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Research Paper

Efficacy of Lamivudine or Entecavir against Virological Rebound after Achieving HBV DNA Negativity in Chronic Hepatitis B Patients

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Abstract

Nucleos(t)ide analogues (NAs) lead to viral suppression and undetectable hepatitis B virus (HBV) DNA in some individuals infected with HBV, but the rate of virological rebound has been unknown in such patients. We examined the prevalence of virological rebound of HBV DNA among NA-treated patients with undetectable HBV DNA. We retrospectively analyzed 303 consecutive patients [158 entecavir (ETV)- and 145 lamivudine (LAM)-treated] who achieved HBV DNA negativity, defined as HBV DNA < 3.7 log IU/mL for at least 3 months. They were followed up and their features, including their rates of viral breakthrough, were determined. Viral rebound after HBV DNA negativity was not observed in the ETV-group. Viral rebound after HBV DNA negativity occurred in 38.7% of 62 HBe antigen-positive patients in the LAM-group. On multivariate analysis, age was an independent factor for viral breakthrough among these patients (P = 0.035). Viral rebound after HBV DNA negativity occurred in 29.1% of 79 HBe antigen-negative patients in the LAM-group. Differently from LAM, ETV could inhibit HBV replication once HBV DNA negativity was achieved. In contrast, LAM could not inhibit HBV replication even if HBV negativity was achieved in the early phase. Attention should be paid to these features in clinical practice.

Key words: Entecavir, HBeAg, HBV DNA, Lamivudine, Virological rebound.

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major health problem and one of the risk factors for the development of hepatocellular carcinoma (HCC) worldwide [1,2]. Chronic HBV infection has been linked epidemiologically to the development of HCC for more than 30 years [3]. To date, the mechanism of HBV-related hepatocarcinogenesis is not clear. Although effective vaccine exists for preventing HBV

infection [4], acute liver failure due to HBV or acute exacerbation of chronic hepatitis B is also a life-threatening disease [5,6].

Positivity for hepatitis B e antigen (HBeAg), which in serum indicates active viral replication in hepatocytes, is associated with an increased risk of HCC [7]. Chronic HBV carriers with high-titer viremia are also at increased risk for HCC [8]. The risk for cirrhosis and that for HCC increase significantly with increasing HBV DNA levels [9, 10]. Thus, it cannot be overstated that HBV DNA should be directly suppressed to prevent the development of HCC.

There are several nucleos(t)ide analogues (NAs) for the treatment of chronic hepatitis B [11]. Currently, the Japanese national health insurance system approves lamivudine (LAM) and entecavir (ETV) as first-line therapy for treatment-naïve patients with chronic hepatitis B, although some patients are treated with standard interferon-alfa or peginterferon-alfa-2a [6,12]. In general, LAM, the first oral NA available for the treatment of chronic hepatitis B, is associated with high rates of drug-resistance, with ~76% after 8 years of treatment [13,14]. ETV is found to be superior to LAM from the point of view that ETV is stronger than LAM and that resistance to ETV is rare, about 1.2% after 5 years of ETV treatment [14,15].

The aim of this study was to determine the efficacy and the rates of virological rebound after achieving HBV DNA negativity in the use of ETV or LAM in clinical practice. Our study showed that ETV could inhibit HBV replication if HBV DNA negativity had been achieved, but LAM was unable to inhibit HBV replication even if HBV negativity was achieved in the early phase.

MATERIALS AND METHODS

Patients and Study Design

This was a retrospective analysis comparing the rates of virological rebound in patients treated with ETV versus those in patients treated with LAM. A total of 303 patients were examined from Chiba University Hospital, Chiba, Japan, and 4 affiliated hospitals between the period of January 2000 and December 2011. NAs-naïve chronic hepatitis B patients daily receiving 0.5 mg of ETV (ETV group, N=158) or receiving 100 mg of LAM (LAM group, N=145) with undetectable HBV DNA (< 3.7 log IU/mL) for three months were enrolled. Some of the included patients had been previously reported [12, 16]. All patients had serum hepatitis B surface antigen (HBsAg) detectable for at least 6 months, regardless of their HBeAg status. They were negative for hepatitis C virus and human immunodeficiency virus antibodies. This study was approved by the Ethics Committee of Chiba University, Graduate School of Medicine (No. 977).

Definition of Virological Rebound of HBV

We defined virological rebound as $\geq$ 3.7 log IU/mL for at least 3 months after achieving undetectable HBV DNA.

Monitoring of HBV DNA, Serum Liver Function Tests and Hematological Tests

The primary outcome of this study was the virological rebound. Patients were followed up at least every 3 months to examine physical status and to monitor liver biochemistry and virology. All clinical laboratory tests including hematological data, biochemical data, and HBV serologies were performed at the Central Laboratory of Chiba University Hospital. HBsAg, HBeAg and anti-HBe antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [17]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al [18]. HBV DNA was measured by transcription-mediated amplification (TMA) assay, COBAS Amplicor HBV Monitor assay, or COBAS TaqMan (Roche Diagnostics, Branchburg, NJ, USA). The clinical efficacy of NAs was assessed as the proportion of patients achieving HBV DNA negativity, defined as an HBV DNA level of < 3.7 log IU/mL.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD). Differences were evaluated by Student's *t*-test, chi-square test, or Fisher's exact test. $P < 0.05$ was considered statistically significant. Variables with $P < 0.05$ at univariate analysis were retained for multivariate logistic-regression analysis. For all tests, two-sided P-values were calculated and the results were considered statistically significant at $P < 0.05$. Statistical analysis was performed using the Excel-statistics program for Windows, version 7 (SSRI, Tokyo, Japan).

RESULTS

A total 303 patients were recruited into either the ETV group (n = 158) or the LAM group (n = 145), with a follow-up period of 33.7 $\pm$ 11.3 months (28.6 $\pm$ 11.3 months or 39.3 $\pm$ 31.4 months, respectively). Baseline demographic and laboratory data are summarized in Table 1. There were no differences in age, gender, HBV DNA, alanine aminotransferase (ALT) levels, ultrasound findings/presence of cirrhosis, and periods from the initial administration of ETV or LAM to

undetectable HBV DNA, between the ETV and LAM groups, although the proportion of HBeAg-positive patients in the ETV group (55%) tended to be higher than that in the LAM group (44%).

Virological Rebound

The patient flow and outcome are summarized in Figure 1. We excluded 9 patients, whose HBeAg status at baseline was unknown, from this analysis. When comparing the baseline characteristics of patients according to HBeAg status, HBeAg-positive patients were younger, had higher ALT levels and HBV DNA levels, and less cirrhotic findings by ultrasound than HBeAg-negative patients (Table 2). The period from the initial administration of ETV or LAM to the determination of undetectable HBV DNA in the HBeAg-negative group tended to be shorter than that in the HBeAg-positive group (Table 2).

In the ETV group, none of the patients had virological rebound during the follow-up periods. In the LAM group, 24 and 23 patients of 62 HBeAg-positive and 79 HBeAg-negative patients at baseline, respectively, developed evidence of virological rebound. In the 24 HBeAg-positive patients at baseline with virological rebound, 9, 8, 3, 1, 2, and 1 had virological rebound at ≤ 1, $1 \sim \leq 2$, $2 \sim \leq 3$, $3 \sim \leq 4$, $4 \sim \leq 5$, and details unknown, respectively. In the 23 HBeAg-negative patients at baseline with virological rebound, 10, 8, 3, 0, 1, and 1 had virological rebound at ≤ 1, $1 \sim \leq 2$, $2 \sim \leq 3$, $3 \sim \leq 4$, $4 \sim \leq 5$ and details unknown, respectively. Baseline characteristics of patients treated with ETV or LAM according to HBeAg status are shown in Table 3. In the ETV group, the period from the initial administration of ETV to the determination of undetectable HBV DNA in the HBeAg-negative group was the same as that in the HBeAg-positive group (Table 3). In the LAM group, the period from the initial administration of LAM to undetectable HBV DNA in the HBeAg-negative group was shorter than that in the HBeAg-positive group (Table 3). In the HBeAg-positive patients, the period from the initial administration to undetectable HBV DNA in the ETV group was shorter than that in the LAM group (Table 3).

Predictors of Virological Rebound in Patients treated with LAM

To clarify the predictors of virological rebound in patients treated with LAM, we compared the pretreatment factors between patients with and without virological rebound according to HBeAg status (Table 4A & 4B). Univariative analysis showed that age, HBV DNA, ALT levels and the period from the initial administration of LAM to the determination of undetectable HBV DNA in HBeAg-positive patients contributed to the occurrence of virological rebound (Table 4A). Factors significantly associated with virological rebound in HBeAg-positive patients treated with LAM by univariate analysis were also analyzed by multivariate logistic regression analysis. Virological rebound was attained independently of age in HBeAg-positive patients treated with LAM (Table 4C). In HBeAg-negative patients, no significant factors contributing to virological rebound could be found (Table 4B).

