Original article

Tumor Marker Levels before and after Curative Treatment of Hepatocellular Carcinoma as Predictors of Patients Survivals

Atsushi Nanashima[1], Naota Taura[2], Takafumi Abo[1], Tatsuki Ichikawa[2], Ichiro Sakamoto[3], Takeshi Nagayasu[1], and Kazuhiko Nakao[2]

[1]Division of Surgical Oncology and Department of Surgery, [2]Department of Gastroenterology and Hepatology, and [3]Department of Radiology, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki, 8528501 Japan

Short title: Prediction based on change in HCC marker

Correspondence: Dr. Atsushi Nanashima,
Division of Surgical Oncology and Department of Surgery, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, JAPAN.
Tel: +81-95-819-7304, Fax: +81-95-819-7306
E-mail: a-nanasm@nagasaki-u.ac.jp

Abstract

α-fetoprotein (AFP) is used as a marker for hepatocellular carcinoma (HCC), which is influenced by hepatitis. Protein induced vitamin K absence or antagonist II (PIVKA-II) is a sensitive diagnostic marker. Changes in these markers after treatment may reflect curability and predict outcome. We conducted an analysis of prognosis in 470 HCC patients who received curative treatments, and examined relationship between changes in AFP and PIVKA-II levels after 1-month of treatment in 156 patients. Subjects were divided into three groups according to changes in both levels: 1) normal (L) group before treatment, 2) normalization (N) or 3) decreased but still above normal level or unchanged (ANU) group after treatment. High AFP and PIVKA-II levels were significantly associated with poor tumor-free and overall survival. Presence of large size and advanced stage were significantly associated with prevalence of DU group. Overall survival in the AFP-L group was significantly better than those of other groups and overall survival in PIVKA-II-L and N groups were significantly better than those of PIVKA-II-ANU groups. Combination of changes in AFP- ANU and PIVKA-II- ANU group showed the worst tumor-free and overall survivals. Multivariate analysis identified high pre-treatment levels of AFP and PIVKA-II and combination of AFP- ANU and PIVKA-II- ANU as significant determinants of poor tumor-free and overall survival, particularly in patients who underwent hepatectomy. We conclude that high levels of AFP or PIVKA-II after treatment for HCC did not sufficiently reflect curative efficacy of treatment and reflected a poor predictor of prognosis in HCC patients.

Key words Hepatocellular carcinoma, PIVKA-II, AFP, Post-curative treatment change, Survival

Introduction

Hepatocellular carcinoma (HCC) is a common malignant disease worldwide following an increase of viral hepatitis and steatohepatitis (1, 2) and patient prognosis has recently improved thanks to advances in treatment modalities (3, 4). For early diagnosis of HCC and estimation of the biological malignant behavior or patient prognosis, sensitive tumor markers are necessary. The α-fetoprotein (AFP) has been used as a classical marker for hepatocellular carcinoma (HCC) (5). However, AFP levels are often high in patients with chronic hepatitis or cirrhosis. (6) Protein induced by vitamin K absence or antagonist II (PIVKA-II; des-gamma-carboxy prothrombin) was proposed as specific markers for the diagnosis of HCC, evaluation of tumor aggressiveness and prognosis. (7, 8) PIVKA-II has been commonly used for the diagnosis in HCC in Japan and has been also adopted worldwide since 2000 (9, 10). Recently, analysis of the combination of tumor markers was proposed for accurate diagnosis of HCC by the Japan Guideline for HCC Diagnosis and Treatment (11, 12). Based on our preliminary experience in patients with resectable HCC(13, 14), preoperative PIVKA-II is a useful marker for predicting postoperative tumor recurrence and prognosis, while AFP does not closely correlate with clinical outcome. Furthermore, other reports indicated that normalization of initially high PIVKA-II correlated with better prognosis (13, 14). High AFP and PIVKA-II levels are associated with tumor recurrence or poor survival after any treatments for HCC and these markers might be useful to predict tumor recurrence during the post-treatment follow-up (15). As described above, changes in sensitive markers may indicate eradication of HCC. In patients with high levels of tumor markers, complete resection

or necrosis of HCC should result in normalization of tumor markers within the half life of these markers, only a couple of days (14, 16). A few reports, including our preliminary studies, indicated that changes of serum levels of AFP and PIVKA-II correlate with prognosis (14, 17, 18). However, to date, there is no consensus regarding the value of post-treatment evaluation of tumor markers. To understand the significance of changes of tumor biomarkers, it is necessary to examine these biomarkers in patients who have undergone curative treatments, including hepatectomy or complete ablation therapy.

We hypothesized that: *1)* high levels of AFP and PIVKA-II are poor predictors of survival of patients with HCC, and *2)* these HCC sensitive markers return to normal levels immediately after curative treatment of HCC, and *3)* that such normalization reflects better prognosis. Measurement of one or more of these markers before and after treatment should be useful for evaluating the curability of HCC. To test our hypothesis, we examined in this retrospective study the AFP and PIVKA-II levels in patients with HCC before and shortly after hepatectomy and local ablation therapy. We then evaluated the relationship between preoperative levels of these markers as well as changes in these markers with tumor relapse and survival.

Materials and Methods

Patient demographics

Data of 470 patients with HCC were collected. These patients were diagnosed at the Division of Surgical Oncology, Department of Radiology and the Department of Gastroenterology and Hepatology, Nagasaki University Hospital (NUH) between 1988 and October 2009. Patients with remnant viable tumor or untreatable tumor after treatment were excluded from this study. All hepatic tumors were completely resected without macroscopic exposure of the amputated section of the liver. After examination at 1-2 months after primary treatment, the patients were followed-up by measuring serum levels of AFP and PIVKA-II every 3 months, and enhanced computed tomography of the liver was obtained every 6 months for at least the first 5 years after hepatectomy to find out tumor recurrence. In 470 patients, changes of these markers at 1-2months after treatments were examined in 156 patients (33%), which were available samples but not randomly selected.

The minimum follow-up period after hepatic resection of HCC was 12 months (range, 12-178 months). Fifty-one of 470 (11%) patients who survived were lost to follow-up, 129 patients died of cancer, 20 patients died of liver-associated diseases and 25 patients died of unrelated diseases. Their data were censored at the last date because they were not known to be cancer-related death. The 261 (56%) patients developed tumor recurrence after treatment. The study design was approved by the Ethics Review Board of NUH including collection of data from the medical records, which were also obtained from the associated hospitals mentioned above.

