

Differences in the impact of prognostic factors for hepatocellular carcinoma over time

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The aim of the present study was to evaluate the prognostic significance of serum markers that reflect tumor progression, liver function, or liver fibrosis in patients with hepatocellular carcinoma (HCC), focusing on how their impact changes over time after diagnosis. Alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP), albumin-bilirubin (ALBI) score, aspartate aminotransferase to platelet ratio index (APRI), and FIB-4 index were measured at the time of initial non-recurrent HCC diagnosis in 1669 patients between 1997 and 2016. Survival rates after diagnosis were compared after stratifying patients by these markers. Time-dependent receiver-operating characteristics (ROC) analysis was carried out to assess how these markers predict patient survival or death. Serum AFP and DCP levels, ALBI score, and APRI and FIB-4 index were strongly correlated with HCC progression, liver function, and degree of liver fibrosis, respectively. Survival rates after diagnosis were significantly different when patients were stratified by these markers. In the time-dependent ROC analysis, AFP and DCP had a high prognostic impact within 3 years of diagnosis but the impact decreased thereafter. In contrast, APRI and FIB-4 index had higher prognostic impact 10 years after diagnosis. ALBI score had a high prognostic impact throughout the study period. Time-dependent ROC analysis clearly showed changes in the prognostic importance of serum markers based on the duration after diagnosis. Whereas the prognostic impact of tumor progression markers was strong in the short term, liver fibrosis markers had higher prognostic impact long after diagnosis. Liver function had constant prognostic impact on patient survival after diagnosis.

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide. It is currently the second most common cause of cancer-related death in the world.^(1,2) Although the outcome of patients with most cancers is usually influenced by tumor progression, liver function at diagnosis also affects the prognosis of patients with HCC. Because most HCC develop in the presence of liver cirrhosis, impaired liver function will cause poor prognosis. Furthermore, it will limit treatment options, which also influence patient survival. In addition to these prognostic factors, the degree of fibrosis in the background liver where HCC develops is an additional factor that can affect the prognosis of patients with HCC, because it is associated with a high potential of HCC recurrence. Several studies have reported the influence of liver fibrosis on recurrence and survival rates in patients with HCC, especially when patients have undergone curative treatment.^(3–5)

Several serum markers are associated with HCC progression, liver function, or liver fibrosis and, consequently, the prognosis of patients with HCC. They include tumor markers for HCC such as AFP and DCP for HCC progression,^(6–8) the recently reported ALBI score for liver function,^(9–11) and APRI and FIB-4 index for liver fibrosis.^(12,13)

Although these markers are reportedly associated with prognosis in patients with HCC,^(7–11,14,15) it is unclear whether

these markers similarly affect the prognosis of patients with HCC in the short term and long term after diagnosis. Therefore, in the present study, we evaluated the impact of serum prognostic markers of HCC, including tumor markers, liver function markers, and liver fibrosis markers, on patient outcomes after diagnosis, while focusing on the timing of when each factor has an impact.

Materials and Methods

Patients. A total of 1669 patients were diagnosed with primary, non-recurrent HCC at our institution between 1997 and 2016, all of whom were included in this study. The diagnosis of HCC was based on appropriate imaging characteristics in the American Association for the Study of Liver Diseases guidelines.^(16,17) In patients who underwent hepatic resection and had HCC specimens available, the diagnosis of HCC was confirmed based on pathology. All patients underwent imaging studies including contrast-enhanced computed tomography or magnetic resonance imaging in addition to ultrasonography, and the progression of HCC was evaluated at diagnosis. Decisions regarding treatment for each individual patient were based on Japanese treatment guidelines for HCC.⁽¹⁸⁾

The study protocol was approved by the institutional review board and was in compliance with the Helsinki Declaration. Informed consent was waived for this retrospective study.