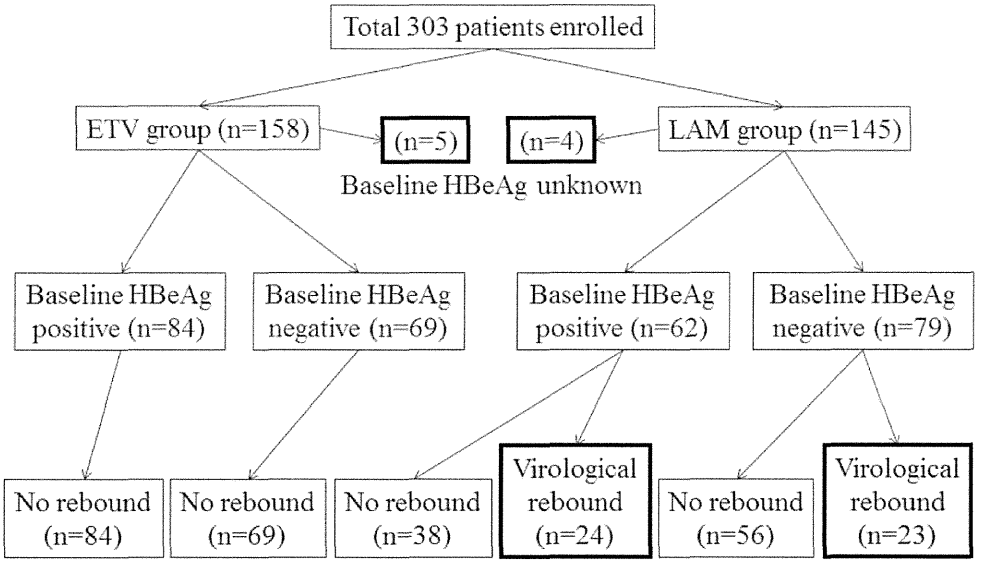

Figure 1. Study design and patient flow for both groups.

Table 1. Baseline characteristics of patients treated with entecavir (ETV) or lamivudine (LAM).

	Total	ETV group	LAM group	*P*-values
Number	303	158	145	
Age (years)	51 ± 12	51 ± 12	50 ± 12	*N.S.*
Gender (male)	205	101	104	*N.S.*
HBeAg (+)	146	84	62	*0.079*
HBV DNA (log IU/mL)	6.5 ± 1.5	6.6 ± 1.7	6.4 ± 1.3	*N.S.*
ALT (IU/L)	203 ± 280	187 ± 290	220 ± 266	*N.S.*
US: Cirrhosis (+)	113	56	57	*N.S.*
Periods to undetectable HBV DNA (months)	10.0 ± 18.2	8.5 ± 11.9	11.8 ± 23.3	*N.S.*

Data are expressed as mean ± SD. ETV group, patients receiving 0.5 mg of ETV daily; LAM group, patients receiving 100 mg of LAM daily; *P*-values, *P*-values between ETV and LAM groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 2. Baseline characteristics of patients according to HBeAg status.

HBeAg	Positive group	Negative group	*P*-values
Number	146	148	
Age (years)	46 ± 12	55 ± 11	*< 0.001*
Gender (male)	101	97	*N.S.*
HBV DNA (log IU/mL)	7.2 ± 1.1	5.8 ± 1.4	*< 0.001*
ALT (IU/L)	257 ± 332	156 ± 211	*0.002*
US: Cirrhosis (+)	41	70	*< 0.001*
Periods to undetectable HBV DNA (months)	11.0 ± 18.1	7.4 ± 14.4	*0.063*

Data are expressed as mean ± SD. *P*-values, *P*-values between HBeAg-positive and HBeAg-negative groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 3. Baseline characteristics of patients treated with entecavir (ETV) or lamivudine (LAM) according to HBeAg status.

	ETV group		LAM group	
HBeAg	Positive	Negative	Positive	Negative
Number	84	69	62	79
Age (years)	48 ± 12	56 ± 11*	44 ± 11 ##	54 ± 11**
Gender (male)	53	45	48	52**
HBV DNA (log IU/mL)	7.5 ± 1.1	5.7 ± 1.5*	6.9 ± 1.1$	5.9 ± 1.3**
ALT (IU/L)	219 ± 325	159 ± 246	309 ± 334	154 ± 174**
US: Cirrhosis (+)	25	29	16	41
Periods to undetectable HBV DNA (months)	8.3 ± 10.5	7.3 ± 11.0	15.0 ± 24.7$$	7.5 ± 16.9#

Data are expressed as mean ± SD. HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *$P < 0.001$, compared to HBeAg-positive of ETV group; **$P < 0.001$ and #$P = 0.034$, compared to HBeAg-positive of LAM group; ##$P = 0.041$, $$P = 0.001$ and $$$P = 0.027$, compared to HBeAg-positive of ETV group.

Table 4A. Predictors of virological rebound in patients treated with lamivudine (LAM). (A) Comparison of HBeAg-positive patients with or without virological rebound by univariate analysis.

Virological rebound	No	Yes	*P-values*
Number	38	23	
Age (years)	42 ± 11	49 ± 11	*0.019*
Gender (male)	30	17	*N.S.*
HBV DNA (log IU/mL)	6.9 ± 1.2	6.8 ± 0.9	*N.S.*
ALT (IU/L)	379 ± 377	196 ± 205	*0.037*
US: Cirrhosis (+)	7	9	*N.S.*
Periods to undetectable HBV DNA (months)	20.6 ± 29.1	4.1 ± 3.1	0.009

Data are expressed as mean ± SD. *P*-values, *P*-values between patients with or without virological rebound groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 4B. (B) Comparison of HBeAg-negative patients with or without virological rebound by univariate analysis.

Virological rebound	No	Yes	*P-values*
Number	56	22	
Age (years)	54 ± 11	54 ± 10	*N.S.*
Gender (male)	40	12	*N.S.*
HBV DNA (log IU/mL)	5.9 ± 1.4	5.9 ± 1.0	*N.S.*
ALT (IU/L)	163 ± 179	137 ± 163	*N.S.*
US: Cirrhosis (+)	30	11	*N.S.*
Periods to undetectable HBV DNA (months)	7.3 ± 14.8	3.1 ± 2.1	*N.S.*

Data are expressed as mean ± SD. *P*-values, *P*-values between patients with or without virological rebound groups; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; US, ultrasound findings; *N.S.*, no statistically significant difference.

Table 4C. (C) Factor associated with virological rebound among HBeAg-positive patients treated with LAM by multivariate analysis.

Factor	Category	Odds ratio	95% CI	*P-value*
Age ≤ 44.5 (years)	(+/-)	0.222	0.0547-0.9023	0.0354

DISCUSSION

To date, there is not much data regarding virological rebound after achieving HBV DNA negativity in the use of ETV or LAM. A recent report supported the merit of the change from LAM to ETV [14]. This study concluded that prior optimal viral suppression with ETV did not confer any significant advantage for patients who switched to LAM.

The present study revealed that ETV could suppress HBV replication after achieving HBV DNA negativity, although additional longer follow-up studies will be needed. On the other hand, LAM could not suppress HBV replication even after achieving HBV DNA negativity (Figure 1), although most cases with virological rebound were observed within 2 years of the start of LAM medication. We could not check the emergence of YMDD motif mutations [19] in all of the cases because the present study was performed as part of regular clinical practice. Of 2 of the HBeAg-positive patients at baseline with virological rebound, one showed YVDD motif (50%). In 4 of the HBeAg-negative patients at baseline with virological rebound, one YVDD motif (25%) and three YIDD motifs (75%) were seen. Virological rebound may not mean the emergence of NA-resistance mutations [12].

We do not know the reason why virological rebound was attained independently of age in HBeAg-positive patients treated with LAM. HBeAg to anti-HBe antibody seroconversions were found in 20 and 11 patients with and without virological rebound, that is, the HBeAg to anti-HBe antibody seroconversion rates were similar in the two groups (data not shown), although the number of study patients seemed small in the present study. Further studies might be needed. In any event, it might be important to consider the LAM-to-ETV switch in HBeAg-positive patients treated with LAM, although some of our patients in the LAM group remained HBV-negative throughout the observation period.

In the present study, 95.3% (122 of 128), 82.3% (14 of 17) and 89.2% (25 of 28) had an adherence rate >90% [16] in ETV-treated, LAM-treated with virological rebound and LAM-treated patients without virological rebound, respectively. These results supported our previous study that viral breakthrough associated with poor adherence could be a more important issue in the treatment with especially stronger NAs, such as ETV [12,16], although we cannot ensure durable HBV negativity after NAs are discontinued. We and others reported that HBeAg could impair both innate and adaptive immune responses to promote chronic HBV infection [16,20,21]. Of interest, the virological rebound with the use of LAM seemed unrelated to the HBeAg status, suggesting that it was dependent on resistant mutation.

Recently, other effective antiviral therapies such as peginterferon [22,23] and tenofovir [24,25] were reported to be useful for the control of HBV infection. These drugs might also be candidates for treating virological rebound. Fung et al. [14] reported that prior optimal viral suppression with ETV did not confer any significant advantage for patients who switched to LAM. Our results also supported the previous studies that ETV was much more efficient than LAM [26-29]. In conclusion, ETV could inhibit HBV replication if HBV DNA negativity had been achieved. In contrast, LAM could not inhibit HBV replication even if HBV negativity was achieved in the early phase. Attention should be paid to these features in clinical

practice.

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CONFLICT OF INTEREST

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ABBREVIATIONS

ALT: alanine aminotransferase; ETV: Entecavir; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; LAM: lamivudine; NA: nucleos(t)ide analogue.

