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Title	The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma
Author(s)	Chuma, Makoto; Hige, Shuhei; Kamiyama, Toshiya et al.
Citation	Journal of Gastroenterology, 44(9), 991-999 https://doi.org/10.1007/s00535-009-0093-z
Issue Date	2009-09
Doc URL	https://hdl.handle.net/2115/39478
Rights	The original publication is available at www.springerlink.com
Type	journal article
File Information	JG44-9_p991-999.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma

Makoto Chuma[1], Shuhei Hige[1], Toshiya Kamiyama[2], Takashi Meguro[3], Atsushi Nagasaka[4], Kazuaki Nakanishi[2], Yoshiya Yamamoto[5], Mitsuru Nakanishi[1], Toshihisa Kohara[1], Takuya Sho[1], Keiko Yamamoto[1], Hiromasa Horimoto[1], Tomoe Kobayashi[1], Hideki Yokoo[2], Michiaki Matsushita[2], Satoru Todo[2], and Masahiro Asaka[1].

Departments of [1]Gastroenterology and Hematology and [2]General Surgery, Hokkaido University, Sapporo, Japan

[3]*Department of Internal Medicine, Hokkaido Gastroenterology Hospital, Sapporo, Japan*

[4]*Department of Gastroenterology, Sapporo Municipal Hospital, Sapporo, Japan*

[5]*Department of Gastroenterology, Hakodate Municipal Hospital, Hakodate, Japan*

Running head: Hepatocellular carcinoma recurrence

Footnote

Address reprint requests to: Makoto Chuma, M.D., Ph.D.; Department of Gastroenterology and Hematology, Hokkaido University, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan; TEL: (81)11-716-1611; FAX: (81)11-706-7867; E-mail: mchuuma@med.hokudai.ac.jp

ABSTRACT

Background. Prediction and prevention of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) recurrence is an important clinical issue. We investigated whether HBV DNA level and antiviral therapy are associated with HCC recurrence. ***Methods.*** This retrospective study involved 103 patients who underwent hepatic resection or radiofrequency ablation for initial HCC. Patients were divided into four groups. Thirty had high serum HBV DNA levels (>4 $\log_{10}$ copies/mL) and had not received antiviral therapy (high virus group; HVG). Thirty-four had low HBV DNA levels (≤4 $\log_{10}$ copies/mL) and had not received antiviral therapy (low virus group; LVG). Twenty received antiviral therapy after HCC developed (therapeutic group A, TG-A). Nineteen received antiviral therapy before HCC developed (therapeutic group B, TG-B). ***Results.*** Cumulative HCC recurrence rates at 3 years in the HVG, LVG, TG-B, and TG-A were 71.1%, 42.2%, 42.3%, and 52.0%, respectively. Recurrence rates differed significantly between the HVG and LVG ($P = 0.016$), and between the HVG and TG-B ($P = 0.008$). Recurrence rate in the TG-A was marginally lower than in the HVG ($P = 0.10$). On multivariate analysis, high serum hepatitis B virus DNA levels (hazard ratio: HR, 2.67; 95% CI, 1.31 - 5.47; $P = 0.007$) and absence of antiviral therapy (HR, 2.57; 95% CI, 1.34-4.94; $P = 0.005$) were independent risk factors for

hepatocellular carcinoma recurrence. ***Conclusion.*** HBV DNA level and antiviral therapy are associated with HCC recurrence. For patients with high HBV DNA levels, antiviral therapy before the development of HCC is important for prevention of recurrence.

Key words: hepatocellular carcinoma, hepatitis B virus, recurrence, antiviral therapy

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third leading cause of death from cancer worldwide. [1, 2] Hepatic resection or transplantation can provides a complete cure, [3, 4] and radiofrequency ablation (RFA) is also recognized as a curative treatment option. [5, 6] Recent remarkable advances in curative treatment have improved the prognosis of patients with HCC, but these techniques remain unsatisfactory because of a high post-treatment recurrence rate. [7, 8] Previous studies have noted that factors contributing to recurrence include: tumor size, number, and differentiation; vascular invasion; levels of alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist II (PIVKA-II); gender; alcohol consumption; hepatitis C virus (HCV) infection; hepatic reserve; and degree of liver fibrosis. [8-12]

Only a few recent studies have evaluated hepatitis B virus (HBV) replication status as a predictor of HCC recurrence, [13-15] and interpretation of their results was complicated by use of antiviral therapy. Since HBV DNA level is reduced by antiviral agents, HBV DNA level at the time of HCC treatment differs significantly between patients who have received antiviral therapy and those who have not. To determine whether HBV DNA level at the time of HCC treatment is associated with HCC recurrence, it is therefore

necessary to exclude patients who have received antiviral therapy after the development of HCC.

Furthermore, the efficacy of antiviral therapy in reducing the risk of HBV-related HCC recurrence is far from clear. Three anti-HBV agents are predominantly used in Japan. Lamivudine is a nucleotide analog that inhibits reverse transcriptase, ameliorates hepatitis, and improves histologic findings in the liver during long-term treatment by inhibiting the replication of HBV. [16, 17] Furthermore, lamivudine is considered to slow the progression of severe liver disease to cirrhosis as well as to HCC. [17-19] Adefovir dipivoxil and entecavir are potent inhibitors of HBV DNA polymerase which have been shown to be safe and effective for the treatment of patients with chronic hepatitis B infection (CHB) that does not respond to lamivudine. [20, 21] With regard to lowering the risk of HCC recurrence, the literature contains only one report for lamivudine [22] and none for adefovir dipivoxil or entecavir.