Measurement of tumor markers

A 4-mL peripheral blood sample was collected from each patient before and 1-2 months after treatment. The sample was then centrifuged at 3,000 rpm (1,000 × *g*) for 10 minutes. PIVKA-II was assayed by an enzyme-linked immunoassay using *Eitest* ® *PIVKA-II* (Sanko Junyaku Co., Tokyo, Japan). The normal value of AFP for HCC in our hospital is less than 20 ng/mL. The reported normal value of PIVKA-II is <40 mAU/mL (13). As an increase of these markers were correlated with patient prognosis, the level of these markers before treatments were divided into 3 groups as : <20ng/mL, 20-200ng/mL, >200ng/mL for AFP, and <40mAU/mL, 40-400mAU/mL, >400mAU/mL for PIVKA-II according to previous study (13, 14). Elevated levels of AFP and PIVKA-II were defined as those exceeding the above levels. Patients were divided into three groups based on changes in these markers after treatment: 1) both markers lower than the above cut-off levels both before and after treatment (the L group), 2) normalization of elevated markers (i.e., levels of both markers above the cut-off values returned to within the normal range after treatment, the N group), 3) marker levels decreased relative to pre-treatment level but were still above the normal ranges, or tumor marker was unchanged or increased (the ANU group). Finally, combination of changes (the L and N groups vs. the ANU group) in both tumor markers was analyzed for patient survivals.

The tumor-related factors were related to histopathological examination of the resected specimen. For assessment, we used the histopathological factors and curability by hepatectomy of *the Liver Cancer Study Group of Japan by the Classification of Primary Liver cancer (*19).

Statistical analysis

Differences in categorical data between groups or prevalence were assessed by the chi-square, Fischer's exact or Dunnet's multiple comparison tests. Differences in continuous data between groups were evaluated by the Student's *t*-test or Mann-Whitney test. The disease-free interval and overall survival were calculated using the Kaplan-Meier method, and differences between groups were tested for significance using the log-rank test. Multivariate analysis was performed using Cox's proportional hazards regression modeling. A two-tailed *P* value of <0.05 was considered significant. Statistical analyses were performed using the SAS software (Statistical Analysis System Inc., Cary, NC).

Results

Patient demographics

The mean age of the patients at the time of surgery was 66.4±9.8 years (range, 23-88 years), and there were 342 males (73%) and 128 females. The background liver abnormalities included chronic hepatitis in 187 (40%) patients, cirrhosis in 270 (57%), and normal liver in 13 (3%), associated with hepatitis virus B (n=122), hepatitis virus C (n=253), both hepatitis B and C (n=19), alcoholic disease (n=6) or others (n=70). According to the Child-Pugh classification, 405 (86%) patients were classified as A and 65 as B and C. The pathological tumor node metastasis (TNM) stage of HCC according to the Liver Cancer Study Group of Japan [21] was stage I in 139 (30%), stage II in 196 (42%), stage III in 93 (20%) and stage IVA in 42 (8%). The treatment included surgical resection (n=331, 70%), thermal ablation by radio frequency or microwave ablation (n=114, 24%), alcohol injection (n=25, 6%). The surgical resections included partial hepatic resections (n=322) and whole liver transplantation (n=9).

Relationship between preoperative tumor marker levels and incidence of tumor recurrence/post-treatment survival

A significantly high incidence of tumor recurrence and poor tumor-free survival after treatment was noted in patients with high pre-treatment levels of AFP and PIVKA-II (Table 1 and Fig. 1A). The median recurrence-free survival rate of HCC patients with normal AFP level (mean survival period (MSP): 1,754 days) was significantly higher than that of patients with high AFP level ($p<0.01$). Furthermore, the 3- and 5-year

tumor-free survival rates of HCC patients with normal AFP level were significantly higher than those of patients with high AFP level.

The median tumor-free survival rates of HCC patients with normal PIVKA-II level (MSP: 1,385 days) and with PIVKA-II level between 40-400 mAU/mL (MSP: 1,546 days) were significantly higher than that of patients with high PIVKA-II level (>400 mAU/mL, MSP: 770 days) ($p<0.01$). The 3- and 5-year recurrence-free survival rates of HCC patients with normal PIVKA-II level or with PIVKA-II level of 40-400 mAU/mL were significantly higher than those of patients with high PIVKA-II level exceeding 400 mAU/mL, respectively ($p<0.01$).

The mean overall survival rates of patients with normal levels of AFP and PIVKA-II were significantly higher than that of patients with high levels (Fig. 1B). The MSP of HCC patients with normal AFP level was significantly longer than that of patients with high AFP levels ($p<0.01$). The 5- and 8-year overall survival rates of HCC patients with normal AFP level were significantly higher than those of patients with higher AFP level. The MSP of HCC patients with normal PIVKA-II level and those with PIVKA-II level of 40-400 mAU/mL were significantly longer than that in patients with high PIVKA-II level >400 mAU/mL ($p<0.01$). The 5- and 8-year overall survival rates of HCC patients with normal PIVKA-II level were significantly higher than those of patients with high PIVKA-II level ($p<0.01$).

Figures 2A and B show the differences in disease-free and overall survival for each treatment. The hepatectomy group had a better prognosis in comparison with ablation therapy. Therefore, we examined the survival analysis associated with changes of tumor markers for each treatment.

Relationship between clinicopathological parameters and serum level of tumor markers or changes in tumor markers

Among all 470 patients, 229 (51%) and 286 (67%) had higher than normal ranges of AFP and PIVKA-II, before treatment, respectively. Serum levels over 200 ng/ml of AFP and over 400 mAU/ml were 107 and 129 patients before treatment, respectively. With respect to post-treatment changes in AFP level, 60 patients were classified in the L group, 49 in the N group and 45 in the ANU group. With respect to post-treatment changes in PIVKA-II level, 22 patients were classified in the L group, 68 in the N group and 60 in the ANU group.

Tables 2 and 3 show the relationship between various clinicopathological parameters and changes in tumor markers. Larger tumor size and advanced TNM stage were significantly associated with the incidence of ANU groups for both AFP and PIVKA-II. The rate of surgical resection was significantly higher in the L and N groups of both AFP and PIVKA-II. The percentages of patients who developed tumor recurrence according to AFP level were 45% of the L group, 71% of the N group and 51% of the ANU group. The percentages of patients who developed tumor recurrence according to PIVKA-II level were 36% of the L group, 72% patients of the N group and 45% of the ANU group. There were no significant differences in these distributions between groups with respect to changes in AFP; however, tumor recurrence rate in group L of PIVKA-II was significantly lower than those in group N or ANU of PIVKA-II.

Relationship between disease-free and overall survival rates and changes in serum tumor markers

Tumor-free survival rate according to changes in AFP in the ANU groups was significantly lower than those in the L or N group (Fig. 3A)($p<0.05$) and tumor-free survival rate according to changes in PIVKA-II in the ANU groups tended to be lower than those in the L or N group but not statistically significant (Figure 3B). The tumor-free survival rate of patients who underwent hepatectomy in the ANU group were significantly lower than those in other groups according to changes in AFP but not in PIVKA-II (Table 4). However, there were no significant differences in patients who underwent thermal ablation. The overall survival rate of the AFP-L group was significantly better than those of AFP-other groups ($p<0.05$) and the overall survival rates of the PIVKA-II-L and N groups were significantly better than those of the PIVKA-II- ANU group (Fig. 4A and B). In patients who underwent hepatectomy, the overall survival rate of the AFP-L group was significantly better than those of AFP-other groups ($p<0.05$) and the overall survival rates of the PIVKA-II-L and N groups were significantly better than those of the PIVKA-II- ANU groups (Table 5). In patients undergoing thermal ablation, the overall survival rates in the L group was significantly better than that in the N or ANU groups according to changes in AFP but not in PIVKA-II.

