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Postoperative changes in protein-induced vitamin K absence or antagonist II levels after hepatectomy in patients with hepatocellular carcinoma: relationship to prognosis

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Abstract

Background. α-Fetoprotein (AFP) has been used as a marker for hepatocellular carcinoma (HCC). However, AFP levels are often high in patients with chronic hepatitis or cirrhosis. Protein-induced vitamin K absence or antagonist II (PIVKA-II) is more sensitive for the diagnosis of HCC and prediction of patient survival. Changes in these markers after treatment may reflect treatment curability and patient outcome. *Methods.* We conducted a retrospective analysis of prognosis of 63 HCC patients with high preoperative levels of AFP and PIVKA-II who underwent hepatectomy and examined the relationship between postoperative changes in both markers at 1 month and patient survival. Subjects were divided into three groups according to changes in these tumour markers after hepatectomy: normalization (N) group, decreased but still above the normal level (D) group and unchanged (U) group. *Results.* There were no significant differences in the numbers of patients who developed tumour recurrence between changes in AFP and PIVKA-II. Survival analysis showed no significant differences in tumour-free and overall survivals between groups with respect to AFP level. The PIVKA-II-N group showed significantly better tumour-free and overall survival compared with the D and U groups ($p < 0.01$). Multivariate analysis that included other prognostic factors identified changes in PIVKA-II level as a significant and independent prognostic factor associated with overall survival. *Discussion.* Although changes in AFP did not correlate with patient prognosis, normalization of PIVKA-II was significantly associated with good patient survival after hepatectomy. Normalization of PIVKA-II after hepatectomy reflected the efficacy of treatment and is a suitable predictor of prognosis in HCC patients.

Key Words: *Hepatocellular carcinoma, hepatectomy, PIVKA-II, AFP*

Introduction

Sensitive tumour markers are useful for accurate diagnosis of solid tumours. α-Fetoprotein (AFP) has been used as a marker for hepatocellular carcinoma (HCC) [1–4]. However, AFP levels are often high in patients with chronic hepatitis or cirrhosis [5], therefore it is difficult to accurately evaluate tumour aggressiveness. Protein induced by vitamin K absence or antagonist II (PIVKA-II) is more specific for the diagnosis and evaluation of tumour aggressiveness and patient prognosis compared with AFP [5–7]. In patients with resectable HCC, our group and other investigators reported that preoperative PIVKA-II was also a useful marker for predicting intrahepatic spread, tumour recurrence and patient prognosis, while AFP was not closely related to clinical outcome [8–11].

We hypothesized that PIVKA-II and other sensitive tumour markers for HCC would return to normal levels immediately after the complete resection of HCC, and that such normalization would reflect improvement of patient prognosis. To test our hypothesis, we selectively examined in the present study 63 patients with HCC who had high AFP and PIVKA-II levels before hepatic resection. We evaluated the relationship between changes in both markers after hepatectomy and patient survival. Based on our results, we also assessed the usefulness of these tumour markers post-hepatectomy for the evaluation of treatment efficacy.

Materials and methods

Data were collected from 94 patients with HCC during surgery on patients who were admitted to the Division of Surgical Oncology, Department of Translational Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences (NUGSBS)

