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IGF-II Producing Hepatocellular Carcinoma Treated with Sorafenib: Metabolic Complications and a Foresight to Molecular Targeting Therapy to the IGF Signal

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Key Words
IGF-II · Hepatocellular carcinoma · Sorafenib · Metabolic complications · Molecular targeting therapy · IGF signal · Hypoglycemia

Abstract
Hypoglycemia is a rare paraneoplastic manifestation of patients with neoplasms. Hypoglycemia can be induced by several causes, including an aberrant increase of hypoglycemic agents and adrenal insufficiency. Sorafenib is the first agent to demonstrate a survival benefit in the treatment of advanced hepatocellular carcinoma (HCC). This small molecule inhibits serine/threonine kinase RAF in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature and decreases tumor growth and angiogenesis. In this paper, we report a case of HCC who was treated with sorafenib and showed severe hypoglycemia. This hypoglycemia might be induced by two causes, both adrenal insufficiency as an adverse effect of sorafenib and activation of the insulin-like growth factor (IGF) signal by excessive secretion of incompletely processed precursors of IGF-II. Although the IGF signal is suggested to be involved in aberrant growth of HCC in some cases, there is no other report showing the influence of sorafenib on HCC with active IGF signal. Unfortunately, the effect of sorafenib was limited in the present case. However, emerging drugs that directly inhibit the IGF signal can be expected to be highly effective in the treatment of HCC with hypoglycemia.

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Introduction

Hypoglycemia is a rare but well-known paraneoplastic manifestation of patients with neoplasms, including hepatocellular carcinoma (HCC), which is referred to as non-islet cell tumor-induced hypoglycemia (NICTH) [1]. Excessive secretion of incompletely processed precursors of insulin-like growth factor-II (termed the 'big' IGF-II) has been suggested to cause NICTH. The IGF signal is involved in both glucose metabolism and cellular proliferation [2]. The 'big' IGF-II excessively stimulates both IGF-I and the insulin receptor, inducing hypoglycemia and tumor growth. In the era of molecular-targeted therapy, agents targeting the IGF signal are being developed to treat lung and pancreatic cancers [3]. Although this signal is suggested to be involved in aberrant growth of HCC [4], clinical trials using these agents against HCC have been initiated only recently.

Sorafenib is the first agent to demonstrate a survival benefit in the treatment of advanced HCC [5]. This small molecule inhibits serine/threonine kinase RAF in tumor cells and tyrosine kinases vascular endothelial growth factor receptor (VEGFR)/platelet-derived growth factor receptor (PDGFR) in the tumor vasculature, decreasing tumor growth and angiogenesis.

In this paper, we report a case of HCC that showed severe hypoglycemia and was treated with sorafenib. Since RAF is one of the downstream components of the IGF signal, sorafenib may be effective against tumors with an activated IGF signal. Although the effect was limited in the present case, emerging drugs that directly inhibit the IGF signal can be expected to be highly effective in the treatment of HCC with NICTH.

Case Report

A 77-year-old male patient with HCC was referred to the authors' hospital. As he had no previous episodes of liver disorders, no imaging procedures had been performed. In February 2010, he was first admitted to another hospital due to bleeding gastric ulcers induced by non-steroidal anti-inflammatory drugs. During hospitalization, an abdominal CT scan showed multiple liver tumors and multiple lung nodules (fig. 1a, b). Based on elevated serum AFP (897 ng/ml) and typical CT scan images as HCC, he was diagnosed as advanced HCC and referred to our hospital in March 2010.

Administration of sorafenib was initiated at a dosage of 800 mg b.i.d. On day 7, pleural effusion was detected and his serum potassium concentration was elevated to 5.5 mEq/l. His general condition declined and he was unable to stand by day 11. On day 14, he was hospitalized because of hyperpotassemia (6.7 mEq/l) and hypoglycemia (27 mg/dl). Hyperpotassemia improved by the administration of an intravenous drip infusion of glucose and furosemide, but hypoglycemia continued at a level of 40 mg/dl. Although the basal levels of adrenal hormones were normal, ACTH and cortisol did not increase at the time of hypoglycemia. This suggested that the relative adrenal insufficiency exerted some influence on the hypoglycemia. We started to administer a short-acting corticosteroid (hydrocortone), and the blood glucose level increased rapidly to around 150 mg/dl.