By the survival analysis according to combination of changes in AFP and PIVKA-II, AFP-L, -N and PIVKA-II-L, -N group showed the best tumor-free survivals and AFP- ANU and PIVKA-II- ANU group showed the worst tumor-free survivals in all patients (Fig. 5A) ($p<0.05$). These differences of tumor-free survival

tended to be observed in ablation therapy but were not significant (Table 6). Regarding overall survival, AFP-LN and PIVKA-II-LN group showed the best survivals and AFP- ANU and PIVKA-II- ANU group showed the worst survivals in all patients (Table 6) ($p<0.05$). This tendency was also observed in patients who underwent hepatectomy or ablation therapy. There were no significant differences of survivals between AFP- ANU and PIVKA-II L, -N *vs.* AFP-L, -N and PIVKA-II-ANU.

Table 7 shows the significant prognostic factors, including changes in AFP and PIVKA-II levels, for all patients, associated with tumor-free and overall survival rates, identified by univariate analysis. With respect to the tumor-free survival rate, multiple tumors, large tumor size, advanced tumor stage, poor liver function, pre-treatment high AFP or PIVKA-II levels, PIVKA-II- ANU group, and combination of AFP- ANU or PIVKA-II- ANU were significantly associated with poor survival based on univariate analysis ($p<0.05$). Multivariate analysis showed that larger tumor size, increase in AFP level, high PIVKA-II levels before treatment, and combination of AFP- ANU and PIVKA-II- ANU were significantly associated with poor tumor-free survival ($p<0.05$). With respect to overall survival, multiple tumors, large tumor size, advanced tumor stage, poor liver function, pre-treatment high levels of AFP or PIVKA-II, AFP- ANU group, PIVKA-II- ANU group, combination of AFP- ANU and PIVKA-II- ANU, and ablation group were significantly associated with poor overall survival ($p<0.05$). Multivariate analysis showed that larger tumor size, PIVKA-II-ANU group, combination of AFP- ANU and PIVKA-II- ANU, and ablation group were significantly associated with poor survival ($p<0.05$).

As a difference in overall survival between hepatectomy and ablation therapy was observed, the prognostic factors in patients who underwent hepatectomy were examined. Table 8 shows the significant prognostic factors, including changes in AFP and PIVKA-II levels after hepatectomy, associated with tumor-free and overall survival in HCC patients who underwent hepatectomy. With respect to tumor-free survival, the presence of multiple tumors, large size tumor, advanced tumor stage, viral etiology, poor liver function, and pre-treatment high levels of AFP or PIVKA-II were correlated significantly with poor survival ($p<0.05$). Multivariate analysis identified large tumor size, poor liver function, and pre-treatment high levels of AFP and PIVKA-II level as significant poor prognostic factors for tumor-free survival. With respect to overall survival, the presence of multiple tumors, large tumor size, advanced tumor stage, poor liver function, pre-treatment high levels of AFP or PIVKA-II, PIVKA-II- ANU group and combination of AFP- ANU and PIVKA-II-ANU were significantly associated with poor survival ($p<0.05$). Multivariate analysis identified only advanced tumor stage and poor liver function as significant poor prognostic factors for overall survival.

Discussion

Specific HCC markers, such as PIVKA-II or AFP L3 fraction, in combination with AFP level, are commonly used in Japan for the diagnosis of HCC or evaluation of tumor aggressiveness (6-15,17,18, 20, 21). High values of these markers reflect patient prognosis after any treatment (13-15, 17, 18, 22). We previously reported that HCC staging including preoperative PIVKA-II level is an independent prognostic marker in HCC patients who undergo hepatectomy. (13,23) Monitoring of PIVKA-II might be useful for predicting tumor recurrence after hepatectomy at earlier period compared to AFP level (24, 25) and our pilot study also showed that changes in PIVKA-II significantly correlated with prognosis in a small number of HCC patients undergoing hepatectomy. (14) It is possible that sensitive tumor markers return to normal levels soon after curative treatment. Therefore, we focused in the present study on changes in these markers at an early postoperative period testing a large number of HCC patients who were treated by curative treatments to clarify usefulness of these markers and their changes. The usefulness of measuring tumor biological markers can be evaluated after curative treatments, including hepatectomy or complete ablation therapy. In the present results, patient survival between hepatectomy and ablation therapy was significantly different and, therefore, we examined survival analysis in each treatment. Survival analysis in patients who underwent chemotherapy or other palliative treatments were not examined in this study.

In our pilot study, (14) we excluded HCC patients with normal preoperative levels of AFP/PIVKA-II because changes in these markers could not be measured and thus their prognosis was not evaluated. In the present study, we examined HCC patients

with low and high preoperative levels of both AFP and PIVKA-II. The cut-off levels of markers were set up according to the normal range and previous reports on the relationship between tumor markers and prognosis in HCC. (5, 7, 8, 13, 14, 26) The number of patients with normal preoperative PIVKA-II levels was less than that of patients with normal AFP level. This finding suggests that AFP level could reflect inflammatory activity associated with chronic hepatitis.(6) The results of the present study showed that high preoperative levels of both tumor markers correlated with tumor recurrence rate and that HCC patients with high levels of both markers before treatment were also associated with poor disease-free and overall survival rates, in agreement with previous reports. (15, 17, 18, 27, 28) Furthermore, survival analysis showed that a high preoperative level of AFP or PIVKA-II was also associated with better survival. Patients with AFP levels of 20-200 and >200 ng/mL had similar tumor-free and overall survival rates. On the other hand, the tumor-free and overall survival rates of patients with PIVKA-II levels of 40-400 mAU/mL were better than in those with levels ≥400 mAU/mL. This result indicates that PIVKA-II level of ≥400 mAU/mL is a predictor of poor prognosis and that level is more sensitive than AFP in predicting tumor recurrence or prognosis.(23-25, 29) In the present study, changes of both AFP and PIVKA-II levels were focused to evaluate effectiveness of any treatments because half-life of these serum markers was limited within a few weeks.(16) We hypothesize that L group showed the lowest malignancies in all groups before treatments and, in the N group, the tumor was mostly disappeared by the effective treatments. In the ANU group, viable or active HCC might remain and HCC of ANU group might be remained and had the most aggressive malignancies in the

study design. The results of analysis of relationship with clinicopathological parameters showed that advanced HCC and poor liver function correlated with the AFP- ANU group. In the AFP- ANU group, remnant liver dysfunction might be influenced the high level of postoperative AFP (30) as well as tumor malignancies. Poor liver function and active hepatitis might progress to tumor recurrence through multicentric carcinogenesis of HCC.(31) Interestingly, changes in PIVKA-II were also related to advanced tumors and poor liver function in the present study. The relationship between PIVKA-II and liver dysfunction has been examined extensively because the metabolism of vitamin K is to a large extent dependent on liver function.(32) Our results showed that changes in PIVKA-II levels after treatment tended to reflect tumor recurrence, compared with changes in AFP level. This finding suggests that analysis of PIVKA-II at follow-up is more reliable for prediction of tumor recurrence after treatment compared with AFP. While, Nobuoka et al. reported that postoperative AFP level is useful to predict recurrence after hepatectomy by comparison with postoperative PIVKA-II level.(33)