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between 1996 and September 2003. Sixty-three of 94 patients (65%) with preoperatively high AFP and PIVKA-II levels were selected in the present study. Patients with remnant tumour after hepatectomy were excluded from this study. The mean age of the patients at the time of surgery was 64 ± 8 years (range 45–81 years), and they comprised 55 males and 8 females. The background liver abnormalities included chronic viral hepatitis in 37 (58%) patients, cirrhosis in 24 (38%) and normal liver in 2 (4%), associated with hepatitis virus B ($n=27$), hepatitis virus C ($n=35$), or both hepatitis B and C ($n=1$). According to the Child-Pugh classification, 60 patients were classified as A and 3 as B. The pathological tumour node metastasis (TNM) stage of HCC according to the Liver Cancer Study Group of Japan [12] was stage I in 2 (3%), stage II in 29 (46%), stage III in 26 (41%) and stage IVA in 6 (10%). Prior to surgery for HCC, three patients were treated with chemoembolization, although such treatment failed to completely control the growth of the tumours. After surgery, none of the patients received adjuvant chemotherapy. The operative procedures included resection segments 2–4 ($n=11$), resection segments 1–4 ($n=1$), resection segments 5–8 ($n=12$), resection segments 4–8 ($n=1$), resection segments 5, 8 ($n=4$), resection segments 7, 8 ($n=6$), resection segments 5, 6 ($n=4$), resection segments 6, 7 ($n=7$), resection segments 2, 3 ($n=3$), resection segment 2 ($n=1$), resection segment 4 ($n=2$), resection segment 5 ($n=1$), resection segment 6 ($n=3$), resection segment 7 ($n=3$) and resection segment 8 ($n=4$) [13]. All hepatic tumours were completely resected without macroscopic exposure of the remaining liver to the amputated section. After checking at 1 month post-hepatectomy, the patients were followed up by measuring serum levels of AFP and PIVKA-II every 3 months, and enhanced computed tomography (CT) of the liver was carried out every 6 months for at least the first 5 years after hepatectomy to rule out tumour recurrence.

The minimum follow-up period after hepatic resection of HCC was 6 months. Two of 63 (3.1%) patients who survived were lost to follow-up and their data were censored at the last date that they were known to be alive. Of the 43 (61%) patients who developed tumour recurrence after hepatectomy (in liver, $n=42$ and bone, $n=1$), 20 were treated with chemoembolization, 11 received ablation therapy, 1 underwent re-resection, and 11 patients received no additional therapies. For the whole group, 27 (43%) patients died of cancer and none died by other diseases in the present study. The study design was approved by the Ethics Review Board of NUGSBS including collection of data from the NUGSBS database, which were also provided by the associated hospitals described above.

In the early morning, a 4-ml peripheral blood sample was collected from each patient before and 1 month after hepatectomy. The sample was then centrifuged at 3000 rpm (1000 *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 in our hospital is <20 ng/ml. The normal value of PIVKA-II has been determined to be <40 mAU/ml [11]. Elevated levels of AFP and PIVKA-II were defined as those exceeding the above levels. In the present study, the pre-hepatectomy levels of AFP and PIVKA-II of all 63 patients were higher than the normal ranges. Patients were divided into three groups based on changes in these markers after hepatectomy as follows: normalization of these markers (i.e. return to within the normal range, the N group), marker levels decreased relative to preoperative level but were still higher than the normal ranges (D group), and tumour marker did not change (U group).

Distribution of time to treatment since 1 January 1990 was compared between groups by the Wilcoxon rank-sum 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. 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, USA).

Results

High AFP level was observed in 67 patients and high PIVKA-II level was observed in 65 patients. The mean survival period in HCC patients with a normal AFP level (2298 days) was significantly greater than that in patients with a higher AFP level exceeding 20 ng/ml (1350 days) ($p<0.01$). The 1-, 3- and 5-year survival rates in HCC patients with a normal AFP level were 93%, 83% and 70% and those in patients with a higher AFP level were 82%, 56% and 47%, respectively. The overall survival in patients with a normal AFP level was significantly greater than that in patients with a higher AFP level. With respect to PIVKA-II, the mean survival period in HCC patients with a normal PIVKA-II level (2378 days) was significantly greater than that in patients with a higher PIVKA-II level exceeding 40 mAU/ml (1540 days) ($p<0.01$). The 1-, 3- and 5-year survival rates in HCC patients with normal PIVKA-II level were 97%, 86% and 78% and those in patients with higher PIVKA-II level were 85%, 62% and 51%, respectively. The overall survival in patients with a normal PIVKA-II level was significantly greater than that in patients with a higher PIVKA-II.