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Research Paper

Adherence to Medication Is a More Important Contributor to Viral Breakthrough in Chronic Hepatitis B Patients Treated with Entecavir Than in Those with Lamivudine

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Abstract

Viral breakthrough is related to poor adherence to medication in some chronic hepatitis B patients treated with nucleos(t)ide analogues (NAs). Our study aimed to examine how adherence to medication is associated with viral breakthrough in patients treated with NAs. A total of 203 patients (135 ETV and 68 LAM) were analyzed in this retrospective analysis. Physical examination, serum liver enzyme tests, and hepatitis B virus marker tests were performed at least every 3 months. We reviewed medical records and performed medical interviews regarding to patients' adherence to medication. Adherence rates <90% were defined as poor adherence in the present study. Cumulative viral breakthrough rates were lower in the ETV-treated patients than in the LAM-treated patients (*P<0.001*). Seven ETV-treated (5.1%) and 6 LAM-treated patients (8.8%) revealed poor adherence to medication (*P=0.48*). Among ETV-treated patients, 4 (3.1%) of 128 patients without poor adherence experienced viral breakthrough and 3 (42.8%) of 7 patients with poor adherence experienced viral breakthrough (*P<0.001*). Only 3 of 38 (7.8%) LAM-treated patients with viral breakthrough had poor adherence, a lower rate than the ETV-treated patients (*P=0.039*). Nucleoside analogue resistance mutations were observed in 50.0% of ETV- and 94.1% of LAM-treated patients with viral breakthrough (*P=0.047*). Viral breakthrough associated with poor adherence could be a more important issue in the treatment with especially stronger NAs, such as ETV.

Key words: Adherence, Entecavir, Lamivudine, Hepatitis B, Viral Breakthrough.

INTRODUCTION

Two billion people have been exposed to hepatitis B virus (HBV), and 350-400 million people remain chronically infected worldwide. In Japan, the prevalence of HBV carriers is estimated at ~1% of the pop-

ulation, but HBV is a major health issue because it causes acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1, 2].

Lamivudine (LAM) is a reverse-transcriptase inhibitor of HBV DNA polymerase that possesses excellent profile of safety and tolerability and causes inhibition of viral replication. LAM was the first nucleos(t)ide analogue (NA) to be approved for antiviral treatment of hepatitis B patients [3, 4]. Entecavir (ETV), a deoxyguanosine analogue, is a potent and selective inhibitor of HBV replication. The in vitro potency of ETV is 100- to 1,000-fold greater than that of LAM, and it has a selectivity index (concentration of drug required to reduce viable cell number by 50% [CC_{50}] / concentration of drug required to reduce viral replication by 50% [EC_{50}]) of approximately 8,000 [5, 6]. LAM (until 2005) and ETV (from 2006) have been used as first-line NAs for most patients with chronic hepatitis B in Japan. Most patients with chronic hepatitis B have been undergoing treatment for longer durations, and prolonged treatment is associated with increasing rates of viral breakthrough [7]. It has been reported that not all cases are associated with resistance mutations [8, 9]. We have also reported that some cases of viral breakthrough during ETV treatment were related to poor adherence to medication [10].

Adherence rates are usually lower in patients with long-term treatment regimens, such as for hypertension, than in patients with short-term regimens, such as for gastric ulcers [11]. It has been reported that 74.8% of patients with hypertension were determined to have an adherence rate ≥80% [12], and that 55.3% of patients with chronic hepatitis B had an adherence rate >90% [8].

In the present study, we aimed to investigate whether drug adherence is related to viral breakthrough in chronic hepatitis B patients treated with LAM or ETV. We also investigated the pattern of poor adherence and suggested how adherence to medication could be improved.

MATERIALS AND METHODS

Patients

Two hundred seventy-five NA-treated naïve patients (185 ETV- and 90 LAM-treated patients), who were admitted to Chiba University Hospital between April 2000 and September 2011, were enrolled (Figure 1). Some of these patients had already been included in a previous report [10]. Between November 2011 and April 2012, doctors performed medical interviews of those patients to determine their adherence to medication. Seventy-two patients (50 ETV- and 22 LAM-treated patients) were excluded from this retrospective analysis, because their adherence to medication could not be confirmed. One hundred thirty-five patients were administered 0.5 mg of ETV daily and 68 patients were administered 100 mg of LAM daily (Table 1). In all patients, serum hepatitis B surface antigen (HBsAg) and HBV DNA were positive. All patients had negative results for hepatitis C virus or human immunodeficiency virus antibodies. Physical examinations, serum liver enzyme tests, and HBV marker tests were performed at least every 3 months. The study was carried out in accordance with the Helsinki Declaration, and was approved by the Ethics Committee of Chiba University, Graduate School of Medicine (No. 977).

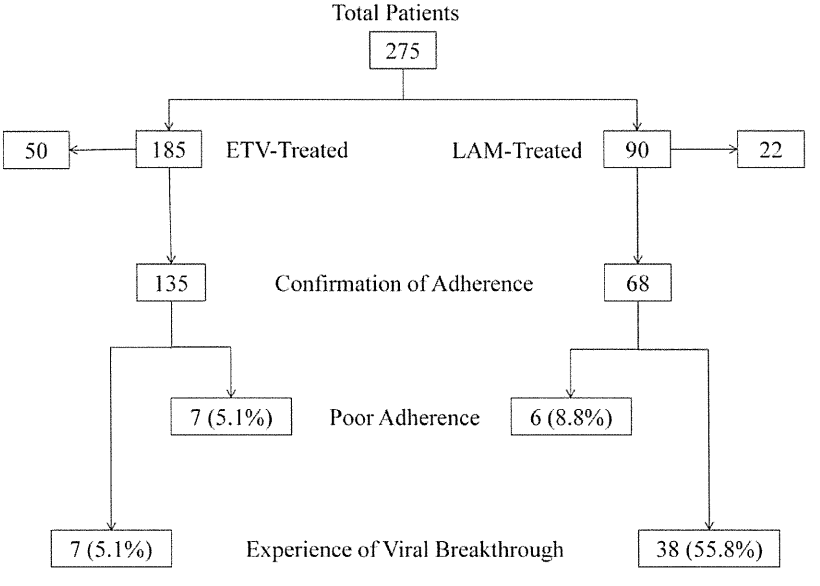


Figure 1. Patients, adherence rates, and the prevalence of viral breakthrough in this study. ETV, entecavir; LAM, lamivudine.

Table 1. Baseline characteristics of patients.

	ETV	LAM	P-values
Number of cases	135	68	
Age (years)	51.7 ± 11.7	45.5 ± 12.1	<0.001
Gender (male/female)	83/52	49/19	0.135
HBeAg (+/-)	64/71	45/23	0.011
Genotype (A/B/C/unknown)	0/11/78/46	1/6/57/4	0.427
HBV DNA (log IU/mL) (≤5.0/> 5.0/unknown)	27/108/0	3/55/10	0.009
ALT (IU/L)	161 ± 195	353 ± 394	<0.001
Platelets ($\times 10^4/mm^3$)	16.3 ± 5.9	16.9 ± 7.0	0.556
APRI	2.49 ± 4.19	6.52 ± 6.98	<0.001
Follow-up period (months)	26.9 ± 21.6	49.0 ± 39.7	<0.001

ETV, entecavir; LAM, lamivudine; HBeAg, hepatitis B e antigen; N.D., not determined; HBV DNA, hepatitis B virus deoxyribonucleic acid; ALT, alanine aminotransferase; APRI, aspartate aminotransferase platelet ratio index. Continuous variables are expressed as mean ± standard deviation.

Blood examinations

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, and platelet counts were reviewed in the present study. We also calculated the aspartate aminotransferase platelet ratio index [APRI: AST (IU/L)/ 35/platelet count ($10^3/\mu L$) x 100], which is significantly correlated with the staging of liver fibrosis, with a higher correlation coefficient than platelet count or AST level alone [13].

Detection of HBV markers

HBsAg, hepatitis B e antigen (HBeAg) and anti-HBe antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan)[14]. HBV genotype was determined by ELISA (Institute of Immunology, Tokyo, Japan) [15]. HBV DNA was measured by Roche Amplicor PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan).

Follow-up period

The follow-up period ended when the NA was switched to another NA or another NA was added, or it was discontinued for various reasons.

Definition of adherence to medication

To obtain information regarding adherence to medication, we reviewed medical records. We also interviewed patients about their adherence to medication. We expressed the rate of adherence to medication as a percentage calculated by the number of days of taking a pill divided by the follow-up period (days). Adherence rates <90% were defined as poor adherence in the present study.

Definition of viral breakthrough

Viral breakthrough was defined as an increase of ≥ 1 log IU/mL in serum HBV DNA level from nadir.

Sequence analysis of HBV DNA

The YMDD motif was analyzed by PCR-ELMA in sera of patients who had experienced viral breakthrough, as reported by Kobayashi et al [16]. HBV polymerase/reverse transcriptase (RT) substitutions were also analyzed in sera of ETV-treated patients who had experienced viral breakthrough. Briefly, HBV DNA was extracted from 100 μL of sera using SepaGene (Sanko Junyaku, Tokyo, Japan). Nested PCR was performed using LA Taq polymerase (Takara Bio, Otsu, Shiga, Japan) under the following conditions: 5-min denaturation at 94°C, 35 cycles with denaturation at 94°C for 40 s, annealing at 58°C for 1 min, and extension at 68°C for 1.5 min [2]. An 862 base-pair fragment (nt 242-1103) containing the polymerase RT domain was amplified on the PCR Thermal Cycler Dice Model TP600 (Takara Bio). The primers for the first PCR were 5′-CAG AGT CTA GAC TCG TGG-3′ (sense, nt 242-258) and 5′-GGC GAG AAA GTG AAAGCC-3′ (antisense, nt 1103-1086). The PCR product was sequenced using the primers: 5′-TGG CTC AGT TTA CTAGTG CC -3′ (nt 668-687) and 5′-GGC ACT AGT AAA CTGAGC CA-3′ (nt 687-668), and these primers were also used for the second PCR. To prepare the sequence template, PCR products were treated with ExoSAP-ITR (Affymetrix, Inc., Santa Clara, CA, USA), and then sequenced using the BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Tokyo, Japan). Sequences were performed with Applied Biosystems 3730xl (Life Technologies) [17].