In this study, by strict classification of patients into groups, comparison of cumulative HCC recurrence rates between the groups, and multivariate analysis, we aimed 1) to clarify the influence of HBV DNA level in the absence of antiviral therapy on recurrence of HCC, and 2) to clarify the influence of antiviral therapy on the risk of HCC recurrence.

Patients and methods

Patients

Between January 2001 and December 2007, a total of 196 patients who were diagnosed with HBV-related HCC at our liver unit underwent hepatic resection or RFA as a primary treatment. HCC was diagnosed based on the American Association for the Study of Liver Disease (AASLD) guidelines.[23] All patients were positive for serum hepatitis B surface antigen (HBsAg) for at least 6 months before their diagnosis of HCC and were negative for antibodies to hepatitis C and human immunodeficiency virus. In this retrospective study, of the 196 patients who were assessed initially, 103 fulfilled the following criteria and were enrolled (hepatic resection; 52 patients, RFA; 51 patients). Inclusion criteria were: (i) hepatic resection or RFA for initial HCC treatment; (ii) three or fewer lesions, each 3 cm or less in diameter; (iii) no extra-hepatic metastasis or vascular invasion; (iv) curative treatment and no local recurrence after treatment; (v) no recurrence 3 months after treatment; (vi) liver function of Child-Pugh class A or B; (vii) no excessive alcohol intake (>65 g/day); and (viii) no evidence of any other active neoplastic site**.**

Since HBV DNA level reduced to ≤4 $\log_{10}$ in all cases after administration of antiviral agents (lamivudine, adefovir dipivoxil, or entecavir), the patients were divided into four groups (Figure 1).

(1) Thirty patients had consistently high serum HBV DNA levels (>4 $\log_{10}$ copies/mL) during serial examinations from the time of HCC diagnosis to recurrence (designated high virus group; HVG). These patients did not receive antivirals.

(2) Thirty-four patients had consistently low serum HBV DNA levels (≤4 $\log_{10}$ copies/mL) during the serial follow-up (low virus group; LVG). These patients did not receive antivirals.

(3) Twenty patients had high serum HBV DNA levels (>4 $\log_{10}$ copies/mL) when HCC was diagnosed and received antiviral therapy after the development of HCC (therapeutic group-A, TG-A). Seventeen patients received antiviral therapy within 1 month after HCC treatment. The remaining three patients received antiviral therapy from diagnosis of active viral hepatitis B; the intervals between HCC treatment and the commencement of nucleotide analogue in these three patients were 12, 15, and 22 months.

(4) Nineteen patients received antiviral therapy before the development of HCC (therapeutic group-B, TG-B). In these patients, HBV DNA level was high (>4 $\log_{10}$

copies/mL) at commencement of the antiviral agents but low (≤4 $\log_{10}$ copies/mL) at HCC diagnosis.

As shown in Figure 1, first, to determine whether HBV DNA level was associated with HCC recurrence, we selected the patients who had not received antiviral therapy (HVG plus LVG) and then compared cumulative HCC recurrence rates between the HVG and LVG. We then performed univariate and multivariate analysis of the hazard ratios for the recurrence of HCC in these patients. Second, to determine whether antiviral therapy was associated with lower risk of HCC recurrence, we selected the patients who had high serum HBV DNA levels when they had not received antiviral therapy (HVG plus TG-A plus TG-B) and then compared cumulative HCC recurrence rates between the HVG and TG-A and between the HVG and TG-B. We then performed univariate and multivariate analysis of the risk factors for recurrence of HCC in these patients.

Initial work-up and follow-up

The initial evaluation included a complete medical history and physical examination, focusing on the symptoms and signs often associated with HCC or chronic liver disease. All patients were tested at baseline for HBsAg, antibody to HBsAg, hepatitis B e

antigen (HBeAg), antibody to HBeAg (anti-HBe), serum levels of alanine aminotransferase (ALT), albumin, bilirubin, AFP, and PIVKA-II, prothrombin time (PT), and complete blood cell counts. HBV DNA was quantified by polymerase chain reaction (PCR) assay (Amplicor HBV monitor assay, Roche Diagnostics, Manheim, Germany). The lower limit of detection of the assay was 2.6 log copies/mL.

During follow-up, clinical evaluations and biochemical tests were performed every 1 - 3 months. Patients underwent triphasic computed tomography of the liver every 3 months. The endpoint used in this study was the recurrence of HCC.

Antiviral therapy

In the TG-A, seven patients received lamivudine only (100 mg/day). Entecavir alone (0.5 mg/day) was used in five patients. Adefovir dipivoxil (10 mg/day) was used together with lamivudine to suppress lamivudine-resistant hepatitis B virus (HBV) in eight patients. In the TG-B, five patients received lamivudine only (100 mg/day). Entecavir (0.5 mg/day) was used in six patients; four of these patients were switched from lamivudine to entecavir. Adefovir dipivoxil (10 mg/day) was used together with lamivudine in eight patients.

Statistical analysis

Cumulative HCC recurrence rate was calculated by the Kaplan-Meier method and differences were compared by the log-rank test.

Univariate and multivariate analysis of the risk ratios for the recurrence of HCC were performed using Cox's proportional hazards regression analysis. The risk factors examined included gender, age, HBeAg status, ALT (>35 IU/L vs. ≤35 IU/L), platelet count (>120 x 10^3 /μL vs. ≤120 x 10^3 /μL), PT (>70% vs. ≤70%), albumin (>3.5 mg/dL vs. ≤3.5 mg/dL), bilirubin (>1.2mg/dL vs. ≤1.2mg/dL), liver fibrosis (cirrhosis vs. no cirrhosis), tumor differentiation (well and moderately differentiated vs. poorly differentiated), AFP (>20 ng/mL vs. ≤20 ng/mL), PIVKA-II (>40 mAU/mL vs. ≤40 mAU/mL), tumor size (>2 cm vs. ≤2 cm), tumor number (single vs. multiple), and initial treatment (hepatic resection vs. RFA). HBV DNA level (>4 $\log_{10}$ copies/ml vs. ≤4 $\log_{10}$ copies/ml) was added to these factors when analyzing the influence of HBV DNA level, and antiviral therapy (received vs. not received) was added when analyzing the influence of antiviral therapy. Differences between the two groups were analyzed using the log-rank test. All P values were two-tailed, and those <0.05 were considered

significant. Statistical analysis was performed using Stat View software (version 5.0; SAS Institute Inc., Cary, NC, USA).