Measurement of tumor markers and calculation of ALBI score, APRI and FIB-4 index. Pretreatment laboratory data, including tumor marker levels, were measured at the time of diagnosis. Serum AFP levels were measured using microchip capillary electrophoresis and a liquid-phase binding assay on a μ TAS-Wako i30 auto-analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan).⁽¹⁹⁾ Serum DCP levels were determined using an enzyme immunoassay (Eitest PIVKA-II kit; Eisai Co., Ltd, Tokyo, Japan).^(20–22)

The ALBI score was calculated based on serum ALB and T-Bil levels using the following formula⁽⁹⁾:

$$\text{ALBI} = (\log_{10} \text{T-Bil}[\mu\text{mol/L}] \times 0.66) + (\text{ALB}[\text{g/L}] \times -0.085),$$

where 1 mg/dL = 17.1 μ mol/L for T-Bil and 1 g/dL = 10 g/L for ALB. The values were stratified into three categories according to previously reported cut-offs,⁽⁹⁾ resulting in three grades: grade 1 (better liver function, ≤ -2.60), grade 2 (> -2.60 and ≤ -1.39), and grade 3 (poorer liver function, > -1.39).

The APRI and FIB-4 index were calculated as follows^(12,13,23):

$$\text{APRI} = (\text{AST}[\text{IU/L}] / \text{upper limit of normal AST}[\text{IU/L}]) \times 100 / \text{platelet count} [10^9/\text{L}] \text{ and}$$

$$\text{FIB-4 index} = \text{AST}[\text{IU/L}] \times \text{age} [\text{years}] / \text{platelet count} [10^9/\text{L}] \times \text{ALT}[\text{IU/L}]^{1/2}.$$

Because AFP and DCP have logarithmic distributions, \log_{10} AFP and \log_{10} DCP were used for analyses. The median value was used as the cut-off between high and low AFP, DCP,

APRI, and FIB-4 index, respectively, when comparing survival or recurrence rates. Regarding liver function, ALBI grades were used for patient grouping.

Statistical analysis. Differences in percentages between groups were analyzed using the chi-squared test. Differences in means of quantitative values were analyzed using the Mann–Whitney *U*-test. Date of HCC diagnosis was defined as time zero for the calculation of survival rates. Survival was defined as time from diagnosis to death, or to last follow up if death had not occurred. Patients who died were not censored. Surviving patients were censored. The Kaplan–Meier method was used to calculate survival rates, and the log–rank test was used to analyze differences in survival.

Cox proportional hazards models were used for univariate and multivariate analysis of factors related to survival and recurrence. Factors analyzed were age, sex, Child–Pugh class,⁽²⁴⁾ tumor size, number of tumors, portal vein invasion, and serum markers for tumor progression, liver function, and degree of liver fibrosis reflected by AFP, DCP, ALBI score, APRI, and FIB-4 index. Univariate analysis was first carried out. Variables that reached statistical significance ($P < 0.05$) in the univariate analysis were subsequently included in the multivariate analysis. Time-dependent ROC analysis was done to evaluate the performance of serum markers in predicting patient survival or death based on the duration since diagnosis.

Statistical analysis was carried out using JMP statistical software, version 11.0 (Macintosh version; SAS Institute, Cary, NC, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria),⁽²⁵⁾ designed to provide statistical functions frequently used in biostatistics. The R package “survivalROC” was used for performance assessment in time-dependent ROC curve estimation. All *P*-values were derived from two-tailed tests, with $P < 0.05$ considered to indicate statistical significance.

Table 1. Characteristics of the study patients ($n = 1669$)

Age (median, IQR, years) (mean \pm SD)	70 (63–75), 68.7 \pm 9.5
Male/female	1181 (70.8)/488 (29.2)
Etiology of HCC (HBV/HCV/HBV + HCV/non-HBV, non-HCV)	239 (14.3)/1108 (66.4)/17 (1.0)/305 (18.3)
Child–Pugh class (A/B/C)†	1181 (70.8)/365 (21.9)/123 (7.4)
ALT (mean \pm SD, IU/L)	55.2 \pm 51.9
AST (mean \pm SD, IU/L)	69.8 \pm 62.4
Albumin (mean \pm SD, g/dL)	3.59 \pm 0.63
Total bilirubin (mean \pm SD, mg/dL)	1.19 \pm 1.17
Platelet count (mean \pm SD, $\times 1000/\text{mL}$)	138 \pm 83
Tumor size (≤ 2 cm/ > 2 cm and ≤ 5 cm/ > 5 cm)	560 (33.6)/665 (39.8)/444 (26.6)
No. tumors (single/multiple)	1025 (61.4)/644 (38.6)
Portal vein invasion (absent/present)‡	1387 (83.1)/282 (16.9)
BCLC staging (0/A/B/C/D)	275 (16.5)/731 (43.8)/203 (12.2)/307 (18.4)/153 (9.2)
Milan criteria (within/without)	982 (58.8)/687 (41.2)
Degree of liver fibrosis in non-cancerous tissue (F1/F2/F3/F4)§	20 (5.5)/56 (15.4)/96 (26.4)/192 (52.7)
AFP (median, IQR, ng/dL), \log_{10} AFP (mean \pm SD)	20.3 (6.5–173.6), 1.67 \pm 1.22
DCP (median, IQR, ng/dL), \log_{10} DCP (mean \pm SD)	62 (20–862), 2.20 \pm 1.10
ALBI score	–2.36 (–2.76 – –1.86), –2.27 \pm 0.64
ALBI grade (1/2/3)	602 (36.1)/898 (53.8)/169 (10.1)
APRI (median, IQR) (mean \pm SD)	1.2 (0.7–2.1), 1.70 \pm 1.66
FIB-4 index	5.09 (3.03–7.94), 6.21 \pm 4.54
Initial treatment (resection/LAT/TACE/others/none)	566 (33.9)/277 (16.6)/470 (28.2)/102 (6.1)/254 (15.2)