Statistical analysis

Statistical analyses were performed using SAS 9.3 Software (SAS Institute, Cary, NC, USA). Continuous variables were expressed as mean ± standard deviation and were compared by Student's t-test or

Welch's t-test. Categorical variables were compared by chi-square test or Fisher's exact probability test. The Kaplan-Meier method was used to calculate viral breakthrough rates. Baseline was taken as the date when the first dose of LAM or ETV was taken. Statistical significance was considered at a *P-value* < *0.05*.

RESULTS

Baseline characteristics of patients

Baseline characteristics of patients are shown in Table 1. In ETV-treated patients, the age was higher, the prevalence of HBeAg-negative patients was higher, HBV DNA was lower, ALT levels were lower, and APRI was lower (ie., liver fibrosis was milder) than in LAM-treated patients. HBV genotype C was dominant in both groups. The follow-up period in ETV-treated patients was shorter than that in LAM-treated patients, based on the fact that ETV was a newer drug and many ETV-treated patients had started treatment more recently.

Adherence to medication, and viral breakthrough between ETV- and LAM-treated patients

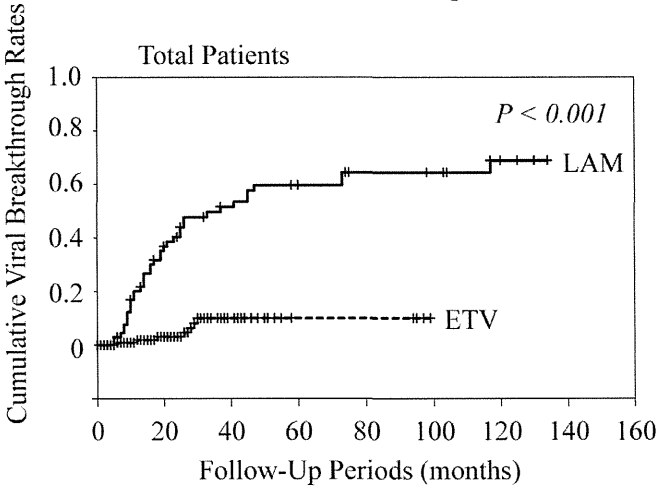
Most patients presented good adherence to medication in the present study. Seven ETV-treated (5.1%) and 6 LAM-treated patients (8.8%) had poor adherence (Figure 1). The number of patients with poor adherence was not significantly different between the ETV- and LAM-treated groups (*P=0.48*). The characteristics of the 13 patients with poor adherence are shown in Table 2. Cumulative viral breakthrough rates were lower in the ETV-treated patients than in the LAM-treated patients (*P<0.001*) (Figure 2).

Viral breakthrough in HBeAg-positive and -negative patients

Among the LAM-treated patients, cumulative viral breakthrough rates in HBeAg-positive patients at baseline (n=45; 25.0% at 1 year, 55.1% at 3 years, and 67.0% at 5 years) were similar to those in HBeAg-negative patients at baseline (n=23; 9.5% at 1 year, 38.2% at 3 years, and 44.4% at 5 years; *P=0.16*). Among the ETV-treated patients, cumulative viral breakthrough rates in HBeAg-positive patients at baseline (n=64; 2.2% at 1 year, 18.1% at 3 years, and 18.1% at 5 years) were also similar to those in HBeAg-negative patients at baseline (n=71; 1.6% at 1 year, 1.6% at 3 years, and 1.6% at 5 years; *P=0.050*).

Among the LAM-treated patients who were HBeAg-positive at baseline, cumulative viral breakthrough rates in patients who converted to HBeAg-seronegative were lower than those in patients who maintained HBeAg seropositivity (*P<0.001*) (Figure 3). All LAM-treated patients who did not become HBeAg-seronegative experienced viral breakthrough. Among the ETV-treated patients who were positive for HBeAg at baseline, conversion to HBeAg seronegativity did not affect the rate of viral breakthrough (*data not shown*).

There were no differences in HBV viral loads at study entry between HBeAg-positive patients with and without viral breakthrough. There were also no differences in HBV viral loads between HBeAg-negative patients with and without viral breakthrough.



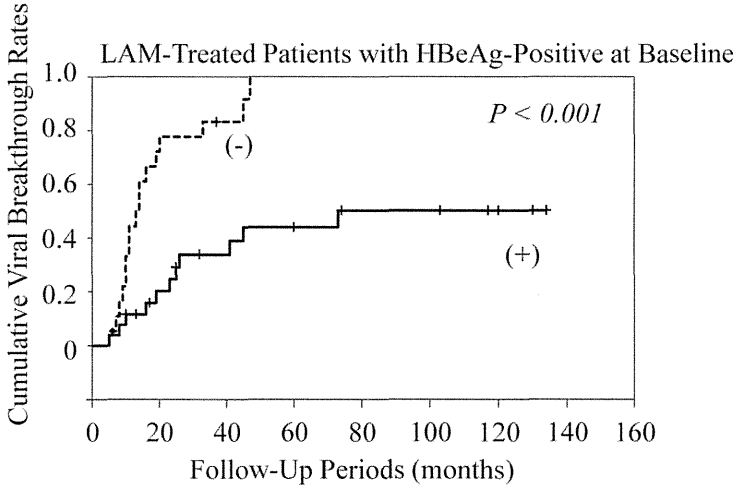
	Drug	Baseline	12	24	36	48	60
Number of Patients	LAM	68	52	34	27	20	19
	ETV	135	95	69	34	19	5

Figure 2. Cumulative viral breakthrough rates. ETV, entecavir; LAM, lamivudine.

Table 2. Patients with poor adherence to medication.

Case	Drug	Adherence rate (%)	Age (years)	Gender	Genotype	HBeAg	HBV DNA (log IU/mL)	ALT (IU/L)	APRI	HBeAg-seronegative	HBV DNA negativity	VT	Duration of treatment before VT (months)	Resistance mutations	Treatment after VT	Clinical outcome
1	ETV	50	55	F	B	-	3.8	16	0.33	N.A.	+	+	6	-	ETV	good
2	ETV	75	49	M	C	+	7.3	107	1.60	+	+	+	28	+	LAM+ADV	good
3	ETV	85	38	M	C	+	6.9	59	2.80	-	+	+	29	N.D.	ETV	good
4	ETV	80	39	M	C	+	5.8	51	0.63	+	+	-	N.A.	N.A.	ETV	good
5	ETV	85	37	F	C	+	6.9	160	2.25	+	+	-	N.A.	N.A.	ETV	good
6	ETV	85	66	M	N.D.	+	7.7	68	0.95	-	-	-	N.A.	N.A.	ETV	good
7	ETV	85	38	M	C	+	6.5	478	7.94	-	+	-	N.A.	N.A.	ETV	good
8	LAM	50	47	F	C	+	6.5	455	2.54	+	+	+	45	-	LAM	good
9	LAM	80	36	M	C	+	7.0	110	4.25	+	+	+	41	+	LAM+ADV	good
10	LAM	85	23	M	C	+	>7.6	161	3.53	-	+	+	11	-	cessation	flare
11	LAM	85	32	M	C	+	>7.6	343	1.30	+	+	-	N.A.	N.A.	LAM	good
12	LAM	85	54	F	C	-	4.1	196	2.68	N.A.	+	-	N.A.	N.A.	LAM	good
13	LAM	85	36	M	C	+	6.7	1576	15.78	+	+	-	N.A.	N.A.	LAM	good

Cases 2 and 3 had already been included in a previous report.[10] HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid, ALT, alanine aminotransferase; APRI, aspartate aminotransferase platelet ratio index; VT, viral breakthrough; ETV, entecavir; LAM, lamivudine; ADV, adefovir; F, female; M, male; N.D., not determined; N.A., not available; HBeAg-seronegative, conversion to HBeAg-seronegative after administration of a nucleoside analogue; HBV DNA negativity, achieving HBV DNA negativity after administration of a nucleoside analogue; flare, fluctuating ALT after treatment after VT.



	HBeAg-Seronegative	Baseline	12	24	36	48	60
Number of Patients	(-)	19	10	4	3	0	0
	(+)	26	22	17	13	11	11

Figure 3. Cumulative viral breakthrough rates in lamivudine (LAM)-treated patients with HBe antigen (HBeAg)-positive at baseline. (-), maintaining HBeAg seropositivity; (+), conversion to HBeAg-seronegative.