Results

Baseline clinical characteristics

Baseline characteristics at the time of initial HCC treatment for the four groups are summarized in Table 1. The mean follow-up period for all patients was 40 (12 to 92) months. There were no significant differences among the four groups with regard to gender; age; HBeAg; levels of ALT, PT, albumin, PIVKA-II, AFP, or bilirubin; platelet count; Child-Pugh score; tumor size; tumor number; stage of HCC; initial HCC treatment; or follow-up period. However, there was a significant difference with respect to HBV DNA level among the four groups. Median HBV DNA levels in the HVG (5.9 log copies/mL, range 4.1 - 7.6) and TG-A (6.0 log copies/mL, range 4.1 - 8.1) were significantly higher than those in the LVG (< 2.6 log copies/mL, range < 2.6 - 3.6) and

TG-B (< 2.6 log copies/mL, range < 2.6 - 4.0) (P = 0.005).

Overall recurrence rate

Overall, 56 (54.4%) of 103 patients had a recurrence of HCC, with the mean period until recurrence from initial treatment being 34.7 ± 22.7 months (range 7.0 – 67.0). The estimated recurrence rates at 1 and 3 years after curative treatment were 16.5% and 53.0%, respectively (Fig. 2).

Comparison of cumulative HCC recurrence rates between the HVG and LVG

To clarify the influence of HBV DNA level on HCC recurrence, we selected the patients who had not received antiviral therapy (the HVG and LVG), and compared cumulative HCC recurrence rates in these two groups. The cumulative HCC recurrence rates in the HVG and LVG were 26.7% and 9.4% at 1 year and 71.1% and 42.2% at 3 years, respectively. There were significant differences regarding the recurrence rates of HCC between the HVG and LVG (P = 0.016) (Fig. 3). The follow-up period for cases in which no recurrence was detected was 12-89 months in the HVG and 15-92 months in

the LVG.

Multivariate analysis of risk factors for HCC recurrence in the absence of antiviral therapy

To evaluate the factors that affected recurrence after curative treatment, the 16 variables of interest shown in Table 2 were included in the analysis. In the multivariate analysis, only high serum HBV DNA level (hazard ratio, 2.67; 95% CI, 1.31 - 5.47; $P = 0.007$) was an independent risk factor for recurrence (Table 2).

Comparison of cumulative HCC recurrence rates between the TG-B and HVG, and between the TG-A and HVG

Next, to clarify the influence of antiviral therapy on the risk of HCC recurrence, we selected the patients who had a high HBV DNA level when they had not received antiviral therapy (the HVG plus TG-A plus TG-B), and compared cumulative HCC recurrence rates between the TG-A and HVG, and between the TG-B and HVG. The cumulative HCC recurrence rates in the TG-B and TG-A were 5.3% and 15.0% at 1

year, and 42.3% and 52.0% at 3 years, respectively. There were significant differences regarding the recurrence rates of HCC between the HVG and TG-B ($P = 0.008$). On the other hand, while recurrence rate was lower in the TG-A than in the HVG, this was not statistically significant ($P = 0.10$) (Fig. 4). The follow-up period for cases in which no recurrence was detected was 12-67 months in the TG-A and 12-58 months in the TG-B.

Analysis of risk factors including antiviral therapy for HCC recurrence

To evaluate the factors that affected recurrence after curative treatment, the 16 variables of interest shown in Table 3 were included in the analysis. In the multivariate analysis, multiple tumors (hazard ratio, 2.81; 95% CI, 1.45-5.42; $P = 0.002$) and absence of antiviral therapy (hazard ratio, 2.57; 95% CI, 1.34-4.94; $P = 0.005$) were independent risk factors for recurrence (Table 3).

Cumulative HCC recurrence for each antiviral agent

Table 4 shows HCC recurrence rates for the antiviral agents used in the TG-A and TG-B. In the TG-A, cumulative HCC recurrence rate at 3 years was 47.9% in patients

who were administered a single agent (lamivudine or entecavir) and 75.0% in patients who were administered two agents (lamivudine plus adefovir). In the TG-B, cumulative HCC recurrence rate at 3 years was 21.7% in patients administered a single agent and 63.5% in those given two agents. In both in the TG-B and TG-A, HCC recurrence tended to be lower with single agents than with two agents, but this trend was not significant ($P = 0.07$ for the TG-B, P = 0.14 for the TG-A).

Discussion

Recurrence of hepatitis B-related HCC is extremely high even after curative treatment, [7, 8] and prediction and prevention of HCC recurrence is therefore an important clinical issue. Several factors are reported to be associated with an increased risk of HCC recurrence after surgical resection or local ablation therapies, including tumor characteristics, such as multiplicity, size, and portal invasion; AFP level; PIVKA-II level; and hepatic functional parameters such as albumin level, PT, and Child-Pugh class. [8-12] In addition, recent studies have suggested that a high viral load is another risk

factor for recurrence. [13-15] However, these studies included patients who had received antiviral therapy and did not fully account for this. Furthermore, the efficacy of antiviral therapy in reducing the risk of HCC recurrence is far from clear. In our study, by clearly categorizing the patients and comparing cumulative HCC recurrence among the groups, we clarified 1) the influence of HBV DNA level in the absence of antiviral therapy on the risk of HCC recurrence, and 2) the influence of antiviral therapy on recurrence of HCC. In patients who had not undergone antiviral treatment, multivariate analysis demonstrated that HBV DNA level >4 $\log_{10}$ copies/mL was an independent factor associated with higher cumulative risk of HCC recurrence after curative treatment.