Percentages are given in parentheses. †Child–Pugh class A includes patients without cirrhosis. ‡Based on imaging studies. §Only in patients who underwent hepatic resection with available resected liver tissue, according to the METAVIR fibrosis scoring system. AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LAT, locoregional ablative therapy; TACE, transcatheter arterial chemoembolization.

	Log ₁₀ AFP	Log ₁₀ DCP	ALBI score	APRI	FIB-4 index
Tumor size					
≤2 cm	1.23 ± 0.73	1.52 ± 0.55	—	—	—
>2 cm and ≤5 cm	1.49 ± 0.97	2.07 ± 0.84	—	—	—
>5 cm	2.53 ± 1.58	3.30 ± 1.15	—	—	—
No. tumors					
Single	1.48 ± 1.10	2.08 ± 1.06	—	—	—
Multiple	1.97 ± 1.33	2.40 ± 1.13	—	—	—
Portal vein invasion†					
Absent	1.44 ± 0.97	1.96 ± 0.92	—	—	—
Present	2.85 ± 1.62	3.41 ± 1.14	—	—	—
BCLC class					
0	1.20 ± 0.75	1.44 ± 0.49	-2.60 ± 0.39	—	—
A	1.37 ± 0.86	1.92 ± 0.85	-2.39 ± 0.55	—	—
B	1.93 ± 1.18	2.45 ± 1.02	-2.30 ± 0.54	—	—
C	2.46 ± 1.59	3.14 ± 1.17	-2.14 ± 0.58	—	—
D	2.21 ± 1.57	2.86 ± 1.30	-1.25 ± 0.54	—	—
Child–Pugh class‡					
A	—	—	-2.58 ± 0.41	—	—
B	—	—	-1.69 ± 0.39	—	—
C	—	—	-1.12 ± 0.45	—	—
Liver fibrosis§					
F1/2	—	—	—	0.94 ± 1.52	3.22 ± 2.53
F3	—	—	—	0.98 ± 0.87	3.71 ± 2.10
F4	—	—	—	1.46 ± 1.20	5.01 ± 3.14

†Based on imaging studies. ‡Child–Pugh class A includes patients without cirrhosis.

§Only in patients who underwent hepatic resection with available resected liver tissue, according to the METAVIR fibrosis scoring system. AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; DCP, des-gamma-carboxy prothrombin.

Results

Characteristics of study patients and serum predictive markers. Table 1 shows the characteristics of all study patients. Median age was 70 years and 70% of patients were male. Etiology of HCC was predominantly HCV infection. Approximately 70% of patients had Child–Pugh class A liver function⁽²⁴⁾ and 60% of patients had BCLC stage 0 or A HCC.⁽²⁶⁾ More than half of the patients underwent hepatic resection or RFA as their initial treatment. Approximately 15% of patients did not undergo treatment for HCC and received best supportive care as a result of severely advanced HCC, deteriorated liver function, or patient refusal to receive treatment.