However, several days later, the patient's morning fasting blood glucose level decreased to around 20 mg/dl. We administrated a longer-acting corticosteroid and the patient also began to have late evening snacks. Although a sufficient amount of cortisol (prednisolone 10 mg/day) was administered, his hypoglycemia continued. We suspected that other factors were involved in the hypoglycemia, but the serum levels of insulin and IGF-I were lower than the normal limits. We assayed the patient's serum using immunoblotting with an anti-IGF-II antibody. The 'big' IGF-II was detected in the serum (fig. 2, lane 2) similarly to the serum of a patient with NICTH (lane 4). Only mature IGF-II was detected in the serum of the normal control (lane 3). Lane 1 was recombinant IGF-II.

By day 14 of sorafenib administration, though the number of lung metastases had increased (fig. 1d), the size of the liver tumors had not changed (fig. 1c) and the tumor marker levels had decreased (AFP from 4,112 to 2,381 ng/ml and PIVKA-II from 4,645 to 952 mAU/ml) (fig. 3). We concluded that sorafenib was effective. The dose of sorafenib was decreased to half (400 mg b.i.d.), and the patient was discharged. Ten days later, he was hospitalized because of unconsciousness caused by hypoglycemia. Though the hypoglycemia improved with treatments, sadly the patient died 6 days later of respiratory failure due to advanced lung metastases.

Discussion

We treated a case of IGF-II producing HCC with sorafenib. Several previous reports have shown NICTH as a rare paraneoplastic manifestation of advanced HCC with a poor prognosis [6]. As far as we are aware, there are no reports describing HCC with NICTH treated with this novel molecular targeted agent, sorafenib. The present case showed interesting endocrine abnormalities such as hypoglycemia and hyperpotassemia due to relative adrenal insufficiency. The possibility that sorafenib suppressed adrenal function must be considered, since there were no other factors known to affect adrenal function such as metastasis to the adrenal glands. No reports have been identified that describe adrenal insufficiency due to sorafenib, while the drug is reported to affect thyroid functions. The possibility that sorafenib played a role in adrenal insufficiency is also supported by the fact that there are some reports of adrenal dysfunction caused by a similar molecular agent, sunitinib, targeting VEGFR/PDGFR [7].

The IGF signal is related to cell proliferation and tumor growth of HCC through the IGF-I receptor [4]. Kaseb et al. [8] reported that lower plasma IGF-I levels are correlated with advanced HCC and poor overall survival. Reactivation of IGF-II, including the 'big' IGF-II, is one of the most frequent mechanisms of IGF signal activation in HCC. Expression of IGF-I may be suppressed by a negative feedback of IGF-II overexpression, resulting in lower plasma IGF-I levels.

The 'big' IGF-II is suggested to induce hypoglycemia through IGF-I and the insulin receptor. Usually, hypoglycemia due to the 'big' IGF-II is not controllable with continuous infusion of glucose. Reduction of tumor volume by surgical operation [9], transarterial chemoembolization or systemic chemotherapy [1] is sometimes effective. Palliative treatments, including administration of hyperglycemic hormones such as corticosteroids and growth hormones, are performed, but the effects are transient and limited.

When the IGF-I receptor is stimulated, the downstream signaling pathways, including PI3K-AKT-TOR and RAF-MEK-ERK, are activated [2]. Sorafenib inhibits the activation of RAF. However, the efficacy of sorafenib was limited in the present case (fig. 3). Several drugs that target the IGF signal are under development [3]. Such drugs directly inhibit intracellular kinase activities or block the binding of IGF to the receptors. We suggest that these agents will likely be effective in NICTH cases. In particular, use of antibodies against IGF-II will probably be selective and safe in such cases.

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Disclosure Statement

The authors have no competing interests to disclose.