Univariate analysis of data of all patients on changes in both tumor markers showed no difference in tumor-free survival rate, irrespective of treatment modality. With respect to overall survival, HCC patients of the AFP- and PIVKA-II-L groups had the best prognosis while multivariate analysis showed that patients of the PIVKA-II- ANU group had the poorest survival. This tendency was similar in HCC patients undergoing hepatectomy. However, multivariate analysis did not identify changes in PIVKA-II as a significant prognostic factor. On the other hand, tumor-free survival correlated significantly with high baseline PIVKA-II levels. In this regard, a

previous study reported that normalization of PIVKA-II correlated with good prognosis.(14) While, Nobuoka et al. stressed the significance of postoperative AFP in relation to posttreatment recurrence of HCC.(34), Kanazumi *et al*. reported that serum PIVKA-II level decreased within 2 weeks after effective surgery (25) and suggested that PIVKA-II level higher than the normal range might reflect remnant HCC. In the present study, we speculated that normalization of PIVKA-II could reflect a satisfactory outcome with cure of HCC based on the result of survival analysis. Considered together with the results of the present study, we propose that patients with high PIVKA-II levels after hepatectomy (e.g., patients of the PIVKA-II- ANU groups) should receive adjuvant therapy to control invisible remnant HCC cells, to improve survival. By results of AFP and PIVKA-II, we attempted to combine both markers for survival analysis. Eventually, combination of AFP- ANU and PIVKA-II- ANU was the worst predictor of tumor-free and overall survivals, respectively. In case of diagnosis for HCC, combination of AFP and PIVKA-II was recommended to diagnose tumor malignancy by the Japan's guideline for HCC at present.(12) Recent studies showed that AFP L3 fraction is a better specific marker for HCC than AFP level.(11, 35) However, since the significance of AFP L3 fraction has not yet been clarified, (36) it is necessary to examine this marker for better assessment of patient prognosis.

For HCC patients who underwent thermal ablation, changes in AFP and PIVKA-II also tended to be associated with tumor-free and overall survival rates. Pretreatment AFP or PIVKA-II levels were associated with survival, though such correlation could not be confirmed by multivariate analysis. Previous studies also showed that high levels of AFP and PIVKA-II were associated with survival.(7,8,

13-15, 17, 18,21-23) Thus, in HCC patients with high levels of AFP or PIVKA-II level, hepatectomy is a better therapeutic choice especially for those patients with preserved liver function because hepatectomy is the best treatment modality associated with longer survival compared with other treatment modalities, with the exception of liver transplantation.(37)

In summary, we conducted a retrospective analysis of prognosis of 470 HCC patients who received curative treatments, including hepatectomy and ablation therapy, including analysis of the relationship between changes in AFP and PIVKA-II after treatment and patient survival in 156 patients. Compared to changes in AFP, preoperative low levels of PIVKA-II and their normalization after treatment were significantly associated with better survival after hepatectomy based on multivariate analyses, particularly in patients who underwent hepatectomy. Normalization of PIVKA-II levels after treatment reflects the efficacy of the treatment and is a suitable predictor of prognosis in patients who underwent curative treatment for HCC. Careful follow-up and adjuvant chemotherapy is necessary for patients who fail to show normalization of tumor markers at the early period after treatment.

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Figure legends

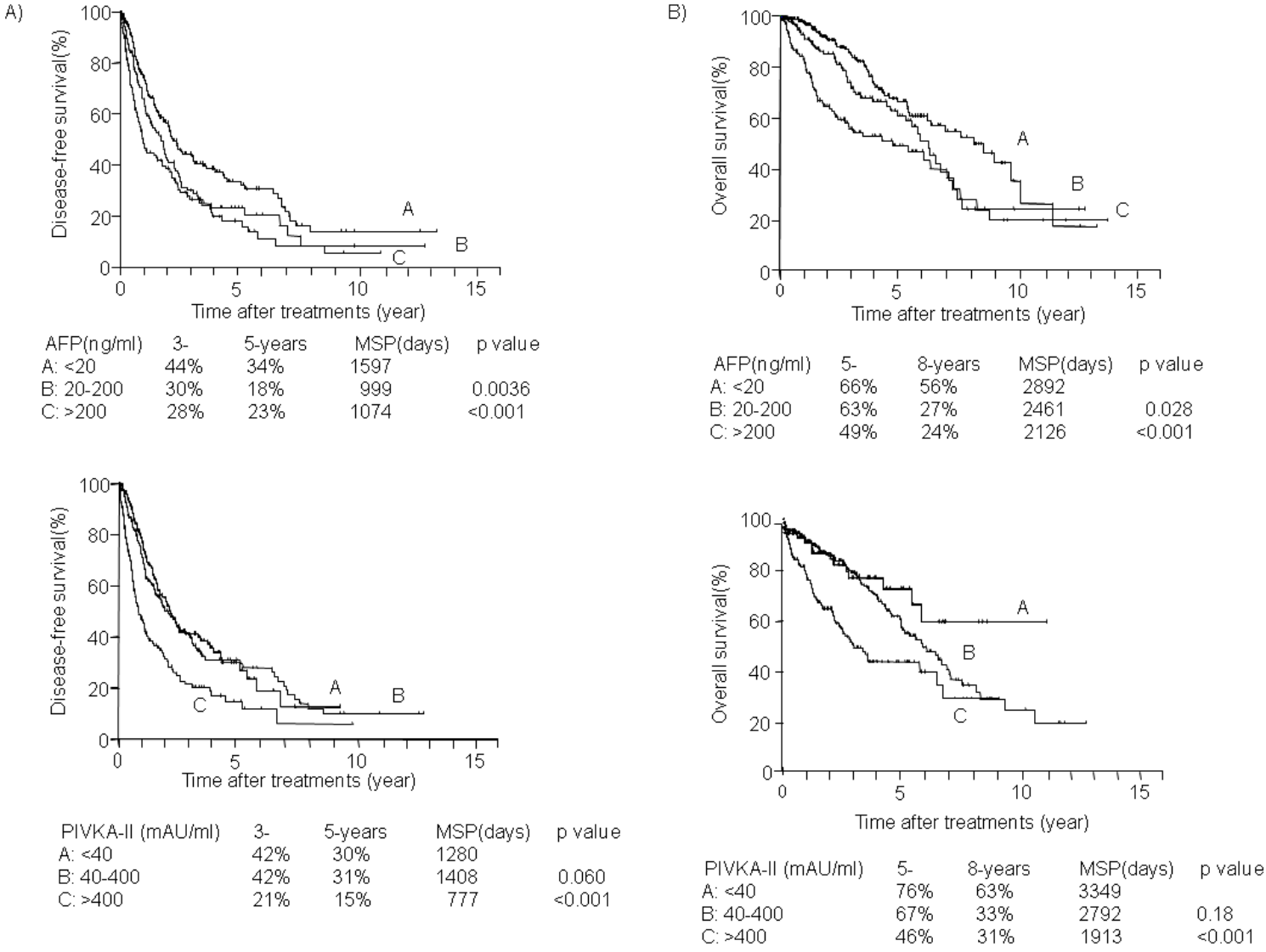


Fig. 1 Post-treatment tumor-free (A) and overall (B) survival rates for each level of preoperative AFP and PIVKA-II.