Levels of AFP and DCP, and ALBI score were compared based on tumor progression and liver function, respectively (Table 2). AFP and DCP levels increased as parameters indicating HCC progression increased, such as tumor size and number of tumors, portal vein invasion, and BCLC class. Patients with BCLC class D HCC did not have higher AFP and DCP level than in patients with BCLC class C HCC, possibly because most patients were categorized as BCLC class D because of Child–Pugh class C liver function. Therefore, ALBI score was markedly higher in patients with BCLC D HCC. ALBI score increased with deterioration of liver function based on Child–Pugh class. APRI, and FIB-4 index were compared based on degree of fibrosis in non-cancerous liver tissue only in patients who underwent hepatic resection with available resected liver tissue (Table 2). APRI and FIB-4 index increased with the progression of liver fibrosis based on METAVIR score.⁽²⁷⁾

Table 2. Association between serum prognostic markers of hepatocellular carcinoma and tumor characteristics, BCLC class, Child–Pugh class, and fibrosis of non-cancerous liver (n = 1669)

Survival rate based on tumor markers, liver function markers, and liver fibrosis markers. Figure 1 shows the comparison of survival rates in patients with HCC after diagnosis when categorized by AFP, DCP, APRI, FIB-4 index, and ALBI grade. Survival rates were significantly different in all categorizations (all, $P < 0.0001$). Based on the shape of survival curves, differences in survival rate between high and low AFP or DCP groups were marked within 10 years of diagnosis but were smaller afterwards. In particular, there was no difference in survival rate between the high and low DCP groups 15 years after HCC diagnosis. In contrast, differences in survival rate between the high and low APRI or FIB-4 index groups were modest within 3 years after diagnosis but gradually increased afterwards, up to 10 years after diagnosis. We found significant constant differences in survival rate after diagnosis when patients were stratified based on ALBI grade.

Univariate and multivariate analyses for factors associated with patient survival after diagnosis revealed all serum markers except for APRI were independently associated with patient survival (Table 3).

Comparison of the impact of serum markers on prognosis using time-dependent ROC analysis. We conducted time-dependent ROC analysis for the prediction of patient survival or death based on duration after HCC diagnosis (Fig. 2). AUROC for tumor markers (AFP and DCP) were highest in the short term but decreased with years after diagnosis. In contrast, AUROC for liver fibrosis markers (APRI and FIB-4 index) were lowest in the short term but increased afterwards; they were higher than AUROC for AFP and DCP 8 years after

