:18	83:37	0.356†
HBV genotype	A:B:C:D:F:G:H		
	17:0:1:1:1:0:1	31:3:6:0:1:1:2	0.372†
Ascites	1/56	2/144	1.000†
Hepatocellular carcinoma	0/62	1/159	1.000†
Acquired immunodeficiency syndrome (AIDS)	10/64	52/162	0.012*,†

b. Comparison of clinical parameters between pre- and post-ART among patients with and without ART	Natural course (without ART)	With ART	*p* value (with vs. without ART)
CD4 count (per μl)			
Start[b]	402.9 ± 180.1	242.5 ± 187.6	0.000*
End[c]	406.4 ± 212.4	398.1 ± 195.9	0.883
p value (start vs. end)	0.893	0.000*	
Albumin (g/dl)			
Start	4.1 ± 0.4	3.8 ± 0.8	0.292
End	3.9 ± 0.8	4.2 ± 0.4	0.025*
p value	0.473	0.001*	
Bilirubin[d] (mg/dl)			
Start	0.7 (0.30, 4.26)	0.5 (0.30, 2.62)	0.138
End	0.5 (0.25, 1.30)	0.9 (0.36, 4.32)	0.000*
p value	0.046*	0.000*	
ALT[d] (IU/l)			
Start	46.0 (15.0, 1418.2)	34.0 (12.8, 1,068.8)	0.120
End	27.0 (9.9, 229.9)	31.5 (12.73, 89.3)	0.713
p value	0.003*	0.000*	
Prothrombin time index (%)			
Start	89.4 ± 13.1	78.8 ± 23.0	0.650
End	78.8 ± 27.3	84.2 ± 16.3	0.531
p value	0.377	0.218	
WBC ($\times 10^3/\mu l$)			
Start	6.1 ± 2.4	4.8 ± 2.1	0.000*
End	5.4 ± 1.4	5.1 ± 1.6	0.404
p value	0.044*	0.247	
Platelet ($\times 10^4/\mu l$)			
Start	22.2 ± 6.5	19.3 ± 6.3	0.010*
End	21.2 ± 6.5	20.8 ± 6.1	0.649
p value	0.204	0.001*	

* $p < 0.05$

† Chi-square test was performed

[a] Two patients with habitual alcohol intake were included in this group

[b] Start of observation period

[c] End of observation period

[d] Means were compared by log transformation because of the nonnormality of the distribution; median and percentiles (5th percentile, 95th percentile) are provided

difference in serum total bilirubin level between the beginning and the end of the observation period [Dbilirubin level = (bilirubin level at the end) − (bilirubin level at the beginning)] in individual patients and compared it between the PI group and the non-PI group. The mean Dbilirubin level in the PI group was 0.5 ± 3.4 mg/dl and that in the non-PI group was −0.2 ± 1.6 mg/dl ($p = 0.250$). The Dbilirubin level in a patient in the PI group who was coinfected with HCV besides HIV/HBV as well was −27.4 mg/dl. Excluding this single outlier, the mean Dbilirubin level was significantly different between the PI and non-PI groups (mean Dbilirubin level 0.8 vs. −0.2; $p = 0.01$).

Discussion

We have summarized here the data from our comprehensive survey of HIV/HBV coinfection in Japan, focusing particularly on the clinical features of the patients and the effect of ART on liver function. As we reported earlier, HIV/HBV coinfection was observed in 6.3 % of Japanese HIV-positive patients [7]. Certain considerations for HBV coinfection are important in HIV patient care.

The major transmission route of HIV was male homosexual contact, which accounted for the infection in about 80 % of the patients; thus, male patients were the majority in the present cohort. The most frequently found genotype of HBV was genotype A, which is infrequent in HIV-negative patients in Japan. Genotype A is often found in the United States, Europe, India, and the west coast of Sub-Saharan Africa [25]. Although the data on HBV subgenotypes were not available in our study, some reports showed that most genotype A strains detected in HIV/HBV-coinfected individuals are of genotype Ae [26]. These findings suggest that HBV infection among Japanese HIV carriers is not caused by the spread of indigenous HBV, such as transmission in the perinatal period, but rather specific strains are circulating among the homosexual population in Japan. Genotypes B and C accounted for more than 96 % of the entire Japanese chronic HBV infection [27, 28]. These findings are compatible with the report that the presumed transmission route of HBV in HIV/HBV-coinfected patients is not from Japanese female partners but from male partners, as shown by Koibuchi et al. [29].