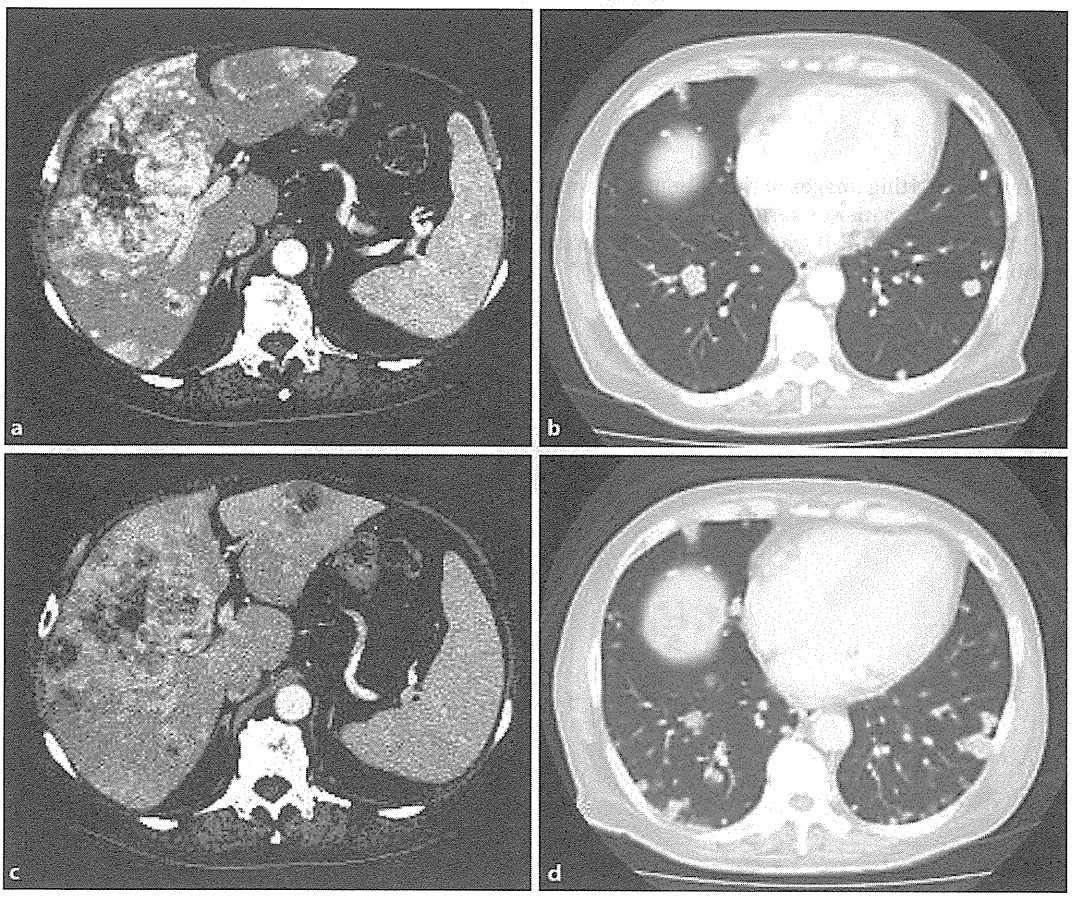

Fig. 1. CT scan images before and after the administration of sorafenib. **a**, **b** Images before therapy. **c**, **d** Images after therapy. By the administration of sorafenib, the size of the liver tumors had not changed (**a** to **c**), but the number of lung metastases had increased (**b** to **d**).

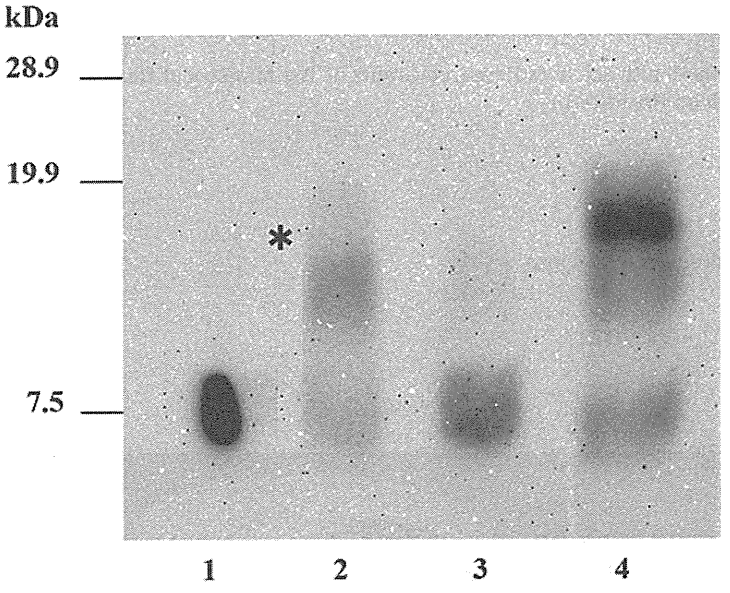

Fig. 2. Immunoblotting images of the patient's serum with an anti-IGF-II antibody. The 'big' IGF-II was detected in the patient's serum (lane 2, asterisk) similarly to the serum of a patient with NICTH (lane 4). Only mature IGF-II was detected in the serum of the normal control (lane 3). Lane 1 is recombinant IGF-II.