Viral breakthrough in patients who achieved, and did not achieve HBV DNA negativity

Among the LAM-treated patients, cumulative viral breakthrough rates in patients who did not achieve HBV DNA negativity were higher than in those who achieved HBV DNA negativity ($P<0.001$) (Figure 4). All patients who did not achieve HBV DNA negativity experienced viral breakthrough. In

contrast, among the ETV-treated patients, cumulative viral breakthrough rates in patients who did not achieve HBV DNA negativity were similar to the rates in those who achieved HBV DNA negativity (*data not shown*).

Correlation between adherence to medication and viral breakthrough

We also compared viral breakthrough rates according to adherence to medication. Among 62 LAM-treated patients who did not have poor adherence, 35 patients (56.4%) experienced viral breakthrough (Figure 5). Among 6 LAM-treated patients with poor adherence, 3 patients (50.0%) experienced viral breakthrough. In LAM treatment, poor adherence did not contribute to viral breakthrough (*P=0.89*). However, among 128 ETV-treated patients who did not have poor adherence, 4 patients (3.1%) experienced viral breakthrough. Among 7 ETV-treated patients with poor adherence, 3 patients (42.8%) experienced viral breakthrough. In the treatment with ETV, poor adherence contributed to viral breakthrough (*P<0.001*).

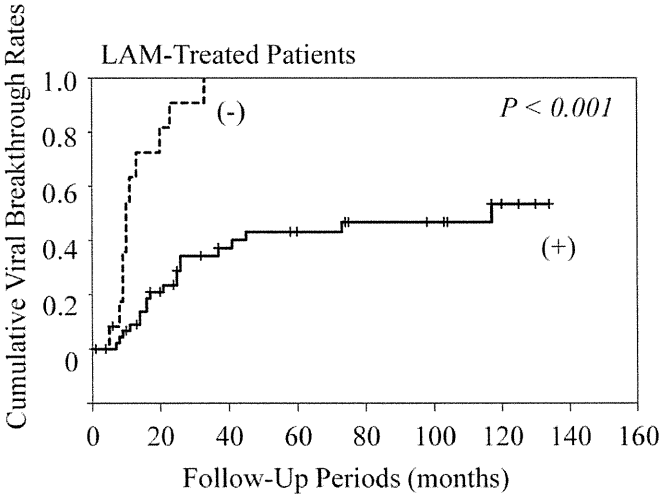
Resistance mutations

Resistance mutations were analyzed in some patients who experienced viral breakthrough. They were analyzed in 34 LAM-treated patients and 4 ETV-treated patients (Table 3). Thirty-two LAM-resistant patients had 10 YVDD, 17 YIDD, and 5 YV/IDD motifs, and 2 ETV-resistant patients had two YVDD motifs. Resistance mutations were not observed in 2 LAM-treated patients (5.8%) and 2 ETV-treated patients (50.0%) (*P=0.047*).

Table 3. Patients with viral breakthrough.

	ETV		LAM	
Adherence rate	≥90%	<90%	≥90%	<90%
Resistance mutation (+)	1	1	31	1
L180M	1	1	N.D.	N.D.
T184A	1	0	N.D.	N.D.
S202G	0	1	N.D.	N.D.
M204V	1	1	9	1
M204I	0	0	17	0
M204V/I	0	0	5	0
M250V	0	0	N.D.	N.D.
Resistance mutation (-)	1	1	0	2

ETV, entecavir; LAM, lamivudine; N.D., not determined. Numbers of amino acid positions were according to Refs. 2 and 10.



	HBV DNA Negativity	Baseline	12	24	36	48	60
Number of Patients	(-)	12	4	1	0	0	0
	(+)	47	39	30	23	19	18

Figure 4. Cumulative viral breakthrough rates in lamivudine (LAM)-treated patients who achieved HBV DNA negativity and those who did not. (-), maintaining HBV DNA positivity; (+), achieving HBV DNA negativity. HBV DNA negativity was unknown in 9 patients because of lack of data.

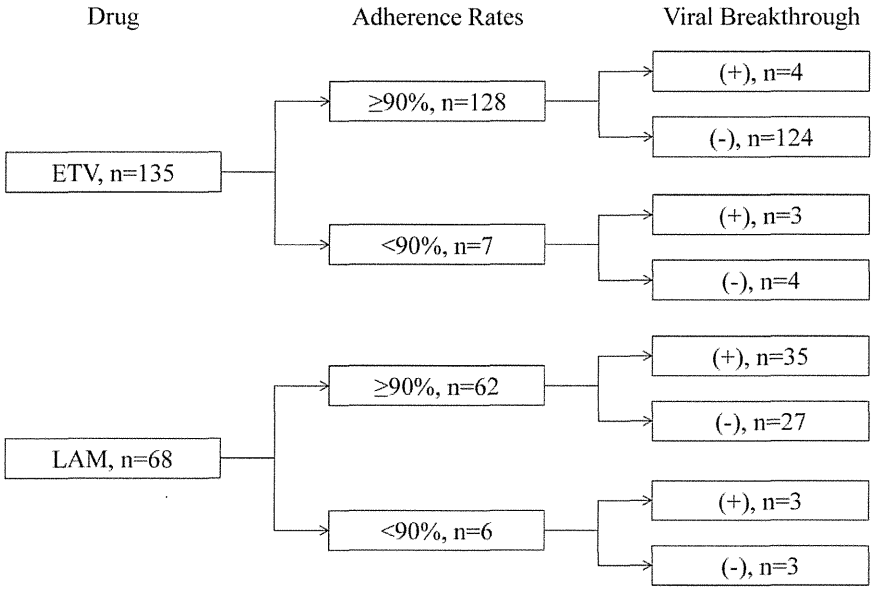


Figure 5. Association between adherence to medication and viral breakthrough.

DISCUSSION

The current study found that ETV-treated patients were not likely to acquire any resistance mutations and experience an ALT flare. Therefore, patients with poor liver residual function, such as liver cirrhosis, were likely to be administered ETV rather than LAM. Unexpectedly, HBsAg loss was observed in 3 of 28 LAM-treated patients without viral breakthrough (10.7%) and in 3 of 118 ETV-treated patients without viral breakthrough (2.5%). Long-term treatment with these drugs might result in HBsAg loss, although several reports have stated that one-year treatment with peg-interferon led to more HBsAg loss than these drugs [18-25].

In the current study, adherence to medication of most patients was excellent. The reasons for this might be as follows: (1) Our setting was a University Hospital, and this may have strengthened their will to succeeded with the treatment; (2) some patients with poor adherence might have been excluded because they did not see a doctor during the interview period; and (3) the rate of adherence to medication was based on patient self-assessment. A previous report showed that adherence might be underestimated by the Medication Event Monitoring System, a system that automatically records whenever a drug bottle is opened, and might be overestimated by pill counting and at interviews [26]. We classified the adherence rate as good at 90% or more, and as poor at less than 90%. However, we could not prove any significant influence of this classification on viral breakthrough as well as resistance mutation.

In the 13 patients with poor adherence (Table 2), we examined the reasons for their failure to take the pills. All 13 patients displayed some carelessness about taking pills. Two ETV-treated patients did not see a doctor and could not take pills continuously for a certain period of time, which particularly appeared to affect their viral breakthrough.

In LAM-treated patients, conversion of HBeAg to seronegative and achieving HBV DNA negativity was one of the important factors for successful treatment (Figures 3 & 4). In contrast, among ETV-treated patients, maintaining HBeAg seropositivity or HBV DNA positivity was not associated with viral breakthrough in the present study. Because of the stronger effect of ETV, it has been reported that long-term ETV treatment leads to a viral response in the vast majority of patients with detectable HBV DNA after 48 weeks [27]. Moreover, in the current study, poor adherence to medication was a major factor of viral breakthrough in the ETV-treated patients, but not in the LAM-treated patients. Ha et al. [9] also reported that medication non-adherence is likely to be a more important contributor to treatment failure than antiviral resistance, especially with new anti-HBV agents such as ETV and tenofovir. In LAM-treated or ETV-treated patients, viral breakthrough without resistance mutations might occur to some degree because of poor adherence to medication. In the present study, in LAM-treated patients, emergence of viral breakthrough with resistance mutations was common. Therefore, viral breakthrough due to poor adherence to LAM might not be important, compared with ETV-treated patients. However, in ETV-treated pa-

tients, viral breakthrough with resistance mutations was rare, and therefore, viral breakthrough due to poor adherence to ETV might be important.

In conclusion, viral breakthrough associated with poor adherence could be an important issue in the treatment with strong nucleoside analogues, such as ETV.

ABBREVIATIONS

ALT: alanine aminotransferase
ETV: entecavir
HBeAg: hepatitis B e antigen
HBsAg: hepatitis B surface antigen
HBV: hepatitis B virus
HCC: hepatocellular carcinoma
NA: nucleos(t)ide analogue
LAM: lamivudine

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Contributors

HK, TK, FI, and OY designed the study. HK, TK, MA, TC, HM, KF, FK, FI, FN and OY saw patients and conducted the interview. HK, TK, WS, and SN analyzed the data. HK and TK drafted the paper and all authors approved the paper.

COMPETING INTERESTS

Dr. Tatsuo Kanda reports receiving lecture fees from Chugai Pharmaceutical, MSD, and Ajinomoto, and Prof. Osamu Yokosuka reports receiving grant support from Chugai Pharmaceutical, Bayer, MSD, Daiichi-Sankyo, Mitsubishi Tanabe Pharma, and Bristol-Myers Squibb.