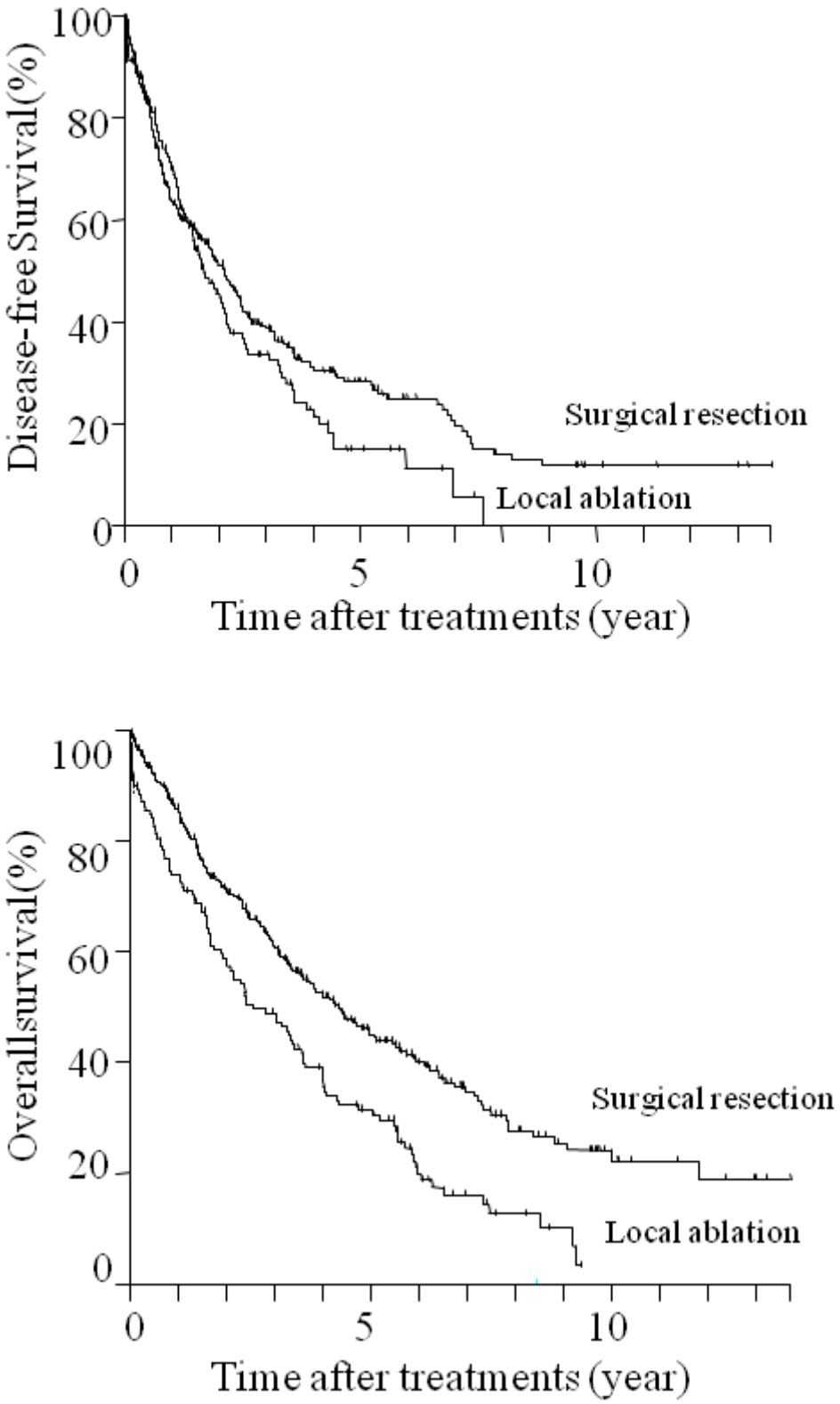
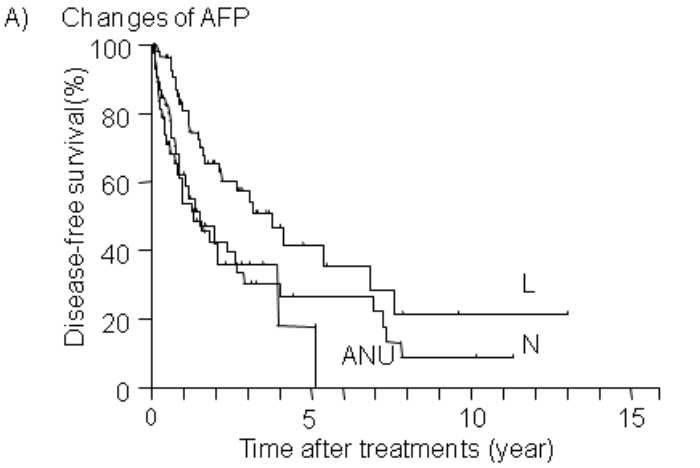
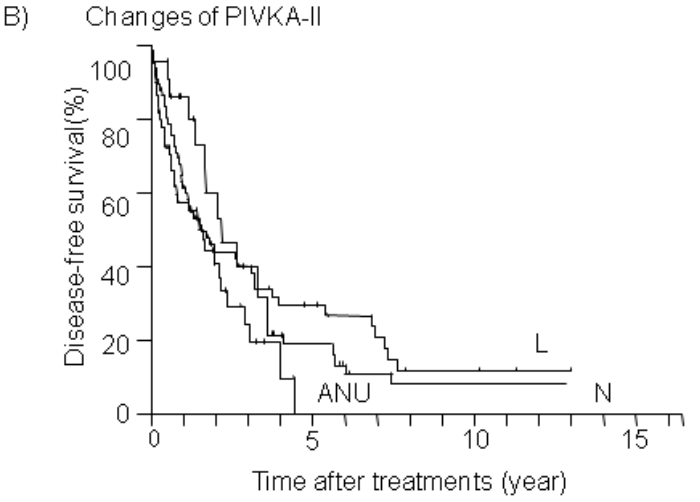


Fig.2 Disease-free and overall survival rates for each treatment modality in the surgical resection and local ablation groups.