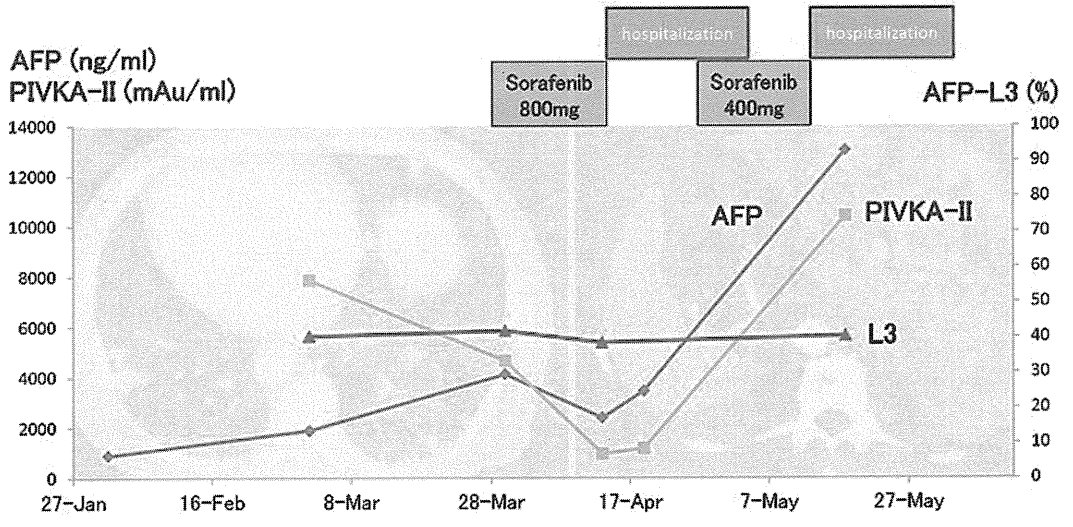

Fig. 3. Changes in the tumor marker levels before and after the administration of sorafenib. By day 14 of sorafenib administration, the tumor marker levels had decreased (AFP from 4,112 to 2,381 ng/ml and PIVKA-II from 4,645 to 952 mAU/ml). However, the effects were transient and the tumor marker levels increased again in spite of the administration of sorafenib.

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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

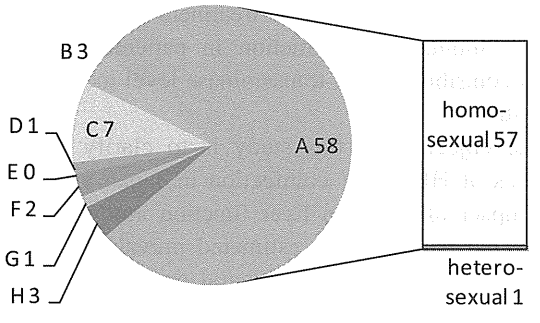

Fig. 1 Hepatitis B virus (HBV) genotype

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 ($\times 10^3$/μl)			
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 ($\times 10^4$ /μl)			
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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Table 1. Characteristics of the Randomized Cohorts and SVR Rates of Heterozygous Genotype rs12979860CT With Additional Genotyping of rs8099917

Random Sample Size	Sample Number	Mean Age ±SD	Male	HCV RNA ≥400.000 IU/mL	Severe Fibrosis	SVR		P-value
						rs12979680CT/ rs8099917TT	rs12979680CT/ rs8099917TG	
10%	96	47±11	58%	69%	55%	48%	36%	0.408
20%	192	48±11	59%	80%	43%	43%	32%	0.379
30%	295	48±11	60%	72%	48%	50%	38%	0.154
40%	396	47±11	63%	66%	55%	57%	39%	**0.012**
50%	474	47±11	60%	68%	53%	56%	37%	**0.003**
60%	588	48±11	58%	71%	52%	57%	35%	**0.0001**
70%	654	47±11	58%	72%	52%	56%	39%	**0.002**
80%	754	48±11	58%	70%	51%	55%	39%	**0.002**
90%	835	48±11	59%	71%	52%	56%	40%	**0.001**
100%	942	48±11	59%	70%	52%	55%	40%	**0.001**

SD, standard deviation; IU, international units; SVR, sustained virological response; $P < 0.05$ considered to be statistically significant.

fibrosis stage on the SVR rates of genotype rs12979860CT/rs8099917TT and rs12979860CT/rs8099917TG (Supporting Table 1). Again, it becomes obvious that the impact of additional genotyping of rs8099917 on the prediction of SVR is improved in patients with heterozygous genotype of rs12979860 who have high baseline HCV RNA levels ($P = 3.7 \times 10^{-5}$), HCV subtype 1a ($P = 3.3 \times 10^{-5}$), or severe fibrosis stages ($P = 0.001$), being female ($P = 0.023$), or of younger age ($P = 0.029$). Thus, the different patient characteristics most likely explain the differences in the SVR rates.