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Gadoxetic acid-enhanced MRI compared with CT during angiography in the diagnosis of hepatocellular carcinoma☆,☆☆

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ABSTRACT

Purpose: To assess the value of gadoxetic acid-enhanced magnetic resonance imaging (MRI) for the pre-therapeutic detection of hepatocellular carcinoma (HCC) using receiver operating characteristic (ROC) analysis with the combination of computed tomography (CT) arterial portography and CT hepatic arteriography (CTAP/CTHA).

Materials and Methods: A total of 54 consecutive patients with 87 nodular HCCs were retrospectively analyzed. All HCC nodules were confirmed pathologically. Three blinded readers independently reviewed 432 hepatic segments, including 78 segments with 87 HCCs. Each reader read two sets of images: Set 1, CTAP/CTHA; Set 2, gadoxetic acid-enhanced MRI including a gradient dual-echo sequence and diffusion-weighted imaging (DWI). The ROC method was used to analyze the results. The sensitivity, specificity, positive predictive value, negative predictive value and sensitivity according to tumor size were evaluated.

Results: For each reader, the area under the curve was significantly higher for Set 2 than for Set 1. The mean area under the curve was also significantly greater for Set 2 than for Set 1 (area under the curve, 0.98 vs. 0.93; $P=.0009$). The sensitivity was significantly higher for Set 2 than for Set 1 for all three readers ($P=.012$, .013 and .039, respectively). The difference in the specificity, positive predictive values and negative predictive values of the two modalities for each reader was not significant ($P>.05$).

Conclusion: Gadoxetic acid-enhanced MRI including a gradient dual-echo sequence and DWI is recommended for the pre-therapeutic evaluation of patients with HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver in adults. It is currently the fifth most common solid tumor worldwide and the third leading cause of cancer-related death. [1] Although patients with advanced HCC have a very poor prognosis, patients with early stage HCC can undergo curative treatments, including surgical resection, liver transplantation or percutaneous ablation techniques; they also have a better prognosis [2]. Therefore, the early diagnosis of HCC can improve the curative treatment outcome [3].

The combination of computed tomography (CT) arterial portography and CT hepatic arteriography (CTAP/CTHA) has improved the detection and characterization of liver tumors, and this method is generally considered to be the most sensitive for the pre-therapeutic assessment of HCC [4–7]. In addition, multiphase CTHA reduces the high false-positive rate for detection of malignant hepatic tumors [8,9]. Despite its high sensitivity, CTAP/CTHA is invasive and costly [6]. Therefore, CTAP/CTHA is not common for pre-therapeutic examination. Nevertheless, there are few reports claiming that the sensitivity of other modalities was superior to that of CTAP/CTHA. Although there are problems, such as cost and invasiveness, until recently it was thought that CTAP with multiphase CTHA was one of the most sensitive modalities for detecting HCC [5,7].

Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (gadoxetic acid disodium, EOB-Primovist; Bayer Schering Pharma, Osaka, Japan) is a recently developed hepatocyte-specific magnetic resonance contrast agent that is excreted equally in the urinary and

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biliary systems [10]. Gadoxetic acid is taken up by hepatocytes, thereby increasing liver parenchymal contrast enhancement. Several studies have suggested that hepatobiliary-phase images improve the detection of HCC compared with dynamic studies alone [11,12] and that gadoxetic acid-enhanced dynamic magnetic resonance imaging (MRI) is similar or superior to multiphasic multidetector CT (MDCT) in the detection of HCC [13–19].

Furthermore, MRI can obtain several sequences, including gradient dual-echo sequences, T2-weighted imaging and diffusion-weighted imaging (DWI) in one examination. There is also evidence that using a gradient dual-echo sequence and DWI improves the detection accuracy for small HCCs [20–22]. These advances in MRI might make it the most sensitive modality for the pre-therapeutic assessment of HCC. Especially, gadoxetic acid-enhanced MRI including a gradient dual-echo sequence and DWI might have greater accuracy, although only one report has compared the diagnostic performance of gadoxetic acid-enhanced MRI and CTAP/multiphase CTHA for detecting HCC to our knowledge [23]. The purpose of this study was to compare the diagnostic performance of gadoxetic acid-enhanced MRI including a gradient dual-echo sequence and DWI with CTAP/multiphase CTHA for detecting HCC.

Table 1
Patient characteristics.

	$n=54$
Age, years	68.8 ± 10.5
Gender, *n* (%)	
Male	40 (74.1)
Female	14 (25.9)
Cause of disease, *n* (%)	
Hepatitis C virus infection	35 (64.8)
Hepatitis B virus infection	11 (20.3)
Hepatitis B and C virus infection	1 (1.8)
Alcoholic hepatitis	4 (7.4)
Autoimmune hepatitis	1 (1.8)
Unknown	2 (3.7)
Biochemical analysis	
Total bilirubin, mg/dl	1.1 ± 0.4
Serum albumin, g/dl	3.7 ± 0.5
Prothrombin time, INR*	1.20 ± 0.12
ICG R_{15}†, %	23.7 ± 13.9
Child-Pugh class, *n* (%)	
A	46 (85.2)
B	8 (14.8)

Values are means ± S.D. unless otherwise specified.
* INR: International normalized ratio.
† ICG R_{15}: Retention rate at 15 min of indocyanine green clearance test.

2. Materials and methods

This retrospective study was approved by our institutional review board and followed the principles of the Declaration of Helsinki and subsequent amendments. Before performing gadoxetic acid-enhanced MRI and CTAP/CTHA, the purpose of the diagnostic imaging was explained to each patient and written informed consent was obtained.

2.1. Patient population

From February 2008 to June 2009, a total of 80 consecutive patients were suspected of having HCC for the first time on the basis of clinical and/or prior sonographic findings and/or dynamic CT and/or gadoxetic acid-enhanced MRI at our institution. Twenty of these patients were considered to have no indication for both resection and local ablation therapy because they had multinodular HCCs, obvious vascular invasion or poor liver function (18 patients underwent transarterial chemoembolization and 1 patient received best supportive care). In the remaining 60 patients who were considered to have an indication for resection or local ablation therapy, CTAP/CTHA was performed for preoperative staging. All of these 60 patients underwent gadoxetic acid-enhanced MRI within 4 weeks before CTAP/CTHA. These examinations were performed as common preoperative practices in patients who had HCC for the first time in our institution. Of these 60 patients, 6 were excluded: 3 had cholangiocellular carcinoma and 3 had no pathological proof of the diagnosis. Thus, 54 consecutive patients were included in this study.

The diagnosis of HCC was proven pathologically. All 54 patients were followed up with dynamic CT or gadoxetic acid-enhanced MRI for 13–42 (mean 28.7) months. Their characteristics are summarized in Table 1. Nine patients were treated with partial surgical resection, and the remaining 45 patients underwent percutaneous radiofrequency ablation. The hepatic function, evaluated according to the Child–Pugh classification, revealed that 46 patients had class A disease and 8 had class B disease.

2.2. Standard of reference

The 54 evaluated patients had a total of 87 HCC nodules, and all of these nodules were confirmed pathologically by surgical resection ($n=13$) or needle biopsy ($n=74$). A total of 41 were well differentiated, 40 were moderately differentiated and 5 were poorly differentiated. The mean number of HCC nodules per patient was 1.59 (range 1–5), with an average nodule size of 18.4 ± 10.8 (range 3–65) mm.

Consensus opinion of the study coordinator and one hepatologist who did not take part in the blind reading determined the final number of HCCs in the remaining part of the liver. All nodules and pseudolesions identified at initial gadoxetic acid-enhanced MRI or CTAP/CTHA were correlated with the findings at follow-up dynamic CT or gadoxetic acid-enhanced MRI by means of lesion-to-lesion analysis. We assumed that there was no HCC when there was no discernible lesion or no change in the size and character of the nodule on dynamic CT or gadoxetic acid-enhanced MRI during the follow-up period. If a growing nodule was identified at dynamic CT or gadoxetic acid-enhanced MRI during the follow-up period, biopsy was subsequently performed. The following were not included in the final number of HCCs: recurrent masses at the time of resection or local ablation margin where no lesion was seen at pre-therapeutic imaging.

2.3. Magnetic resonance imaging

Patients underwent MRI on a 1.5-T scanner (Intera Achieva 1.5 T Nova Dual; Philips Healthcare) using a six-channel phased-array coil as the receiver coil. Before injecting the contrast agent, we obtained T1-weighted dual fast field echo images (TR/TE, 183/2.3/4.6; flip angle, 75°; matrix size, 256×204; field of view, 36×36 cm; section thickness, 6 mm; slice gap, 0 mm). Dynamic fat-suppressed T1-weighted 3D turbo field-echo images (TR/TE, 4/2.1; flip angle, 10°; matrix size, 256×211; field of view, 36×36 cm; section thickness, 7 mm; slice gap, −3.5 mm) were obtained before and 23 s, 80 s, 240 s and 15 min after the administration of contrast agent. Breath-hold multi-shot T2-weighted images (TR/TE, 2000/100; flip angle, 90°; matrix size, 256×198; field of view, 36×36 cm; section thickness, 7 mm; slice gap, 0 mm) and single-shot spin-echo echo-planar diffusion-weighted images (TR/TE, 1000/66.2; flip angle, 90°; matrix size, 128×101; field of view, 36×36 cm; section thickness, 6 mm; slice gap, 0 mm; *b* value, 1000 s/mm^2) were obtained in the interval of the dynamic study. The dose of gadoxetic acid (EOB Primovist; Bayer HealthCare, Osaka, Japan) was 0.025 mmol/kg body weight. The contrast agent was administered at a rate of 2 ml/s through the cubital vein, which was flushed with 20 ml of saline using a power injector. MRI parameters are shown in Table 2.