With regard to the AFP level, 42 patients were classified as the N group, 13 as the D group and 8 as the U group. With respect to the PIVKA-II level, 49 patients classified as the N group, 11 as the D group and 3 as the U group. The 25th, 50th and 75th sample percentiles of the time to treatment according to AFP level was not significantly different between groups

(2.4 years, 4.9 years, 6.1 years for the N group; 2.9, 3.7, 5.4 for the D group; and 1.9, 4.0, 6.0 for the U group). Similarly, the 25th, 50th and 75th sample percentiles of the time to treatment according to PIKA-II level were not significantly different between groups (2.8, 4.0, 5.9 for the N group; 2.5, 4.0, 5.2 for the D group; and 1.8, 2.5, 6.1 for the U group).

The numbers of patients who developed tumour recurrence postoperatively according to AFP level were 26 (62%) patients of the N group, 10 (77%) of the D group and 7 (88%) of the U group. The numbers of patients who developed tumour recurrence postoperatively according to PIVKA-II level were 30 (48%) patients of the N group, 10 (91%) of the D group and 3 (100%) of the U group. There were no significant differences in these groups with respect to changes in AFP and PIVKA-II.

We also examined the relationship between changes in tumour markers and mortality rate. The numbers of patients who died postoperatively were 11 (27%) patients of the AFP-N group, 8 (62%) of the AFP-D group and 8 (100%) of the AFP-U group, 16 (33%) patients of the PIVKA-II-N group, 9 (82%) of the PIVKA-II-D group and 2 (67%) of the PIVKA-II-U group. There were no significant differences in these distributions in these groups with regard to changes in AFP and PIVKA-II.

Tumour-free survival of patients in the AFP-N group tended to be better than those of the AFP-D and AFP-U groups, although the difference was not significant (Figure 1A). The difference in overall survival between N, D and U groups was not significant (Figure 1B). The tumour-free and overall survivals of the PIVKA-II-N group were significantly better than those of the PIVKA-II-D and PIVKA-II-U groups (Figure 2A and B). Table I shows the significant prognostic factors, including changes in AFP and PIVKA-II levels after hepatectomy, associated with tumour-free and overall survival after hepatectomy, identified by the univariate analysis in the present series. Multivariate analysis showed that although the number and size of tumour, and long-term ascites after hepatectomy correlated significantly with tumour-free survival, changes in both tumour markers were not significant factors. On the other hand, the surgical margin of the resected liver and changes in PIVKA-II levels were significantly associated with overall survival after hepatectomy.

Discussion

At present, more specific HCC markers, such as PIVKA-II or the percentage of lens culinaris agglutinin A-reactive fraction of AFP (AFP L3 fraction)