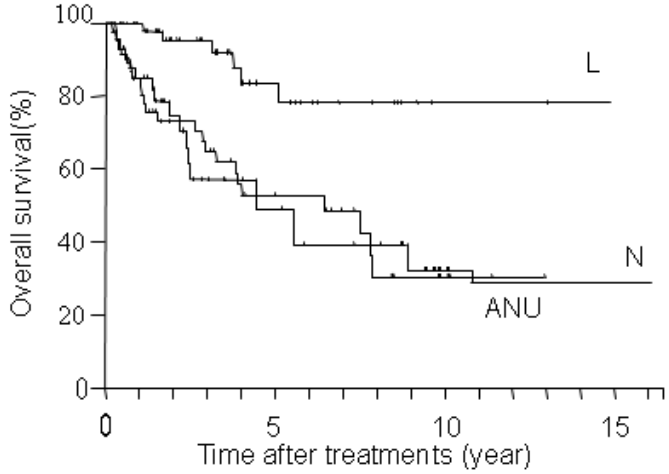
	3-	5-years	MSP (days)	p value
L	57%	42%	1911	
N	30%	27%	1167	0.019
ANU	36%	18%	795	



	3-	5-years	MSP (days)	p value
L	42%	31%	987	
N	18%	18%	1012	0.13
ANU	28%	10%	588	

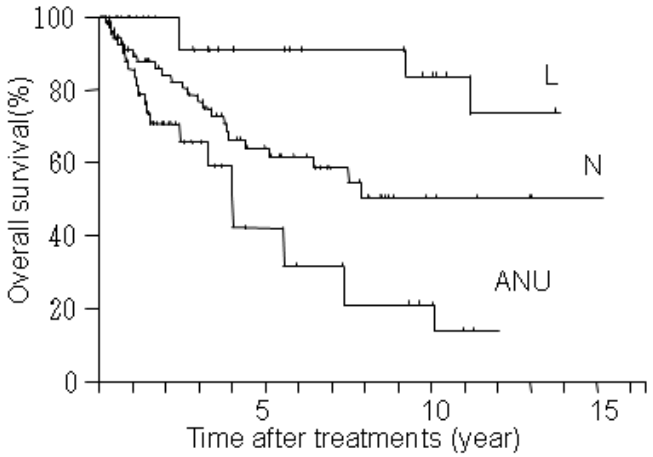
Fig. 3 Disease-free survival rates and mean survival period (MSP) after treatment of HCC according to the three different patterns of changes in levels of tumor markers AFP (A) and PIVKA-II (B). L: both markers lower than the above cut-off levels both before and after treatment. N: normalization of elevated markers (i.e., levels of both markers above the cut-off values before treatment and returned to within the normal range after treatment, the group). ANU: marker levels decreased relative to pre-treatment level but were still above the normal ranges, or the levels of tumor markers was unchanged after treatment.

A) Changes of AFP



	5-	8-years	MSP (days)	p value
L	83%	78%	3993	
N	53%	30%	2379	<0.001
ANU	65%	35%	1841	

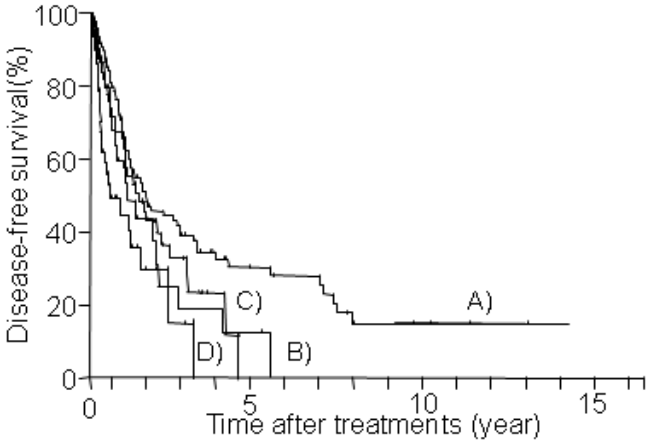
B) Changes of PIVKA-II



	5-	8-years	MSP (days)	p value
L	91%	91%	3290	
N	64%	51%	3035	0.009
ANU	42%	26%	1210	

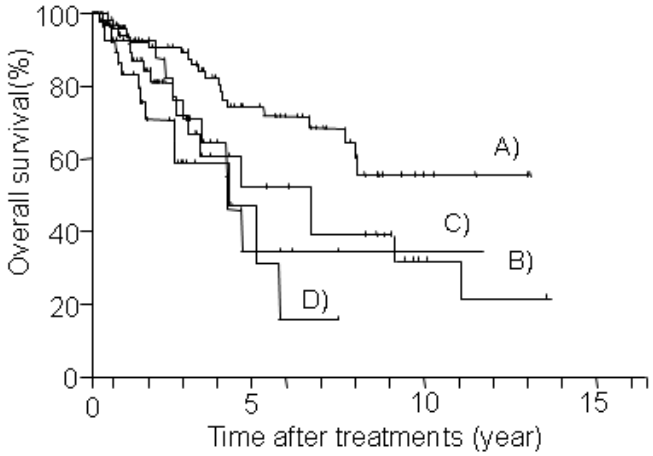
Fig. 4 Overall survival rates after treatment of HCC according to the different patterns of changes in levels of tumor markers AFP (A) and PIVKA-II (B). For definition of the different groups, see Figure 3.

A) Changes of AFP-PIVKA-II



	3-	5-years	MSP (days)	p value
A) AFP-LN, PIVKA-II-LN	37%	30%	1444	
B) AFP-ANU, PIVKA-II-LN	19%	13%	669	0.0074
C) AFP-LN, PIVKA-II-ANU	23%	0%	690	
D) AFP-ANU, PIVKA-II-ANU	15%	0%	435	

B) Changes of AFP, PIVKA-II



	5-	8-years	MSP (days)	p value
A) AFP-LN, PIVKA-II-LN	72%	56%	3360	
B) AFP-ANU, PIVKA-II-LN	52%	39%	2023	0.0014
C) AFP-LN, PIVKA-II-ANU	35%	-	1629	
D) AFP-ANU, PIVKA-II-ANU	32%	16%	1401	

Fig. 5 Disease-free and overall survival rates after all treatment for HCC according to the different combined patterns of changes in levels of AFP and PIVKA-II. For definition of the different groups, see Figure 3.

Table 1 Relationship between pretreatment AFP or PIVKA-II and posttreatment tumor recurrence.

	No recurrence (n=209)	Recurrence (n=261)	P Value
AFP (ng/mL)			
<20	122	95	<0.001
20-200	42	80	
>200	34	73	
Not examined	11	13	
PIVKA-II (mAU/mL)			
<40	83	59	<0.001
40-400	68	89	
>400	41	88	
Not Examined	17	25	

AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K antagonist or agonist

Table 2 Relationship between postoperative changes in AFP and patient demographics, clinicopathological parameters and post-treatment tumor recurrence.