The mechanism for recurrent carcinogenesis associated with HBV in the remaining liver in patients who have undergone curative treatment remains unclear. Both direct and indirect carcinogenic mechanisms are thought to be involved. [24] Active replication of HBV may initiate malignant transformation through a direct carcinogenic mechanism by increasing the probability of viral DNA insertion in or near proto-oncogenes, tumor-suppressor genes, or regulatory elements of cellular DNA. [25, 26] The integration of viral DNA may increase the production of transactivator protein hepatitis B X antigen, which may promote neoplasia of hepatocytes, as well as bind to the p53 tumor-suppressor gene and disrupt its functions. [27, 28] Indirectly, continuing HBV

replication can also induce chronic liver fibrosis and inflammation and mediate alteration in transforming growth factor-beta1 (TGF-β1) and alpha-M production, thereby leading to carcinogenesis. [29, 30] High HBV viral load can induce hepatocarcinogenesis via direct and indirect ways; hence, the risk of multicentric recurrent tumors in the liver remnant is thought to be increased.

Given the strong association between HBV DNA level and cancer recurrence, we next investigated and demonstrated that antiviral therapy is associated with lower risk of HCC recurrence. Multivariate analysis showed that absence of antiviral therapy and number of tumors were the two independent factors associated with higher cumulative risk of HCC recurrence in patients with high serum HBV DNA level after curative treatment. The number of tumors has previously been associated with HCC recurrence. Recently, the efficacy of lamivudine in preventing hepatocellular carcinoma in chronic hepatitis B has been described, [19] and one study demonstrated that lamivudine therapy reduced the recurrence of HCC in patients with chronic hepatitis B. [22] The authors stated that remission of active hepatitis in response to lamivudine therapy may decrease HCC development and metastatic potential. Taken together, these findings suggest that although antiviral therapy itself does not have anticancer effects, it may suppress HCC recurrence directly and indirectly by decreasing HBV viral load. We further showed that

while recurrence rate of HCC was significantly lower in the TG-B than in the HVG, it was only marginally lower in the TG-A than in the HVG. This suggests that, for patients with high serum HBV DNA levels, it is important to give antiviral therapy before HCC develops to prevent HCC recurrence. In addition, we showed that the rate of HCC recurrence was marginally lower for single antiviral agent therapy than for therapy using two agents. The patients who received two agents were unresponsive to lamivudine and had high serum HBV DNA level in the lamivudine-refractory period, despite receiving antiviral therapy. The difference in HBV DNA level between these two modes of therapy may be associated with the difference in rate of HCC recurrence (data not shown). However, we were unable to further evaluate such relationships, as there were few patients in each therapeutic group. Further analysis needs to be performed in a larger population of patients with HBV-related HCC and with a longer follow-up period in order to clarify our findings.

In conclusion, both HBV DNA level and absence of antiviral therapy appear to be associated with HCC recurrence. To prevent HCC recurrence for patients with high serum HBV DNA levels, it seems important to commence antiviral therapy before HCC develops. Large-scale prospective trials are necessary to elucidate the effects of HBV DNA viral load on recurrence after curative treatment and the protective roles of

antiviral therapy.

Figure legends

Fig.1. Classification of patients according to HBV DNA level and use of antiviral therapy. Enrolled patients were divided into four groups according to HBV DNA level and use of antiviral therapy. 1) Patients who did not have antiviral agents and who had consistently high serum HBV DNA levels during the time of HCC development to recurrence (high virus group; HVG). 2) Patients who did not have antiviral agents and had consistently low serum HBV DNA levels (low virus group; LVG). 3) Patients who had high serum HBV DNA levels at development of HCC and who received antiviral therapy after this (therapeutic group-A; TG-A). 4) Patients who received antiviral therapy before HCC developed (therapeutic group-B; TG-B).

Fig. 2. Overall recurrence rate. The estimated hepatocellular carcinoma (HCC) recurrence rates at 1 and 3 years after curative treatment were 16.1 % and 53.2 % respectively.

Fig. 3. Comparison of cumulative hepatocellular carcinoma (HCC) recurrence rates between the high virus group (HVG) and low virus group (LVG). Cumulative HCC recurrence rates at 3 years were significantly higher in the HVG than in the LVG (71.1% vs. 42.2%, $P = 0.016$).

Fig. 4. Comparison of cumulative hepatocellular carcinoma (HCC) recurrence rates

between the antiviral therapy before HCC diagnosis group (TG-B) and the high virus group (HVG), and between the antiviral therapy after HCC diagnosis group (TG-A) and the HVG. The cumulative HCC recurrence rates at 3 years in the TG-B and TG-A were 42.3% and 52.0%, respectively. There were significant differences regarding HCC recurrence rates between HVG and TG-B ($P = 0.008$). On the other hand, although HCC recurrence rate was lower in the TG-A than in the HVG, this difference was not statistically significant ($P = 0.10$).