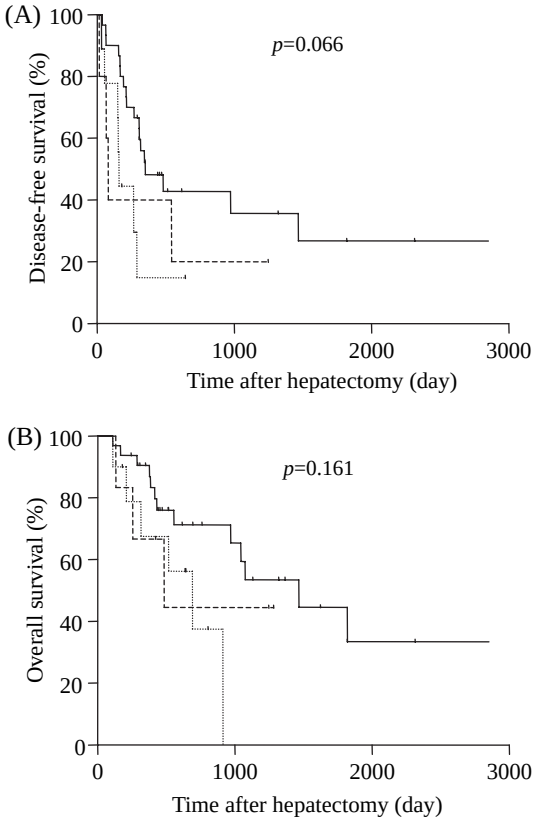

Figure 1. Relationship between postoperative AFP level and patient disease-free (A) and overall (B) survival after hepatectomy. Solid line = normalization group (N group, $n = 42$); large dotted line = D group--postoperative AFP level was less than the preoperative value but still higher than normal ($n = 13$); small dotted line = unchanged group (U group, $n = 8$).

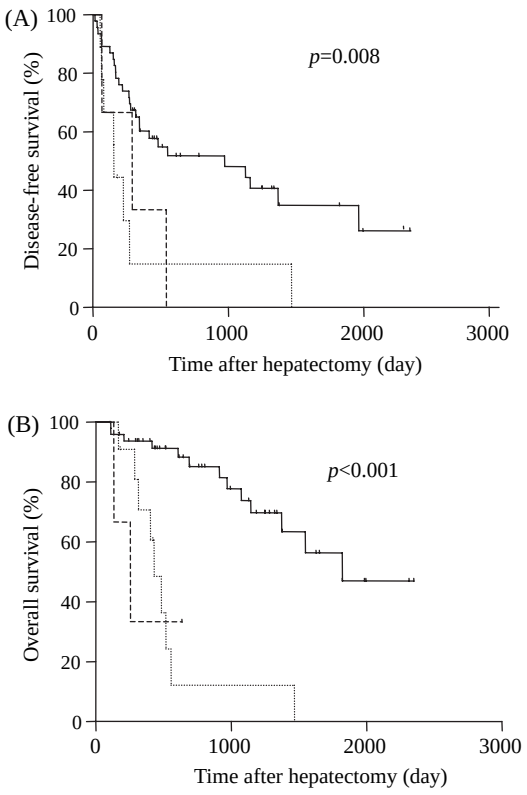

Figure 2. Relationship between postoperative PIVKA-II level and patient disease-free (A) and overall (B) survival after hepatectomy. Solid line = N group ($n = 49$); large dotted line = D group ($n = 11$); small dotted line = U group ($n = 3$).

Table I. Multivariate analysis by Cox's proportional hazard test of prognostic factors influencing tumour-free survival and overall survival

Variable	Tumour-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 tumours multiple vs solitary	1.85 (1.02–3.35)	0.044	2.28 (1.13–4.61)	0.022	2.25 (1.02–4.93)	0.044	1.49 (0.57–3.89)	0.410
Size of tumour $\geqslant$3 cm vs $<$3 cm	2.39 (1.24–4.43)	0.006	3.93 (1.16–13.31)	0.028	3.86 (1.51–9.86)	0.005	4.45 (0.78–25.27)	0.092
Vascular invasion Yes vs No	2.34 (1.21–4.52)	0.011	1.79 (0.85–3.78)	0.129	3.72 (1.57–8.81)	0.003	1.97 (0.72–5.39)	0.187
Surgical margin $<$5 mm vs $\geqslant$5 mm	2.54 (1.30–4.97)	0.006	2.01 (0.98–4.10)	0.559	2.85 (1.19–6.38)	0.019	2.94 (1.07–8.12)	0.037
Intraoperative bleeding $\geqslant$1500 ml vs $<$1500 ml	1.82 (0.87–3.81)	0.113	0.89 (0.35–2.28)	0.816	3.52 (1.53–8.09)	0.003	1.23 (0.41–3.74)	0.711
Long-term ascites Yes vs No	3.09 (1.58–6.01)	$<$0.001	2.38 (1.10–5.13)	0.028	4.40 (1.92–10.08)	$<$0.001	2.26 (0.78–6.49)	0.132
Changes of AFP D, U group vs N group	2.16 (1.10–4.26)	0.027	1.07 (0.42–2.70)	0.891	2.03 (0.85–7.06)	0.088	0.88 (0.28–2.71)	0.819
Changes of PIVKA-II D, U group vs N group	2.38 (1.22–4.62)	0.011	1.64 (0.77–3.49)	0.204	5.51 (2.561–13.04)	$<$0.001	3.76 (1.02–13.88)	0.047

RR, risk ratio; CI, confidence interval.