From that, one possibly may conclude that two SNPs are good in large cohorts but not relevant for clinical practice. However, the idea of large studies is to inform individual clinical practice. Our results derived from a large cohort suggest that algorithms and models that include both rs12979860 and rs809917 as well as baseline parameters and viral factors are informative to guide therapeutic decision making.[3]

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Plasma Lysophosphatidic Acid Levels and Hepatocellular Carcinoma

To the Editor:

We read with interest the article by Mazzocca et al.,[1] showing that serum lysophosphatidic acid (LPA) levels are increased in hepatocellular carcinoma (HCC) patients correlated with tumor burden, while not enhanced in cirrhosis patients. However, we think that their LPA values in serum samples need to be carefully evaluated, because of some technical issues in the measurement of LPA levels in blood samples. First, because LPA is released from platelets, LPA has been measured in plasma but not in serum when evaluating its clinical significance.[2,3] Second, as we previously demonstrated,[4] LPA levels in plasma samples are markedly increased after sample preparation unless the temperature is kept under strict control, potentially because the synthetic enzyme autotaxin (ATX) and the substrate lysophosphatidyl choline coexist in plasma samples to abundantly produce LPA. LPA was once reported as a biomarker of ovarian cancer,[2] but contrary data were later demonstrated, in which a distinct sampling of plasma may explain this discrepancy.[3] Indeed, LPA levels in serum reported by Mazzocca et al. were approximately 10 times higher than the previously reported LPA levels in plasma.[2,3] If their LPA values in serum were increased after sampling similarly in each sample, plasma LPA levels might be correlated with HCC burden as reported. To clarify this, we have newly measured plasma LPA levels in HCC patients,

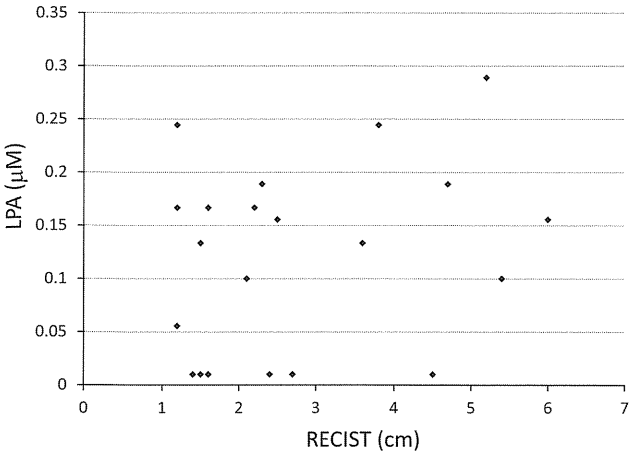

Fig. 1. Plasma LPA levels and HCC burden. Plasma LPA levels, measured in 21 HCC patients (13 males and 8 females; 2 patients with chronic hepatitis B, 15 with chronic hepatitis C, and 4 with non-B non-C chronic liver disease), were not significantly correlated with HCC burden as evaluated by RECIST (Response Evaluation Criteria in Solid Tumors; Spearman rank, $r = 0.158$, $P = 0.4937$). This study was approved by the Institutional Research Ethics Committee and informed consent was obtained for the use of the samples.

and found that they were not correlated with tumor burden, as shown in Fig. 1. Moreover, plasma LPA levels in HCC patients (0.12 ± 0.09 mM, mean ± SD, n = 21), were not different from the previously reported levels in non-HCC patients with chronic hepatitis C (0.10 ± 0.05 mM).[5] Although Mazzocca et al. reported no enhancement of serum LPA levels in cirrhosis patients, we[5] and others[6] previously showed that plasma LPA levels and serum ATX activity were increased in chronic liver diseases in association with fibrosis and cholestatic pruritus, from which HCC frequently arises. Collectively, a role of LPA in HCC should be cautiously analyzed.

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Potential conflict of interest: Nothing to report.

Reply:

Ikeda et al. remark that platelets are a main source of lysophosphatidic acid (LPA) and therefore the interpretation of LPA serum concentrations deserves careful attention. However, the same authors previously reported[1] an inverse correlation between plasma LPA concentrations and the number of platelets in patients with chronic C hepatitis. Therefore, it is possible that in physiologic conditions platelets remain the main source of LPA, while in chronic inflammation such as hepatitis C, liver cirrhosis, or hepatocellular carcinoma (HCC), the platelet contribution to LPA production may likely become less relevant. In our study we analyzed sera for LPA detection in healthy donors, liver cirrhosis, and HCC patients, performing well-standardized procedures of collection for each sample. Thus, the contribution of platelets to the LPA concentration was, in reality, normalized. On the contrary, the authors should consider that even in plasma or whole blood, platelet activation is an extremely difficult problem to deal with and control. For example, prolonged tourniquet application, or twisting of the needle in the vein, are major factors interfering with the function of platelets during blood withdrawal, as reviewed by Ruggeri.[2] Unfortunately, these limitations are common for a number of molecules involved both in cancer and in blood cell biology.[3]