Seventy-five percent of HIV/HBV-coinfected patients received ART with two agents against HBV, and its efficacy against HBV as well as HIV is considered to be high. As recommended by the United States Department of Health and Human Services (DHHS) and the Japanese guidelines on HIV treatment, the initiation of ART with NRTIs with anti-HBV activity as the backbone is indicated for HIV/HBV-coinfected patients regardless of HIV viral load or CD4+ T lymphocyte count [30]. Nucleoside analogues can improve liver function in HBV-monoinfected patients [31]. Our study shows that ART decreased the levels of ALT and albumin in HIV/HBV-coinfected patients. It is noteworthy that the regimen used in ART includes multiple drugs with anti-HBV activity such as lamivudine plus abacavir, which is unusual for HBV-monoinfected patients.

When we compared the characteristics of patients on ART with those not on ART, there were some notable differences in their immune status and liver function. At the beginning of the observation period, patients on ART showed a lower CD4+ T-cell count and poorer liver function. Our study is a retrospective observation, and patients were not grouped randomly. These observations are rather understandable because those who had a low CD4+ T cell count were more likely candidates for ART. Additionally, patients on ART had a longer observation period and were more likely to develop AIDS. These findings are also understandable because the longer the duration of HIV infection, the more likely is the immune system of the patient to deteriorate. Moreover, once ART is started, patients need to visit clinics or hospitals regularly for a long period; in reality, for the rest of their life. Following current recommendations for the initiation of ART for HIV infection, patients with worse immune status are more likely to receive the treatment. These findings can explain our observation.

Our data show that the serum albumin level and platelet count improved in the patients who were on ART. As the regimen of ART usually contains two drugs against HBV, ART suppresses HBV replication, which may lead to an improved liver function, as observed in HBV-monoinfected patients treated with nucleoside analogues [31]. Long-term treatment with lamivudine was shown to regress the fibrosis of the liver [32, 33] and decrease the proportion of patients with hepatocellular carcinoma complication [34]. In view of these findings, ART for HIV/HBV-coinfected patients may markedly improve the prognosis of patients. In our study, only a small number of patients with advanced liver diseases associated with HBV infection such as cirrhosis or hepatocellular carcinoma were observed, which could be attributable in part to the short observation period and the short duration of HBV infection. If we had a longer observational period, we would be able to clarify the difference in clinical course between the ART and non-ART groups, and the actual significance of ART for HIV/HBV-coinfected patients should become clearer.

We found that some parameters related to liver function changed paradoxically, particularly in the ART group. Although the mean serum albumin level, ALT level, and platelet count improved, the mean serum bilirubin level worsened, from 0.5 to 0.9 mg/dl. On the other hand, the serum bilirubin level in the non-ART group decreased. Both changes are statistically significant, which suggests

that the observed hyperbilirubinemia was not associated with HBV activity. The increase in serum bilirubin level is presumably caused by PIs. Hyperbilirubinemia following PI administration was previously reported [35]. Although it is unclear whether hyperbilirubinemia itself may lead to liver injury, PIs should be used carefully particularly for patients with advanced liver diseases.

Our present study has one major limitation; that is, the effect of alcohol on liver function was not analyzed because the history of alcohol consumption could not be obtained in the majority of the studied patients. Excessive alcohol consumption has been found to be an important risk factor for the development of severe hepatic injury in HIV-infected patients with [3] or without HCV coinfection [5]. Our present study showed that among the 26 patients whose history of alcohol consumption was available, only 2 patients were habitual drinkers. The results suggested that the effect of alcohol on liver function is small in HIV/HBV-coinfected patients in Japan.

In conclusion, ART with anti-HBV drugs may retard the progression of liver diseases and prevent liver-related death in HIV/HBV-coinfected patients. Multiple agents with anti-HBV activity seem essential for the efficacy. PIs should be carefully used particularly for patients with advanced liver diseases.

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