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Prognostic significance of a combination of pre- and post-treatment tumor markers for hepatocellular carcinoma curatively treated with hepatectomy

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Background & Aims: Previous studies reported that the combination of three tumor markers for hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP), has the ability to discriminate survival among patients with HCC. In those studies, however, the study population included all patients with various treatment modalities, and tumor markers were measured only before treatment. We investigated the prognostic value of a combination of these tumor markers for HCC, measured before and after treatment, on survival and recurrence in patients treated with hepatectomy.

Methods: A total of 173 patients who underwent hepatectomy for primary, non-recurrent HCC were analyzed. Tumor characteristics, postoperative survival, and recurrence rates were compared according to the number of elevated tumor markers measured before and after treatment.

Results: The correlation between the number of elevated tumor markers before treatment and tumor size, rate of portal vein invasion, and tumor differentiation, respectively, was stronger than that between the number of elevated tumor markers after treatment. In contrast, the number of elevated tumor markers after treatment displayed an excellent ability to discriminate post-treatment survival and recurrence rates compared to that before treatment, and was an independent factor associated with survival and recurrence in multivariate analysis.

Conclusions: The combination of tumor markers measured after hepatectomy has a better discriminatory ability for postoperative survival and recurrence in HCC patients treated with hepatectomy in comparison to the combination of tumor markers measured before treatment.

Keywords: Hepatocellular carcinoma; Tumor markers; Survival; Recurrence; Curative hepatectomy.

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Abbreviations: HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; PIVKA-II, vitamin K absence/antagonist-II; CT, computed tomography; US, ultrasound; MRI, magnetic resonance imaging.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death [1,2]. In Japan, HCC currently represents the third and fifth most common cause of death from cancer in men and women, respectively, [3]. Presently, three tumor markers specific for HCC are used clinically: alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP), which is also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II). The clinical utility of these tumor markers for detection and diagnosis of HCC, for evaluation of tumor progression, and for determination of prognosis has been reported [4–7]. In addition, the combination of these three tumor markers has been indicated as a useful predictor of patient outcome. We previously reported the prognostic significance of the combination of three tumor markers measured at diagnosis on the survival of all patients with HCC [8]. An increase in the number of elevated tumor markers, consisting of AFP, AFP-L3, and DCP, was clearly associated with a decreased survival rate in patients with HCC. In addition, an increase in the number of elevated tumor markers was well correlated with indicators of HCC progression, including the size and number of tumors, and the rate of portal vein invasion. More recently, Kim *et al.* have reported less progression of HCC without the elevation of AFP and DCP, with higher survival rates [9].

However, in these studies, all patients with HCC who underwent various treatment modalities had been included into the study. In addition, the levels of tumor markers were measured at diagnosis and before treatment. The predictive ability of post-treatment *vs.* pretreatment tumor markers has not been evaluated and compared. In the present study, we measured the levels of these three tumor markers both before and after treatment, in patients who underwent hepatectomy with curative intent. We analyzed their relationship with tumor progression, survival, and recurrence after treatment.

Materials and methods

Patients

A total of 828 patients were diagnosed with primary, non-recurrent HCC, between January 2001 and December 2010 at Ogaki Municipal Hospital. Of these

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patients, 264 were treated with hepatectomy. Stored serum samples were available for measurement of the levels of three tumor markers, AFP, AFP-L3, and DCP, before and after hepatectomy in 173 patients. Decisions regarding each patient's course of treatment were made based on the treatment guidelines for HCC in Japan [10]. Anatomical hepatectomy was performed in all 173 patients. HCC tumors were resected with ample margins and enucleation of tumors without adequate margins was not performed. The diagnosis of HCC was confirmed by pathologic examination of resected specimens.

One month after hepatectomy, all patients underwent computed tomography (CT) examination of thorax and abdomen to confirm the absence of residual HCC. All patients were followed-up, for a median of 34.2 months (range, 4.3–122.8 months) until March 2012 at our institution, with ultrasound (US) and CT, or US and magnetic resonance imaging (MRI) every 3–6 months. Regular monitoring of the three tumor markers was performed every 3 months. When an elevation of tumor markers was detected, additional imaging examinations (usually CT or MRI) were performed to check for recurrence. If the presence of recurrence was confirmed, patients underwent treatment for recurrent HCC based on treatment guidelines.

The entire protocol was approved by the hospital institutional review board and carried out in compliance with the Helsinki Declaration.

Measurement of hepatocellular carcinoma tumor markers

Pretreatment tumor markers were measured within 1 week before hepatectomy. Post-treatment tumor markers were measured in the serum sample obtained at the first visit, between 1 and 2 months after hepatectomy. The reported half-lives of AFP and AFP-L3 are 4 days [11] and the half-life of DCP is 60 h [12]. Therefore, the values of post-treatment tumor markers were not influenced by pretreatment tumor marker elevations. Measurements of AFP, AFP-L3, and DCP levels were performed with a microchip capillary electrophoresis and liquid-phase binding assay on the μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) [13]. The cut-off value of 20 ng/ml was used to establish positivity for AFP, as proposed by Oka *et al.* and Koda *et al.* [14,15]. The cut-off value used to establish positivity for AFP-L3 was 5%, based on our previous study [16]. The cut-off value used to establish positivity for DCP was 40 mAU/ml, as proposed by Okuda *et al.* [17]. The number of tumor markers above the cut-off values was calculated as the number of elevated tumors markers, and survival and recurrence rates were analyzed according to the number of elevated tumor markers.