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Table 1. Baseline characteristics of the four groups

Variables	HVG (n = 30)	LVG (n = 34)	TG-A (n = 20)	TG-B (n = 19)
Gender (men/women)	22/8	28/6	14/6	15/4
Age (years) †	55.6±8.3	55.9±8.3	55.7±7.9	54.3±9.2
HBeAg (+/-)	13/17	8/26	10/10	6/13
HBV DNA (log_{10} copies/ml) ††	5.9 (4.1-7.6)	< 2.6 (< 2.6- 3.6)	6.0 (4.1-8.1)	< 2.6 (< 2.6- 4.0)
Genotype (B/C)	1/17	0/11	0/10	0/7
ALT (IU/L) †	37.7±16.8	23.5±9.1	43.1±19.6	27.9±13.8
Platelet count ($x10^3/\mu L$) †	13.5±6.2	14.3±8.3	11.4±4.9	12.0±4.3
PT (%) †	83.7±15.9	85.5±15.7	84.6±14.5	81.7±12.4
Albumin (mg/dL) †	3.9±0.4	3.9±0.4	4.0±0.6	4.1±0.6
Bilirubin (mg/dL) †	0.9±0.5	0.9±0.4	0.9±0.3	0.8±0.3
Child-Pugh score (A/B)	27/3	31/3	17/3	15/4
Liver fibrosis (F1/F2/F3/F4)	3/2/4/16	1/2/2/15	1/0/2/11	1/0/3/7
Differentiation (well/mod/por)	8/12/2	4/17/2	4/6/2	4/4/0
AFP (ng/mL) ††	20.7 (1.0-3387.6)	15.1(1.0-1124.0)	26.7 (2.8-2009.7)	26.0 (1.0-2870.3)
PIVKA-II (mAU/mL) ††	40.0 (8.0-795.0)	33.0 (11.0-403.9)	36.5 (9.0-1651.0)	34.0 (12.0-1162.0)
Tumor size (cm) †	2.1±0.7	1.9±0.7	1.7±0.5	1.8±0.5
Multiple tumors (number, %)	7 (23.3%)	9 (26.5%)	5 (25.0 %)	4 (21.0%)
TNM stage (I/II/III)	10/17/3	12/17/5	9/10/1	6/10/3
Treatment for HCC (OPE/RFA)	16/14	16/18	10/10	9/10
Follow-up period (months)	49.2 (12-89)	55.5 (15-92)	35.5(12-67)	34.0 (12-58)

HVG, high virus group (HBV DNA ≥ 4 log copies/mL); LVG, low virus group (HBV DNA < 4 log copies/mL); TG-A, antiviral therapy group after the development of HCC; TG-B, antiviral therapy group before the development of HCC; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotranferase; PT, prothrombin time; Differentiation, Tumor differentiation; AFP,alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II ; HCC, hepatocellular carcinoma; OPE, hepatic resection; RFA, radiofrequency ablation. † Mean±SD, †† Median (range).

Table 2. Factors affecting HCC recurrence in patients not given antiviral therapy (HVG plus LVG)

Characteristic	Univariate analysis	Multivariate analysis	Hazard ratio (95% CI)
Gender (male)	0.171	-	
Age (≥ 55 y)	0.204	-	
HBe Ag status (positive)	0.433	-	
HBV DNA (≥ 4 log copies/mL)	0.015	0.007 [a]	2.67 (1.31-5.47)
ALT (≥ 35 IU/L)	0.309	-	
Platelet count (< 120 x 10^3/μL)	0.040	0.077	2.05 (0.93-4.51)
PT (< 70%)	0.037	0.191	1.83 (0.74-4.54)
Albumin (< 3.5 mg/dL)	0.382	-	
Bilirubin (≥ 1.2 mg/dL)	0.122	-	
Liver fibrosis (cirrhosis)	0.366	-	
Tumor differentiation (mod,por)	0.703	-	
AFP (≥ 20 ng/mL)	0.336	-	
PIVKA-II (≥ 40 mAU/mL)	0.185	-	
Tumor size (≥ 2 cm)	0.072	-	
Tumor number (multiple)	0.155	-	
Initial treatment (resection vs. RFA)	0.291	-	

HCC, hepatocellular carcinoma; HVG, high virus group (HBV DNA ≥ 4 log copies/mL); LVG, low virus group (HBV DNA < 4 log copies/mL); 95% CI, 95% confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotranferase; PT, prothrombin time; mod, moderately differentiated; ,por, poorly differentiated; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II ; RFA, radiofrequency ablation.

[a] Statistically significant

Table 3. Factors affecting HCC recurrence in patients with high HBV DNA levels (HVG plus TG-A plus TG-B)

Characteristic	Univariate analysis	Multivariate analysis	Hazard ratio (95% CI)
Gender (male)	0.540	-	
Age (≥ 55 y)	0.661	-	
HBe Ag status (positive)	0.075	-	
Antiviral therapy (not received)	0.018	0.005 [a]	2.57 (1.34-4.94)
ALT (≥ 35 IU/L)	0.902	-	
Platelet count (< 120 x 10^3/μL)	0.360	-	
PT (< 70%)	0.341	-	
Albumin (< 3.5 mg/dL)	0.158	-	
Bilirubin (≥ 1.2 mg/dL)	0.392	-	
Liver fibrosis (cirrhosis)	0.490	-	
Tumor differentiation (mod,por)	0.852	-	
AFP (≥ 20 ng/mL)	0.424	-	
PIVKA-II (≥ 40 mAU/mL)	0.229	-	
Tumor size (≥ 2 cm)	0.284	-	
Tumor number (multiple)	0.009	0.002 [a]	2.81 (1.45-5.42)
Initial treatment (resection vs. RFA)	0.851	-	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HVG, high virus group (HBV DNA ≥ 4 log copies/mL); TG-A, antiviral therapy group after the development of HCC; TG-B, antiviral therapy group before the development of HCC; HBeAg, hepatitis B e antigen; 95% CI, 95% confidence interval; ALT, alanine aminotranferase; PT, prothrombin time; PT, prothrombin time; mod, moderately differentiated; ,por, poorly differentiated; AFP, alpha-fetoprotein; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II ; RFA, radiofrequency ablation. [a] Statistically significant