Table 2
MRI Parameters.

	T1-weighted imaging	T2-weighted imaging	Diffusion-weighted imaging	Contrast-enhanced imaging
Magnetic resonance sequence	Dual fast field echo	Spin echo	Spin-echo echo-planar	Three-dimensional turbo field echo
Fat suppression	No	Yes	Yes	Yes
Respiratory triggered	No	No	Yes	No
Repetition time (ms)	183	2000	1000	4
Effective echo time	2.3/4.6	100	66.2	2.1
Flip angle (deg)	75	90	90	10
Number of excitations	1	1	4	1
Matrix size	256×204	256×198	128×101	256×211
Section thickness (mm)	6	7	6	7
Slice gap (mm)	0	0	0	-3.5
Field of view (cm)	36×36	36×36	36×36	36×36
Other			$b=1000$ s/mm^2	Scan delay after administration of gadoxetic acid: 23, 80, and 240 s and 15 min

2.4. CT Arterial portography/CT hepatic arteriography

CTAP/CTHA was performed using an IVR-CT system (Infinix Active; Toshiba Medical Systems) comprising a digital subtraction angiography system (CAS-8000 V/DFP-2000A; Toshiba Medical Systems) and a four-detector MDCT scanner (Aquilion; Toshiba Medical Systems).

The right or left femoral artery was punctured using the Seldinger technique, and a 4-Fr angiographic catheter (Shepherd-hook or Cobra type; Hanako Medical, Tokyo, Japan) was positioned in the proximal superior mesenteric artery (SMA). If the patients had a replacement of the hepatic artery from SMA, we placed the catheter tip in the distal SMA over the junction of replacement using a 2.7-Fr microcatheter system (Progreat; Terumo, Tokyo, Japan). Then, 10 μmol of alprostadil (Palux; Taisho, Tokyo, Japan) was injected into the SMA to increase the portal blood flow immediately before the injection of iohexol (Omnipaque 300; Daiichi Pharmaceutical, Tokyo, Japan). Finally, 30 ml of iohexol was injected, at a rate of 3.0 ml/s, for CTAP.

After CTAP was performed, we placed the catheter tip in the common or proper hepatic artery using the 4-Fr catheter of a coaxial 2.7-Fr microcatheter system. Then, 18 ml of iohexol was injected, at a rate of 2 ml/s, for CTHA. If the patient had replacement of the hepatic arteries (e.g., replacement of the left hepatic artery with the left gastric artery, replacement of the right hepatic artery with the SMA, etc.), we placed the catheter tip in each hepatic artery in turn and repeated CTHA.

The CTAP and CTHA parameters were as follows: 120 kVp, 250 to 280 mA, 2-mm detector collimation, pitch 0.875, reconstruction interval 5.0 mm and a 0.5-s gantry rotation time during a single breath-hold helical acquisition of 8 to 12 s, depending on the liver size. Two- and three-phase imaging were performed for CTAP and CTHA, respectively. The scan delay times after starting the injection were 20 and 35 s for CTAP and 3, 18 and 60 s for CTHA. The CT parameters are also shown in Table 3.

2.5. Image analysis

Two radiologists and one hepatologist with at least 7 years of experience in interpreting magnetic resonance images and CT during angiography images of the liver participated as blinded readers. They reviewed two sets of images (Set 1, CTAP/CTHA; Set 2, gadoxetic acid-enhanced MRI including a gradient dual-echo sequence, T2-weighted imaging, gadoxetic acid-enhanced dynamic study, hepatobiliary phase and DWI) separately, independently, blindly and randomly.

Table 3
CT Parameters.

Parameter	CTAP	CTHA
Tube current (mA)	250–280	250–280
Tube voltage (kVp)	120	120
Rotation time (s)	0.5	0.5
Detector collimation (mm)	2	2
Helical pitch	0.875	0.875
Reconstruction interval (mm)	5	5
Iodine concentration (mg/ml)	300	300
Total amount (ml)	30	18
Injection rate (ml/s)	3	2
Injection artery	Superior mesenteric artery	Common or proper hepatic artery
Scan delay time after start of injection (s)	20, 35	3, 18, 60

To minimize recall bias, the interval between reviews of the two sets was at least 4 weeks. All images were read on an open-source PACS workstation DICOM viewer (OsiriX 64-bit, ver. 3.7.1). For blinded readings, the patient's name, gender, age and all hospital record numbers were removed from the images. Readers knew that the patients were referred for pre-therapeutic assessment of suspected liver malignancy, but did not know anything else about the patients or their final diagnoses.

The image review was conducted on a segment-by-segment basis because the objective was to assess the ability of the readers to identify lesions. To prevent incorrect localization of the lesions by the readers, readers recorded the tumor size and hepatic segmentation according to Couinaud's numbering system. If a lesion was located in two or more segments, the readers described only the segment that contained the greatest part of the lesion. Each reader independently scored each image for the presence of HCC lesions and assigned confidence levels to the observations (1 = definitely negative, 2 = probably negative, 3 = possibly positive, 4 = probably positive, 5 = definitely positive).

For objectivity and reproducibility, the criteria for diagnosing HCC were provided to the readers before the reading session. The following criteria were used. On gadoxetic acid-enhanced MRI, HCC was defined as lesions showing peaks of contrast enhancement in the arterial phase and hypointensity in the portal, delayed or hepatobiliary phases with or without capsular enhancement. In addition, suggestive but inconclusive findings of HCC included (a) mild hyperintensity on T2-weighted MRI, (b) early nodular enhancement without washout, (c) hypointensity in the hepatobiliary phase without early enhancement in the arterial phase, (d) signal loss of the tumor on opposed-phase images compared with in-phase images and (e) a high signal on DWI. Based on the presence of these suggestive findings, the confidence level was adjusted up or down. On CTAP and CTHA, HCC was defined as hypoattenuating lesions on CTAP, hyperattenuating lesions on CTHA and ring-enhancement in the second or third phase of CTHA regardless of tumor size. Suggestive, but inconclusive, findings of HCC on CTAP/CTHA included (f) hypoattenuation on CTAP without hyperattenuation on CTHA. These criteria were given to the readers, but the final diagnostic decision was left up to each reader. Fig. 1 shows the typical appearance of HCC on CTAP/CTHA and on gadoxetic acid-enhanced MRI.

After the image review, all true-positive lesions, all false-positive lesions (confidence level of 3 or more that was *not* confirmed as HCC at imaging follow-up or pathological examination) and all false-negative lesions (confidence level of 1 or 2 that *was* pathologically confirmed as HCC) were retrospectively assessed by the study coordinator and the three readers.