Moreover, Ikeda et al. investigated patients with chronic hepatitis C, in whom the inflammatory response is a key component of the tissue microenvironment. In their study, the fibrotic status was also questionable, due to their choice of statistical method (comparison among groups should be done with Kruskal-Wallis tests), and because of the very limited number of patients (14), further stratified into four different groups, which means the conclusions were affected by low power.[1] In our study,[4] we compared liver cirrhosis versus HCC. In the former case, the inflammation is reduced while the fibrotic response is increased, consequently inducing a different microenvironment response.[5] This could explain why patients with liver cirrhosis display relatively low levels of LPA. In addition, it is conceivable that when HCC develops in cirrhotic liver, LPA levels rise once more, as in cases of active inflammatory states (i.e., viral hepatitis). Another key point is patient selection. Ikeda et al. do not provide any information with regard to the clinical features of the patients, i.e., etiology, BCLC stage, previous therapy, etc., as well as how they calculated the size of the tumor in patients with multifocal disease, for instance. Finally, some differences between Caucasian and Asian patients with HCC are to be expected, since the natural history is completely different in Western and Southeast Asian countries.[6] In our study,[4] we demonstrated that LPA has a role in promoting tumor progression and we did not attempt to speculate about the use of LPA as a clinical biomarker. To validate LPA as a potential biomarker for HCC a different study design is required, as well as first considering the power of the study. The enhancement of serum LPA levels reported by Watanabe et al.[1] referred to a relatively small number of patients with chronic hepatitis C. In addition, the

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CLINICAL STUDIES

Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors

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Keywords
ablation – hepatocellular carcinoma – percutaneous ethanol injection – prognostic factor – recurrence – survival – treatment outcome

Abbreviations
AFP-L3, lectin-reactive AFP; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence intervals; CT, computed tomography; DCP, des-γ-carboxy-prothrombin; HBs-Ag, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

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Abstract

Background: Ethanol injection is the best-known image-guided percutaneous ablation for hepatocellular carcinoma (HCC) and a well-tolerated, inexpensive procedure with few adverse effects. However, there have been few reports on its long-term results. *Aims:* We report a 20-year consecutive case series at a tertiary referral centre. *Methods:* We performed 2147 ethanol injection treatments on 685 primary HCC patients and analysed a collected database. *Results:* Final computed tomography demonstrated complete ablation of treated tumours in 2108 (98.2%) of the 2147 treatments. With a median follow-up of 51.6 months, 5-, 10- and 20-year survival rates were 49.0% [95% confidence interval (CI) = 45.3–53.0%], 17.9% (95% CI = 15.0–21.2%) and 7.2% (95% CI = 4..5–11.5%) respectively. Multivariate analysis demonstrated that age, Child–Pugh class, tumour size, tumour number and serum alpha-fetoprotein level were significant prognostic factors for survival. Five-, 10- and 20-year local tumour progression rates were 18.2% (95% CI = 15.0–21.4%), 18.4% (95% CI = 15.2–21.6%) and 18.4% (95% CI = 15.2–21.6%) respectively. Five-, 10- and 20-year distant recurrence rates were 53.5% (95% CI = 49.4–57.7%), 60.4 (95% CI = 56.3–64.5%) and 60.8% (95% CI = 56.7–64.9%) respectively. There were 45 complications (2.1%) and two deaths (0.09%). *Conclusions:* Ethanol injection was potentially curative for HCC, resulting in survival for more than 20 years. This study suggests that new ablation therapies will achieve similar or even better long-term results in HCC.

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

The most studied percutaneous ablation is ethanol injection. Ethanol injection is a well-tolerated, inexpensive procedure with few adverse effects and has been considered the standard against which any new ablation therapy should be compared (2). Although ethanol injection was introduced into clinical practice in the 1980s (3, 4), few reports of its long-term results have been published (5–8). We report here a 20-year consecutive case series at a tertiary referral centre. This study documents the largest number of ethanol injection treatments at a single institution. Findings in this 20-year experience may be extrapolated to other ablation therapies, such as radiofrequency ablation, in which such long-term outcomes are not yet available (9).