Table 4. Cumulative HCC recurrence according to antiviral agents

TG-A (n = 20)		
Antiviral agents	No. of patients	Recurrence rate (3 years, %)
lamivudine	7	54.3
entecavir	5	20.0
single agent (lamivudine or entecavir)	12	47.9
lamivudine plus adefovir dipivoxil	8	75.0
TG –B (n = 19)		
Antiviral agents	No. of patients	Recurrence rate (3 years, %)
lamivudine	5	0
entecavir	2	0
lamivudine then entecavir	4	50.0
single agent (lamivudine or entecavir)	11	21.7
lamivudine plus adefovir dipivoxil	8	63.5

TG-A, antiviral therapy group after the development of HCC; TG-B, antiviral therapy group before the development of HCC

Figure 1

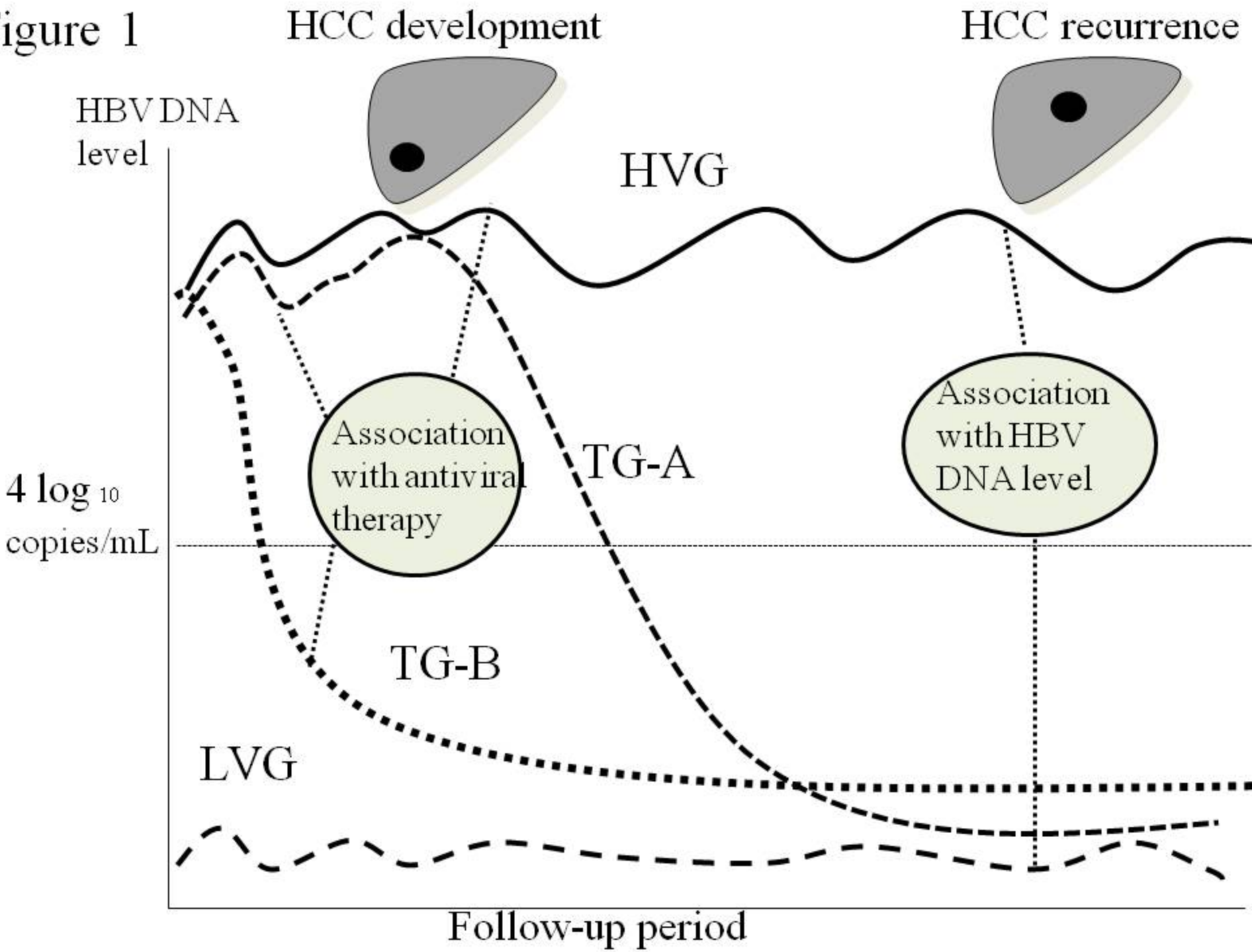

HCC development
HCC recurrence
HBV DNA level
HVG
Association with antiviral therapy
Association with HBV DNA level
TG-A
4 log 10 copies/mL
TG-B
LVG
Follow-up period

Fig. 2.