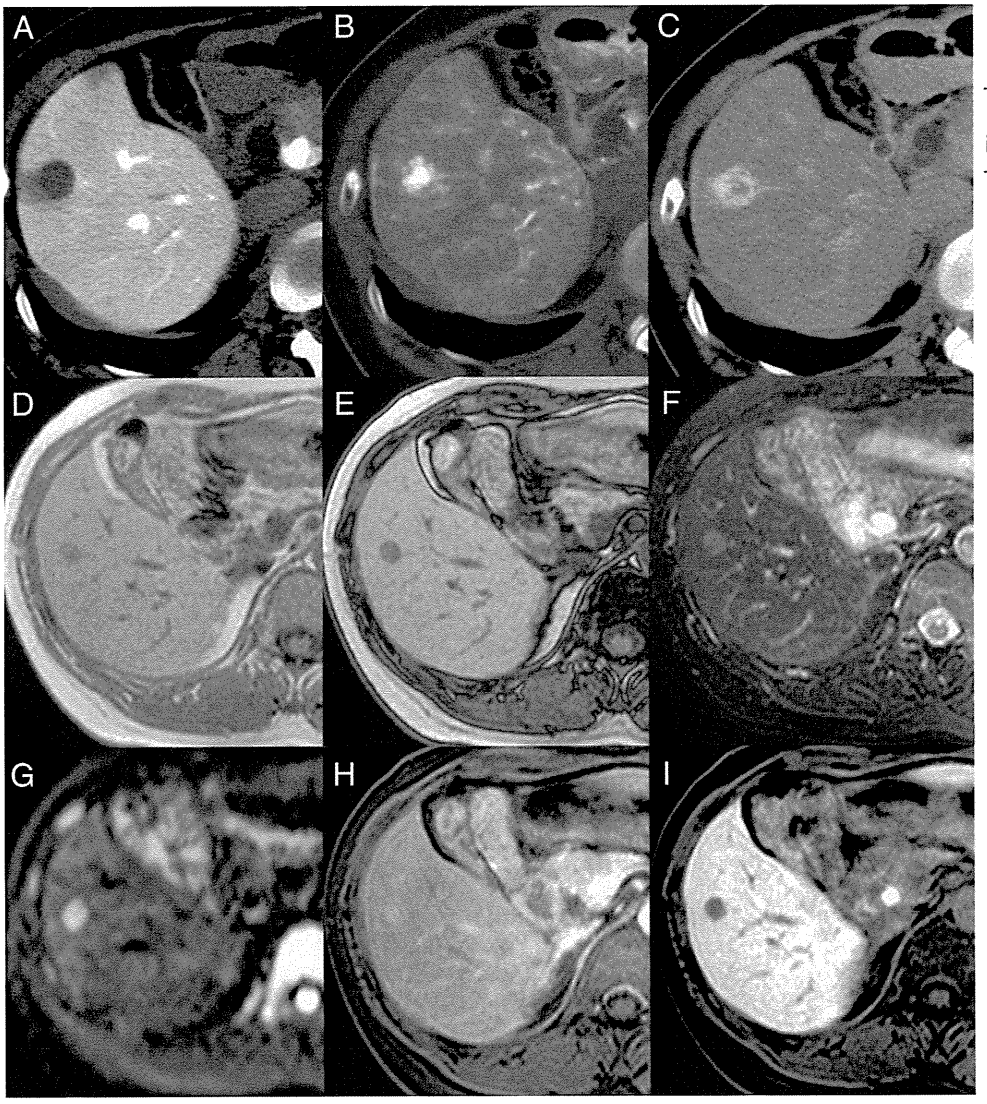

Fig. 1. Typical HCC findings on CTAP/CTHA and on gadoxetic acid-enhanced MRI. These images were from a 48-year-old female with hepatitis B virus infection with typical HCC, which was proven in the resected specimen. The figure shows S5 classical HCC. This HCC nodule was hypoattenuating on CTAP (A), hyperattenuating on CTHA (B, C) and showed corona-like enhancement on second-phase CTHA (C). On gadoxetic acid-enhanced MRI, it showed low intensity on T1WI (D), signal loss of the tumor on opposed-phase images compared with in-phase images (D, E), slightly increased intensity on T2WI (F), very high signal on DWI (G), early enhancement in the early phase (H) and low intensity in the hepatobiliary phase (I).

2.6. Statistical analysis

To evaluate the inter-reader variability in interpreting images, kappa statistics were used to measure the degree of agreement between readers. A kappa value of >0 was considered to indicate a positive correlation. Values up to 0.4 were considered to indicate positive, but poor, correlation; values of 0.41 to 0.75, good correlation; and values of >0.75, excellent correlation.

A receiver operating characteristic (ROC) analysis using statistical software (DBM MRMC 2.2; University of Iowa, Iowa City, IA, USA) was used to determine the accuracy of each evaluation [24,25]. The area under the ROC curve was used to indicate the overall diagnostic performance of each modality and each reader. The sensitivity and positive predictive values for lesion detection with each modality for each reader were calculated for those lesions that were assigned a score of 3 or more.

The relative sensitivities and specificities of each modality were compared using the McNemar test. All statistical analyses were performed using IBM SPSS Statistics 18 (SPSS Japan).

3. Results

3.1. Inter-reader agreement

Excellent ($\kappa = 0.81$–0.88) reader agreement for the detection of HCC nodules was obtained with each technique among the three readers with regard to the presence of lesions (Table 4).

Table 4
Agreement between readers regarding the presence or absence of HCC.

Imaging modality	Readers		
	1 and 2	2 and 3	1 and 3
CTAP/CTHA	0.817	0.817	0.854
Gadoxetic acid-enhanced MRI	0.879	0.881	0.856

Data are expressed as kappa values, which indicate the degree of agreement between readers regarding the presence (conspicuity score of 3, 4 or 5) or absence (conspicuity score of 1 or 2) of lesions.

Table 5
Area under ROC curve values for detection of HCC.

Imaging modality	Reader 1	Reader 2	Reader 3	Mean
CTAP/CTHA	0.94 (0.91–0.97)*	0.92 (0.89–0.96)†	0.93 (0.90–0.96)‡	0.93 (0.90–0.96)§
Gadoxetic acid-enhanced MRI	0.98 (0.96–1.00)*	0.99 (0.98–0.99)†	0.97 (0.94–1.00) ‡	0.98 (0.96–0.99)§

Values in parentheses are the 95% confidence intervals.
The differences in all area under the ROC curve values (each reader and mean) between the two imaging modalities are statistically significant (*P=.0107, †P=.0011, ‡P=.0258, §P=.0009).

3.2. ROC Analysis

Table 5 shows the area under the curve values for the detection of HCC for each modality and each reader. For each reader, the area under the curve was significantly higher for the gadoxetic acid-enhanced MRI set than for the CTAP/CTHA set. In addition, the mean area under the curve was significantly higher for the gadoxetic acid-enhanced MRI set than for the CTAP/CTHA set (area under the curve, 0.98 vs. 0.93; P=.0009).

3.3. Sensitivity

Table 6 shows the sensitivity in terms of HCC detection of each modality, each reader and each tumor size. All HCCs larger than 20 mm were detected with both modalities by all readers. For lesions ≤20 mm, the sensitivity was significantly higher for the gadoxetic acid-enhanced MRI set than for the CTAP/CTHA set for all readers.

3.4. False-negative findings

Only one HCC was not identified by any reader on any image set. Excluding that one, nine HCCs were not detected by any reader on the CTAP/CTHA set, but all of them were identified by at least one reader on the gadoxetic acid-enhanced MRI set (Figs. 2 and 3). There was no HCC that was not detected by any reader on the gadoxetic acid-enhanced MRI set but that was detected on the CTAP/CTHA set.

3.5. False-positive findings and positive predictive value

A total of 39 lesions were judged as false-positive lesions by at least one reader on at least one modality: 13 lesions were noted only on the gadoxetic acid-enhanced MRI set, 18 lesions were noted only on the CTAP/CTHA set and the remaining 8 were noted on both modalities. The positive predictive values of the gadoxetic acid-enhanced MRI set and combined CTAP/CTHA analysis were similar and did not differ significantly (Table 6).

3.6. Specificity and negative predictive value

Table 6 also shows the specificities and negative predictive values of each modality. Both specificities and negative predictive values were high and similar in both modalities, and did not differ significantly.

4. Discussion

Although CTAP/CTHA has several problems, such as cost and invasiveness, it was until recently thought to be one of the most sensitive modalities for HCC detection, especially small tumors [4–7]. Recent advances in other modalities have significantly improved their diagnostic performance, apparently changing the modality that is thought to be the most sensitive and accurate for preoperative evaluation of HCC.

The sensitivity of gadoxetic acid-enhanced MRI for detecting HCC was recently reported to be higher than that of multiphase MDCT [13–19]. In particular, hepatobiliary-phase images improve the diagnostic performance of small HCC compared with dynamic-phase images alone [12,26]. Another advantage is that MRI can provide several sequences in a single examination. The dual fast field-echo sequence improves the detection of small, well-differentiated HCCs because of the detection of small amounts of fat [20], while DWI improves the detection of HCC because it distinguishes it from hypervascular pseudo-lesions [21,22]. Furthermore, gadoxetic acid-enhanced MRI does not require hospitalization and is less invasive than CTAP/CTHA.

To our knowledge, only one report has compared the diagnostic performance of gadoxetic acid-enhanced MRI and CTAP/CTHA for HCC detection. This report showed a marked difference in the diagnostic performance for small HCCs (≤2 cm) between gadoxetic acid-enhanced MRI and CTAP/CTHA using all resected specimens [23]. However, using resected specimens only, we could not assess patients who did not undergo surgical treatment because of their poor liver function; this study included many such patients. In addition, we assessed not only the resected specimens, but also the entire liver. To reduce the weakness in the lack of pathological proof for negative lesions, we used a long follow-up period (mean, 28 months). Another difference is the number of MRI sequences. In our study, a gradient dual-echo sequence and DWI were also used because we use these sequences in clinical practice. These sequences seem to improve not only the sensitivity, but also the confidence level of diagnosis.

Table 6
Sensitivity, specificity, positive predictive values and negative predictive values in the detection of HCC.

		Reader 1		Reader 2		Reader 3	
		CTAP/CTHA	Gadoxetic acid-enhanced MRI	CTAP/CTHA	Gadoxetic acid-enhanced MRI	CTAP/CTHA	Gadoxetic acid-enhanced MRI
Sensitivity	Total (n=87)	85.0 (74/87)	95.4 (83/87)*	82.7 (72/87)	94.2 (82/87)†	83.9 (73/87)	91.9 (80/87)‡
	≤20 mm (n=59)	77.9 (46/59)	93.2 (55/59)§	74.5 (44/59)	91.5 (54/59)¶	76.2 (45/59)	88.1 (52/59)**
	>20 mm (n=28)	100 (28/28)	100 (28/28)	100 (28/28)	100 (28/28)	100 (28/28)	100 (28/28)
Specificity		96.8 (343/354)	95.7 (339/354)	96.6 (342/354)	97.1 (344/354)	96.6 (342/354)	98.0 (347/354)
Positive predictive values		87.0 (74/85)	84.6 (83/98)	85.7 (72/84)	89.1 (82/92)	85.8 (73/85)	91.9 (80/87)
Negative predictive values		96.3 (343/356)	98.8 (339/343)	95.7 (342/357)	98.5 (344/349)	96.0 (342/356)	98.2 (347/354)

Values in parentheses are the numbers used to calculate the percentages.
The differences in the sensitivity of all HCCs and ≤20-mm HCCs between the two imaging modalities for each reader are statistically significant (*P=.012, †P=.013, ‡P=.039, §P=.012, ¶P=.013, **P=.039). The differences in specificity, positive predictive values and negative predictive values between the two imaging modalities for each reader were not statistically significant (P>.05).