Statistical analyses

Differences in percentages between groups were analyzed with the Chi-square test. Differences in mean quantitative values were analyzed with the Mann–Whitney *U* test. Changes in percentages and quantitative values with the increase in the number of elevated tumor markers were analyzed with the Cochran–Armitage test and the Jonckheere–Terpstra test, respectively. Receiver-operating characteristics analyses were performed to determine the cut-offs of the number of elevated tumor markers in order to evaluate the accuracy of prediction of 1-, 3-, and 5-year survivals and recurrences and compare them with the accuracy of elevation of each tumor marker. The date of hepatectomy was defined as time zero for the calculation of survival and recurrence rates. In the analysis of survival rates, patients who died were non-censored and patients who survived were censored. In the analysis of recurrence rates, patients in whom HCC recurred were non-censored, and those in whom HCC did not recur were censored. The Kaplan–Meier method [18] was used to calculate survival and recurrence rates, and the log-rank test [19] was used to analyze differences in survival and recurrence.

The Cox proportional hazards model [20] was used for multivariate analyses of factors related to survival and recurrence. Variables analyzed included age, sex, Child-Pugh class (A/B), tumor size, number of tumors, differentiation of HCC (well-moderately or poorly), growth pattern (expansive growth/infiltrative growth), macroscopic and microscopic portal vein invasion (absent/present), and number of elevated tumor markers (zero, one, two, or three). Data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC, USA). All *p* values were derived from two-tailed tests, with $p < 0.05$ considered to indicate statistical significance.

Results

Characteristics of patients and hepatocellular carcinoma

Table 1 summarizes the pretreatment characteristics of the study patients. This population comprised 136 males and 37 females with a mean age of 67.0 ± 8.8 years. Most (95.4%) patients belonged to Child-Pugh class [21] A. Multiple tumors were present in 16.8% of patients. HCC was well differentiated in 37.0% and portal vein invasion was observed in 23.1% of patients, based on the pathologic examination of resected HCC specimens. Pretreatment AFP, AFP-L3, and DCP were above the specified cut-off levels in 34.7%, 44.5%, and 52.0% of patients, respectively.

Clinical and pathologic characteristics of hepatocellular carcinoma based on a combination of three tumor markers measured before and after hepatectomy

At pretreatment, there were 47 (27.2%) patients with no elevated tumor markers and 57 (32.9%) patients with one, 38 (22.0%) with two, and 31 (17.9%) with three elevated tumor markers. After hepatectomy, 75 (43.3%) patients had no elevated tumor markers, 70 (40.5%) patients had one, 24 (13.9%) had two, and 4 (2.3%) had three elevated tumor markers. Tables 2 shows pretreatment clinical characteristics and pathologic characteristics of the resected HCC specimens according to the number of elevated tumor markers measured before and after hepatectomy. An increase in tumor size was associated with an increase in the number of elevated tumor markers before treatment ($p < 0.0001$). This gradual increase in tumor size according to the number of elevated tumor markers was not observed with post-treatment values ($p = 0.5836$). On pathologic examination, there was a gradual decrease in the rate of well-differentiated HCC ($p < 0.0001$) and of HCC with expansive growth ($p = 0.0010$), and a gradual increase in the rate of HCC with portal vein invasion ($p < 0.0001$) based on the number of elevated tumor markers before treatment. These are not significant when compared with postoperative values (the rate of well-differentiated HCC, $p = 0.3962$, of HCC with expansive growth, $p = 0.3036$, and the rate of HCC with portal vein invasion, $p = 0.0898$).

Post-operative survival rates based on the combination of three tumor markers measured before and after hepatectomy

The survival rates were compared by the elevation of each tumor marker. By comparing tumor markers measured before treatment, we found significant differences in survival rates by elevated AFP, and AFP-L3 levels, but not DCP (Supplementary Fig. 1). By comparing tumor markers measured after treatment, survival rates were significantly lower in patients with elevated AFP, AFP-L3, and DCP levels, respectively (Supplementary Fig. 2). We determined the survival rates of patients after hepatectomy as a function of the number of elevated tumor markers before and after hepatectomy (Fig. 1). The number of elevated tumor markers after treatment provided a better discrimination of survival rates than the number of elevated tumor markers before treatment. The survival rates were higher in patients without elevated tumor markers after treatment, followed by patients with one, two, and three elevated tumor markers, in this order.

We next compared the accuracy of death prediction between each individual tumor marker and the combination of the three (Supplementary Table 1). Higher accuracy in predicting death within 1, 3, and 5 year(s), respectively, was found by combining the three markers than by each individual marker alone.

In multivariate analysis, the number of elevated tumor markers was not associated with specific survival after hepatectomy, when tumor markers measured before treatment were used (Supplementary Table 2). In contrast, it was an independent