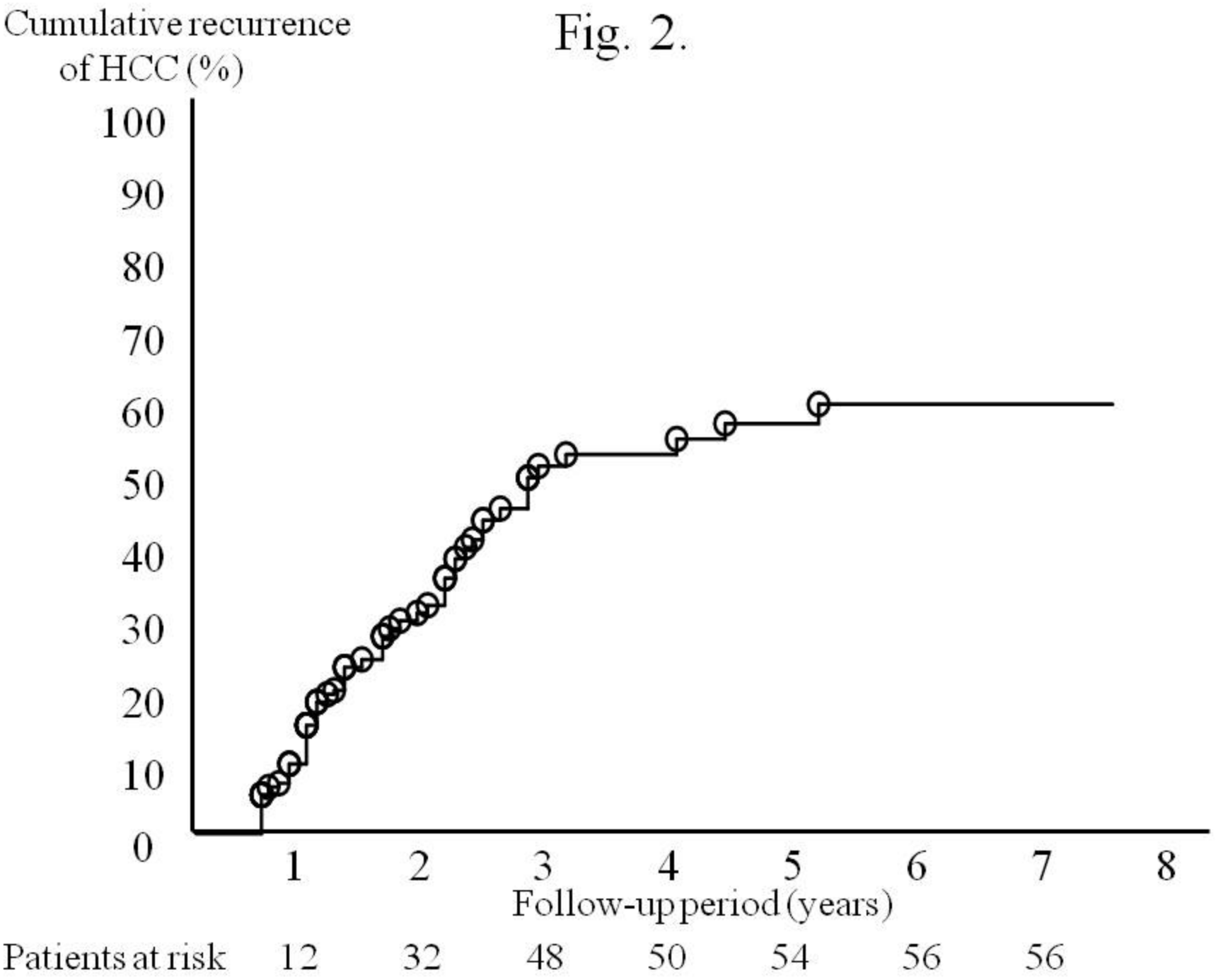

Cumulative recurrence of HCC (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Follow-up period (years)
Patients at risk
12
32
48
50
54
56
56