Patients and methods

Indications for ethanol injection

Ethanol injection was performed in patients satisfying the following criteria: (i) ineligible for resection or transplantation, or had refused surgery; (ii) no extrahepatic metastasis or vascular invasion. Exclusion criteria were as follows: (i) tumour was not visualized

by ultrasonography or not accessible percutaneously; (ii) total bilirubin level ≥ 3.0 mg/dl; (iii) platelet count $<40 \times 10^9$/L; (iv) prothrombin activity <35%; (v) refractory ascites. In general, we performed ethanol injection on patients with Child–Pugh class A or B, with 3 or fewer tumours ≤ 3 cm in diameter. We performed ethanol injection on patients beyond these conditions, however, who were likely to benefit from the procedure for possible cure or prolongation of life. No patients were excluded solely because of tumour location (10). Informed consent was obtained from each patient. This study was conducted according with the Helsinki Declaration of 1975 and approved by the Institutional Review Board.

Patients

In this cohort study, we analysed a prospectively collected computerized database. Between 1985 and 2005, 2735 HCC patients were admitted to the Department of Gastroenterology, University of Tokyo (Fig. 1). At initial hospitalization, 1615 had primary HCC and the remaining 1120 had recurrent HCC. The recurrent HCC patients had undergone therapies other than ethanol injection for primary HCC.

Of the 1615 patients with primary HCC, 1459 (90.3%) underwent percutaneous ablation as the initial treatment, including ethanol injection. The remaining 156 patients received other therapies: transarterial chemoembolization for 123 patients with multinodular or large tumours that could not be treated by ablation therapies; hepatic resection for 18 with good liver function who consented to an operation; chemotherapy for four with vascular invasion or extrahepatic metastasis; and best supportive care for 11 with decompensated cirrhosis or poor general condition.

Of the 1459 patients treated by percutaneous ablation, 685 underwent ethanol injection, 122 underwent microwave ablation, and the remaining 652 radiofrequency ablation. The type of percutaneous ablation performed varied with the date of treatment. We started ethanol injection in December 1985, microwave ablation in October 1995 and radiofrequency ablation in February 1999 (11). Between October 1995 and February 1999, both ethanol injection and microwave ablation were performed. Microwave ablation was chosen for patients who had better liver function and whose tumour was located in a position where the electrode could be inserted and held safely. Since February 1999, both ethanol injection and radiofrequency ablation have been performed. Between April 1999 and January 2001, 232 patients with three or fewer tumours, each ≤ 3 cm in diameter, and Child–Pugh class A or B were entered into a randomized controlled trial (12). Patients outside these inclusion criteria were mostly treated by radiofrequency ablation. After this trial, radiofrequency

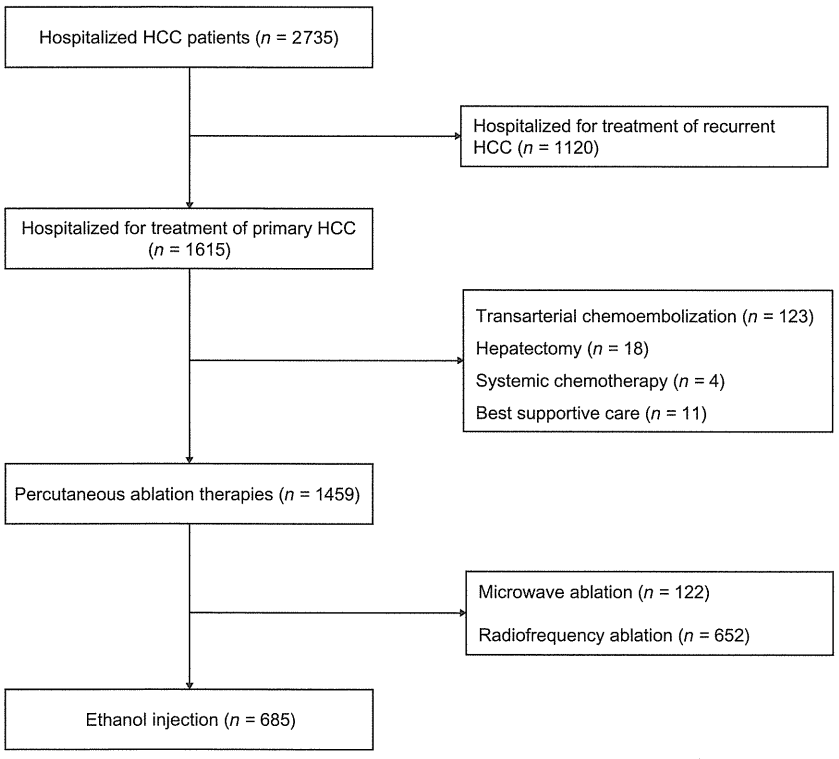

Fig. 1. Flow of patients in this study. HCC, hepatocellular carcinoma

ablation was generally the treatment of choice, and ethanol injection was used only in those unsuitable for radiofrequency ablation: those with either enterobiliary reflux or tumour adhesion to the gastrointestinal tract.