	Group L (n=60)	Group N (n=49)	Group ANU (n=45)	P value
Gender (male / female)	44/16	37/12	28/17	0.31
Age	66 ±8	63 ± 10	66 ± 9	
PIVKA-II (mAU/ml)	97	700	192	
Child-Pugh (A / B/ C)	49/8/3	40/8/1	31/13/1	0.27
Viral status (None / B / C / B&C)	16/10/34/0	8/17/21/3	0/13/31/1	0.31
Number of tumors (solitary/multiple)	48/12	31/18	32/13	0.15
Tumor size (<2/2-5/≥5 cm)	27/25/8	8/19/22	14/18/13	0.002
Japan TNM classification (1/2/3/4a) [a]	26/23/10/1	7/15/17/10	13/15/13/4	0.001
Treatment				
Surgery/ Thermal ablation	34/26	46/3	23/22	0.002
Tumor recurrence (no/yes)	33/27	14/35	22/23	0.35

[a] General Rules for the Clinical and Pathological Study of Primary Liver Cancer [19]

For abbreviations, see Table 1.

Table 3 Relationship between postoperative changes in PIVKA-II and patient demographics, clinicopathological parameters and post-treatment tumor recurrence.

	Group L (n=22)	Group N (n=68)	Group ANU (n=60)	P value
Gender (male/female)	15/7	53/15	40/20	0.33
Age	68 ± 8	64 ± 9	68 ± 9	
AFP (ng/ml) (median)	9	41	34	
Child-Pugh (A / B/ C)	17/5/0	63/5/0	38/17/5	<0.001
Viral status (None / B / C / B&C)	1/3/18/0	10/27/29/2	11/11/36/2	0.13
Number of tumors (solitary/multiple)	18/4	50/18	40/20	0.37
Tumor size (<2/2-5/ ≥5 cm)	17/5/0	8/28/32	20/27/13	<0.001
Japan TNM classification (1/2/3/4a)	15/5/2/0	6/32/22/8	20/16/17/7	<0.001
Treatments				
Surgery/ Thermal ablation	4/18	62/6	38/22	<0.001
Tumor recurrence (no/yes)	14/8	19/49	33/27	0.001

For abbreviations, see Table 1.

Table 4 Three- and five-year disease-free survival rates between groups of hepatectomy and thermal ablation for HCC according to the different patterns of changes in levels of tumor markers AFP and PIVKA-II.

AFP	Disease-free survival rate (%)		Mean survival period (days)	Significance (P value)
	3- years	5- years		
Hepatectomy				
L group	59	42	1967	
N group	29	26	1128	0.049
ANU group	28	14	766	
Ablation				
L group	59	29	1460	
N group	50	0	483	0.062
ANU group	0	0	490	
PIVKA-II				
Hepatectomy				
L group	42	35	1765	
N group	41	22	1128	0.84
ANU group	0	0	365	
Ablation				
L group	53	53	1065	
N group	50	28	1596	0.22
ANU group	37	17	493	

Table 5 Five- and eight-year overall survival rates between groups of hepatectomy and thermal ablation for HCC according to the different patterns of changes in levels of tumor markers AFP and PIVKA-II.

AFP	Overall survival rate (%)		Mean survival period (days)	Significance (P value)
	3- years	5- years		
Hepatectomy				
L group	81	74	3821	
N group	57	29	2315	0.007
ANU group	27	27	1388	
Ablation				
L group	82	86	3083	
N group	100	-	1158	0.018
ANU group	59	30	1544	
PIVKA-II				
Hepatectomy				
L group	100	82	2198	
N group	63	48	2954	0.041
ANU group	30	-	1294	
Ablation				
L group	90	90	3106	
N group	80	80	2700	0.27
ANU group	71	35	1702	

Table 6 Disease-free and overall survival rates between groups of hepatectomy and thermal ablation for HCC according to the different patterns of combination of changes in levels of tumor markers AFP and PIVKA-II.

	Disease-free survival rate (%)		Mean survival period (days)	Significance (P value)
	3- years	5- years		
Hepatectomy				
AFP-L-N,PIVKA-II-L-N	43	33	1501	
AFP-ANU,PIVKA-II-L-N	26	13	608	0.26
AFP-L-N,PIVKA-II-ANU	43	43	923	
AFP-ANU,PIVKA-II-ANU	30	0	450	
Ablation				
AFP-L-N,PIVKA-II-L-N	78	60	1177	
AFP-ANU,PIVKA-II-L-N	60	0	573	0.084
AFP-L-N,PIVKA-II-ANU	60	21	533	
AFP-ANU,PIVKA-II-ANU	48	48	490	

	Overall survival rate (%)		Mean survival period (days)	Significance (P value)
	5- years	8- years		
Hepatectomy				
AFP-L-N,PIVKA-II-L-N	69	50	3107	
AFP-ANU,PIVKA-II-L-N	34	28	1613	0.011
AFP-L-N,PIVKA-II-ANU	39	40	1568	
AFP-ANU,PIVKA-II-ANU	36	36	541	
Ablation				
AFP-L-N,PIVKA-II-L-N	100	100	3354	
AFP-ANU,PIVKA-II-L-N	60	60	1616	0.09
AFP-L-N,PIVKA-II-ANU	100	50	1816	
AFP-ANU,PIVKA-II-ANU	73	37	1493	

Table 7 Multivariate analysis by Cox's proportional hazard test of prognostic factors influencing tumor-free survival and overall survival