Fig. 3

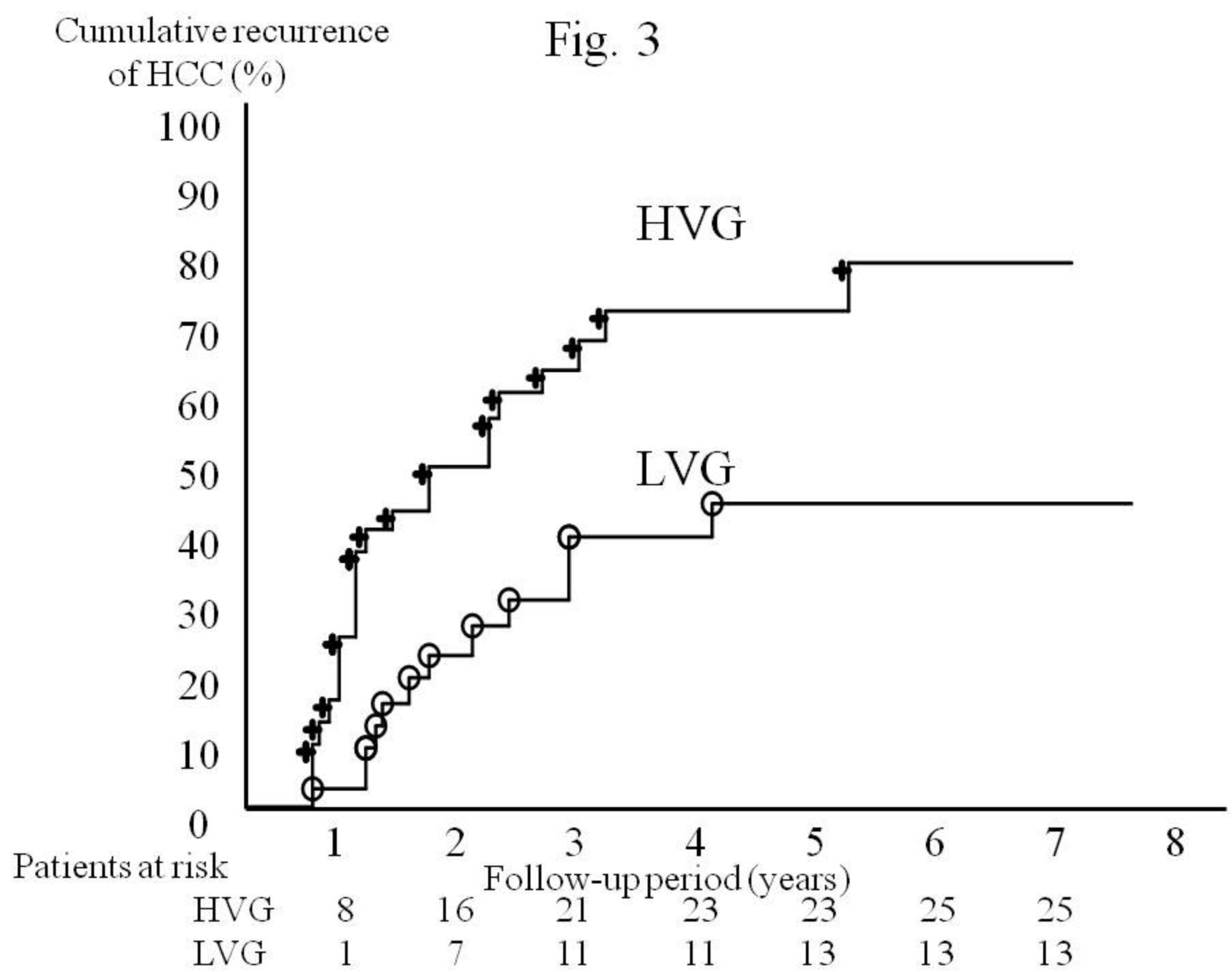
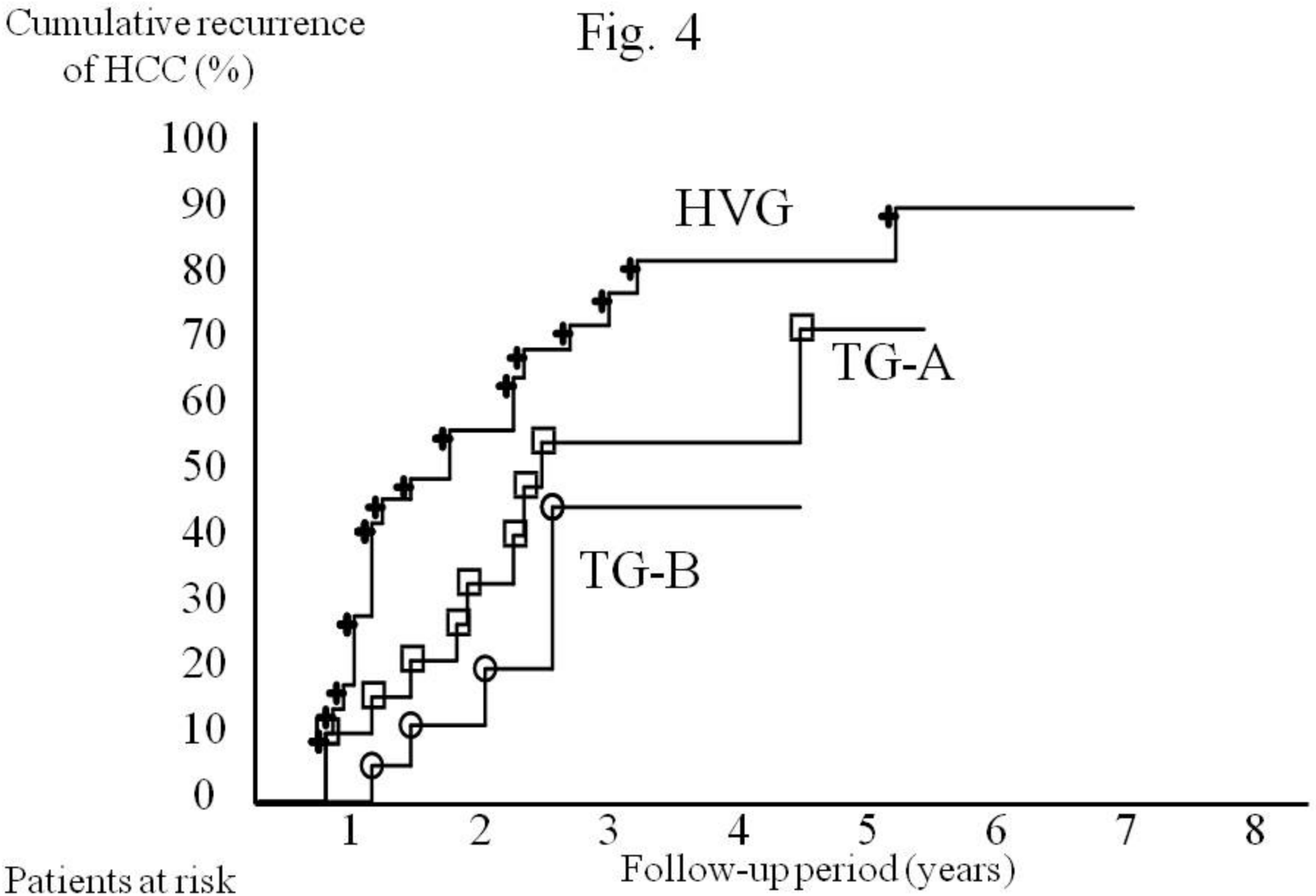

Cumulative recurrence of HCC (%)
100
90
80
70
60
50
40
30
20
10
0
HVG
LVG
1
2
3
4
5
6
7
8
Follow-up period (years)
Patients at risk
HVG 8 16 21 23 23 25 25
LVG 1 7 11 11 13 13 13

Fig. 4



Patients at risk

HVG	8	16	21	23	23	25	25
TG-A	2	5	9	9	11		
TG-B	1	4	7	7			