Hepatocellular carcinoma was diagnosed based on typical imaging findings of early phase enhancement and late phase contrast washout on computed tomography (CT) (13). HCC diagnosis was also confirmed by biopsy in 630 (92.0%) of the 685 patients with primary HCC treated by ethanol injection. A total of 587 (85.7%) were diagnosed as having cirrhosis.

In general, chemoembolization was combined with ethanol injection in patients with either ≥ 4 tumours or those with two or three tumours at least one of which is >3.0 cm in diameter. The combination of chemoembolization with ethanol injection was performed in 186 patients.

Treatment methods

Preoperative planning including ultrasound examination and evaluation of all imaging findings was performed to identify the tumours and to determine the access route. The procedure was performed according to an institutional protocol and under the supervision of experienced physicians who had performed this treatment more than 200 times. The precise techniques of ethanol injection are described elsewhere (12). Briefly, all procedures were performed percutaneously under ultrasound guidance. Artificial pleural effusion or artificial ascites method is much less frequently used in ethanol injection compared with radiofrequency ablation, because the procedure is necessary to be repeated several times. Since 1990, we have used two or three needles to inject ethanol into several sites in one procedure (12). Ethanol injection was performed twice per week. The procedure was repeated until ethanol appeared to have been injected throughout the tumour. To judge a timing to stop repetition of injecting ethanol and to order a CT scan, we considered total volume of injected ethanol and change of echogenicity. The general guideline for the necessary volume of injected ethanol was calculated according to the following numerical expression, $V = (4/3)\ \pi\ (r + 0.5)^3$, where V (in millilitres) is the volume of ethanol and r (in centimetres) is the radius of the tumour; 0.5 is added to provide a safety margin, which is based on the concept that some surrounding liver parenchyma all around the tumour as well as the tumour itself must be ablated (5).

A CT scan was then performed 1–3 days after the procedure to evaluate technique effectiveness (14). Complete ablation was defined as hypoattenuation of the entire tumour. When the presence of unablated tumour portions was suspected, a few more procedures were performed. We did not predefine the number of procedures in a treatment. The ethanol injection treatment was generally continued until CT demonstrated the entire tumour necrosis.

Follow-up

Follow-up investigations consisted of CT, ultrasonography and measurement of serum α-fetoprotein (AFP), des-γ-carboxy-prothrombin (DCP) (since April 1993) levels and lectin-reactive AFP (AFP-L3) (since July 1997) every 4 months. Local tumour progression was defined as appearance of viable tumour touching the original tumour (14) and distant recurrence as emergence of tumour(s) separate from the primary site. Ethanol injection was used for recurrence if the patient still met the indication criteria. If multiple recurrences were not treatable with ethanol injection, chemoembolization was generally performed.

Statistical analyses

This study is a report of a consecutive case series. All ethanol injection treatments performed on primary HCC patients at the Department of Gastroenterology, University of Tokyo between 1985 and 2005 were included. Data are presented as mean ± SD for quantitative variables, and as absolute frequencies for qualitative variables.

A 'procedure' was defined as a single intervention episode that consisted of one or more ablations performed on tumours, and a 'treatment' as the completed effort to ablate tumours. A treatment consisted of several procedures (14). 'Technique effectiveness' rate was defined as the percentage of successfully eradicated macroscopic tumours as evidenced at CT scan after the last procedure (14). In cases in which there was Lipiodol deposit inside the tumour because of the combination of chemoembolization with ethanol injection, we judged that the tumour had been successfully eradicated if it was surrounded with completely non-enhanced tissue in final CT.

Overall survival was calculated in the 685 primary HCC patients. Survival curves were generated using the Kaplan–Meier method. In addition to overall survival, subgroup analyses were performed with clinical characteristics including tumour size, tumour number and Child–Pugh class. Recurrence was evaluated in 591 patients in whom ethanol injection was performed with curative intent. All tumours were treated by ethanol injection in those patients. The remaining 94 patients were excluded from the recurrence analysis because some small tumours had been left untreated by ethanol injection on account of detection failure by ultrasonography. Recurrence rates were calculated using the Gaynor method (15). All time estimates were made from the date of the first ethanol injection. The follow-up was finalized at either death or the last visit to the outpatient clinic before December 31 2010. Transplanted patients were censored from this study at the date of transplantation.

The prognostic relevance of baseline variables (Table 1), the combination of chemoembolization,