[6–8,14,15], relative to AFP level, are usually used for evaluation of HCC aggressiveness. High values of these markers reflect patient prognosis after any treatment modality [8–11,14–17]. Using multivariate analysis, we previously showed that although the prognosis of HCC patients is influenced by the complex of hepatic function, tumour-related factors and treatment-related factors [18–20], HCC staging including PIVKA-II level is an independent prognostic marker in HCC patients who undergo hepatectomy [21]. Monitoring of PIVKA-II is useful for predicting tumour recurrence after hepatic resection for HCC at an earlier period than AFP level [22,23]. PIVKA-II and AFP may decrease after effective treatment of HCC. Therefore, in the present study we focused on changes in these markers at an early (i.e. 1 month) postoperative time point.

In the present study, HCC patients with preoperatively normal AFP/PIVKA-II levels were not included in the present study because changes in these markers could not be evaluated and prognosis of such patients was better [1–4,8–11,21–23]. In the present study, in fact, HCC patients with a normal AFP or PIVKA-II level had a better prognosis than patients with a higher level, respectively. Therefore, we examined HCC patients with high preoperative levels of both AFP and PIVKA-II and all patients underwent macroscopically complete resection. These tumour markers decreased markedly during the 1-month postoperative period in about 90% of the patients. However, the number of patients who showed postoperative normalization of PIVKA-II level was more than that of AFP level. A high level of AFP after hepatectomy might reflect persistent activity of chronic hepatitis [5]. In the present study, patients who showed normalization of PIVKA-II had a good prognosis, although the presence of a high preoperative level of PIVKA-II is known to correlate with poor prognosis [8–11]. On the other hand, patients in the D group (in whom PIVKA-II decreased postoperatively, but the level was still above normal) and U group (no change in the marker level post-operatively) showed poor prognosis after surgery. Kanazumi *et al.* [24] reported that serum PIVKA-II level was decreased within 2 weeks after effective operation and, therefore, a PIVKA-II level higher than than normal range might reflect remnant HCC. In the present series, no additional treatment was provided based only the serum level of tumour markers at 1 month after hepatectomy in the D or U groups. Currently, there are no standardized rules for the selection of specific treatment modality for recurrent tumour detected by imaging studies, e.g. enhanced CT. However, taking into consideration the present results, we suggest that in patients with high PIVKA-II level after hepatectomy, adjuvant therapy such as chemotherapy should be provided to control invisible remnant HCC cells, which could lead to better patient survival.

AFP level after hepatectomy did not correlate with patient survival. Recent studies showed that the percentage of AFP L3 fraction was a better specific marker for HCC than AFP level [14–17]. It is necessary to examine this marker before and after hepatectomy for better assessment of patient prognosis in the next step of our study. If this marker is useful, similar to PIVKA-II, the combination of these tumour markers may be useful for evaluation of tumour aggressiveness.

In summary, we conducted a retrospective analysis of the prognosis of 63 HCC patients who underwent hepatic resection, including examination of the relationship between changes in AFP and PIVKA-II after hepatectomy and patient survival. Compared to change in AFP, normalization of PIVKA-II level was more significantly associated with good patient survival after hepatectomy by univariate and multivariate analyses. Normalization of PIVKA-II level after hepatectomy reflected the efficacy of the treatment and is a suitable predictor of prognosis in HCC patients.

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