Variable	Tumor-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	RR* (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value
*Number of tumors (solitary/*multiple)	1.85(1.38-2.46)	<0.001	1.29 (0.89-1.87)	0.18	2.90(2.06-4.07)	<0.001	1.46 (0.89-2.24)	0.09
Size of tumor								
<2 cm								
2-5 cm	1.73 (1.30-2.30)	<0.001	1.67 (1.08-2.56)	0.020	2.76 (1.86-4.30)	<0.001	1.84 (1.16-2.92)	0.038
≥5 cm	1.45 (1.07-2.43)	<0.001	1.53 (1.06-2.21)	0.025	1.73 (1.16-2.58)	0.006	1.79 (0.91-3.31)	0.13
Japan TNM classification								
1								
2	1.47 (1.14-1.89)	0.003	1.67 (1.12-2.44)	0.011	1.44 (1.02-2.27)	0.045	1.38 (0.82-2.34)	0.67
3	1.75 (1.31-2.33)	<0.001	0.88 (0.54-1.45)	0.62	2.68 (1.65-4.33)	<0.001	1.03 (0.59-1.80)	0.87
4a	2.80 (1.87-4.19)	<0.001	1.26 (0.69-2.33)	0.35	6.50 (3.74-11.3)	<0.001	1.29 (0.94-2.63)	0.06
Liver cirrhosis								
No								
Yes	1.21 (0.94-1.55)	0.14			1.20 (0.86-1.65)	0.28		
Background liver								
Non-viral								
Viral	1.70 (0.98-2.58)	0.064			1.181 (0.69-2.00)	0.55		
Child-Pugh classification								
A								
B or C	1.53 (1.08-2.18)	0.017	1.21 (0.90-1.64)	0.210	1.81 (1.17-2.78)	0.007	2.27 (0.96-5.37)	0.06
Pretreatment AFP								
<20								
≥ 20	1.84 (1.42-2.38)	<0.001	1.77 (1.29-2.43)	<0.001	1.62 (1.08-2.44)	0.020	1.68 (0.56-2.45)	0.12
≥200	1.66 (1.26-2.19)	<0.001	0.90(0.64-1.27)	0.54	1.49 (1.01-2.20)	0.049	3.30 (0.94-11.6)	0.10
Pretreatment PIVKA-II								
<40								
≥40	1.67 (1.23-2.27)	<0.001	1.10 (0.76-1.595)	0.62	1.42 (1.02-2.94)	0.044	1.18 (0.73-1.91)	0.494
≥400	2.31 (1.76-3.04)	<0.001	1.75 (1.23-2.49)	0.002	1.91 (1.30-2.80)	<0.001	1.25 (0.81-1.93)	0.310
Changes of AFP								
L								
N	1.45 (0.78-2.72)	0.244	0.85 (0.48-1.50)	0.56	2.72 (1.36-5.45)	0.005	1.64 (1.28-2.49)	0.022
ANU	1.65 (0.84-3.24)	0.15	0.70 (0.35-1.40)	0.31	1.49 (1.15-2.65)	0.022	1.62 (0.78-2..46)	0.45
Changes of PIVKA-II								
L								
N	1.07 (0.68-1.69)	0.76	1.03 (0.55-1.91)	0.97	1.00(0.46-2.21)	0.95	1.56 (0.89-2.77)	0.19
ANU	1.49 (1.02-2.14)	0.045	1.17 (0.46-2.95)	0.67	2.50 (1.03-6.05)	0.043	4.43 (1.21-21.5)	0.040
Combination of changes of AFP and PIVKA-II								
AFP-L-N and PIVKA-II-L-N								
AFP-ANU and PIVKA-II-L-N	1.94 (1.16-3.22)	0.022	1.69 (0.64-4.22)	0.21	1.29 (0.89-2.12)	0.065	1.65 (0.24-18.8)	0.76
AFP-L-N and PIVKA-II-ANU	1.32 (1.03-1.58)	0.048	0.75 (0.41-1.45)	0.41	1.86 (0.87-3.39)	0.11	0.88 (0.36-1.87)	0.21
AFP-ANU and PIVKA-II-ANU	2.15 (1.35-3.56)	0.001	2.37 (1.24-4.56)	0.018	2.67 (1.44-6.20)	0.012	1.83 (1.05-4.78)	0.036
Treatment								
Hepatectomy								
Ablation	1.10 (0.83-1.47)	0.50			1.55 (1.03-2.35)	0.037	3.15(1.86-5.33)	<0.001

* Risk ratio. For abbreviations, see Table 1.

Table 8 Multivariate analysis by Cox's proportional hazard test of prognostic factors influencing tumor-free survival and overall survival in patients who underwent hepatectomy

Variable	Tumor-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	RR* (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value
Number of tumor								
solitary								
multiple	2.04 (1.49-2.78)	<0.001	1.20 (0.80-1.79)	0.376	2.81(1.95-4.03)	<0.001	1.76 (1.07-2.92)	0.030
Size of tumor								
<2cm								
2-5cm	1.41 (1.02-1.94)	0.036	1.16 (0.82-1.64)	0.401	1.70 (1.15-2.50)	0.008	1.47 (0.95-2.28)	0.091
≥5cm	1.79 (1.20-2.67)	0.004	1.82 (1.14-2.90)	0.013	1.81 (1.14-2.88)	0.012	1.14 (0.62-2.09)	0.676
Japan TNM classification								
1								
2	1.34 (0.87-2.06)	0.189	0.87 (0.53-1.42)	0.579	1.85 (1.27-2.63)	0.001	0.99 (0.51-1.92)	0.983
3	2.50 (1.59-3.94)	<0.001	1.24 (0.69-2.22)	0.477	2.35 (1.31-4.20)	0.004	1.34 (0.61-2.91)	0.465
4a	4.00 (2.35-6.82)	<0.001	1.71 (0.84-3.39)	0.127	5.36 (2.82-10.2)	<0.001	2.38 (0.95-5.98)	0.074
Liver cirrhosis								
No								
Yes	1.22 (0.92-1.62)	0.166			1.19 (0.84-1.69)	0.332		
Background liver								
Non-viral								
Viral	1.89 (1.19-3.00)	0.007	1.68 (1.04-2.70)	0.033	1.40 (0.79-2.49)	0.254		
Child-Pugh classification								
A								
B or C	2.05 (1.28-3.26)	0.003	1.67 (1.02-2.73)	0.041	2.68 (1.60-4.49)	<0.001	2.47 (1.42-4.29)	0.005
Pretreatment AFP								
<20								
≥20-200	1.98 (1.40-2.81)	<0.001	1.86 (1.28-2.72)	0.001	1.84 (1.27-2.67)	0.001	1.15 (0.69-1.91)	0.600
≥200	1.64 (1.21-2.21)	0.001	0.86 (0.59-1.26)	0.428	1.75 (1.21-2.52)	0.003	1.12 (068-1.84)	0.647
Pretreatment PIVKA-II								
<40								
≥40	1.77 (1.17-2.67)	0.007	1.09 (0.68-1.75)	0.726	1.38 (0.83-2.29)	0.211	0.91 (0.50-1.66)	0.762
≥400	2.07 (1.47-2.91)	<0.001	1.46 (0.99-2.14)	0.041	1.84 (1.25-2.71)	0.002	1.21 (0.72-2.04)	0.474
Changes of AFP								
L								
N	1.43 (0.86-2.35)	0.165			1.54 (0.84-2.82)	0.160	0.92 (0.50-1.67)	0.782
ANU	1.41 (0.75-2.65)	0.287			2.04 (1.00-4.16)	0.049	1.21 (0.69-1.93)	0.595
Changes of PIVKA-II								
L								
N	1.01 (0.57-1.80)	0.969			2.56 (1.23-5.26)	0.009	0.91 (0.02-66.4)	0.943
ANU	1.06 (0.59-1.91)	0.842			2.99 (1.47-6.02)	0.002	1.21 (0.052-32.9)	0.524
Combination of changes of AFP and PIVKA-II								
AFP-L-N and PIVKA-II-L-N								
AFP-ANU and PIVKA-II-L-N	1.97 (0.88-4.41)	0.101			1.57 (0.63-3.91)	0.334	1.65 (0.62-4.43)	0.318
AFP-L-N and PIVKA-II-ANU	0.87 (0.44-1.69)	0.672			1.84 (0.85-3.96)	0.124	0.47 (0.09-2.43)	0.371
AFP-ANU and PIVKA-II-ANU	1.19 (0.46-3.09)	0.719			3.69 (1.42-9.59)	0.007	1.03 (0.09-2.43)	0.971

5 * Risk ratio. For abbreviations, see Table 1.