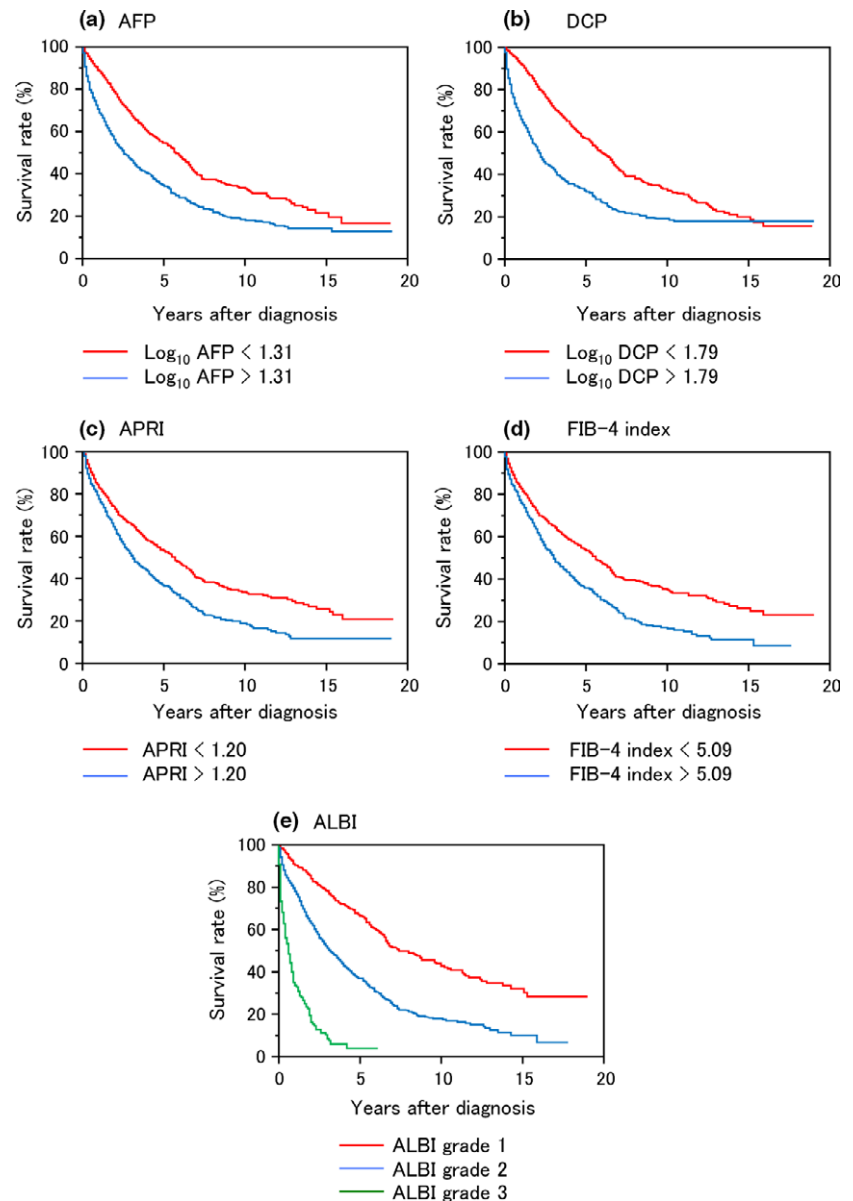


Fig. 1. Overall survival rates of patients with hepatocellular carcinoma (HCC) after diagnosis ($n = 1699$) based on (a) serum alpha-fetoprotein (AFP) level, (b) serum des-gamma-carboxy prothrombin (DCP) level, (c) aspartate aminotransferase to platelet ratio index (APRI), (d) FIB-4 index, and (e) albumin-bilirubin (ALBI) grade at diagnosis.

diagnosis. AUROC for ALBI score, a marker of liver function, was constantly high: it was higher than AUROC for other markers throughout, except for DCP within 2 years of diagnosis. When Child score, a finer classification of Child–Pugh class (score 5 and 6 for Child–Pugh class A, 7, 8, and 9 for class B, and higher for class C), was analyzed, AUROC of Child score was comparable to ALBI score early after diagnosis of HCC but decreased gradually afterward.

Survival and recurrence rates based on tumor markers, liver function markers, and liver fibrosis markers in patients with HCC within Milano criteria who underwent curative therapy. Survival rates were compared between patient groups categorized by AFP, DCP, APRI, FIB-4 index, and ALBI grade at diagnosis in patients with HCC within Milano criteria and who underwent curative therapy (i.e. hepatic resection or RFA) (Fig. S1). The difference in survival rate was modest between groups with high and low AFP ($P = 0.0472$). No difference was found between the high and low DCP groups ($P = 0.3249$). However, survival rates were significantly lower in patients with high APRI or FIB-4 index than in patients with low levels (APRI,

$P = 0.0087$; FIB-4 index, $P = 0.0123$). These differences became marked 2 years after treatment. There were no patients with ALBI grade 3 in patients who underwent curative therapy. Patients with ALBI grade 1 had a significantly higher survival rate than patients with ALBI grade 2 ($P < 0.0001$). When recurrence rates were compared (Fig. S2), no differences were found between groups with high and low AFP ($P = 0.3299$) and DCP ($P = 0.2148$). Recurrence rates were higher in patients with high APRI or FIB-4 index than in patients with low levels (each, $P < 0.0001$). Again, these differences became marked 2 years after treatment.

Discussion

In the present study, we evaluated the prognostic impact of serum markers on HCC progression, liver function, and liver fibrosis focusing on changes in the impact of these factors over time. HCC progression is usually evaluated based on morphological findings in imaging studies, or pathological examination if HCC resection or transplantation was carried out. Liver function is

Table 3. Univariate and multivariate analysis of factors associated with survival in patients with hepatocellular carcinoma (n = 1669)

Factor	Univariate analysis		Multivariate analysis	
	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)
Age	0.0033	1.01 (1.00–1.02)	<0.0001	1.02 (1.01–1.03)
Sex				
Male		1		1
Female	0.0499	0.86 (0.74–1.00)	<0.0001	0.72 (0.61–0.85)
Tumor size				
≤2 cm		1		1
>2 cm and ≤5 cm	<0.0001	1.46 (1.24–1.72)	0.0008	1.35 (1.13–1.61)
>5 cm	<0.0001	4.19 (3.53–4.97)	<0.0001	1.98 (1.52–2.56)
Number of tumors				
Single		1		1
Multiple	<0.0001	2.00 (1.75–2.29)	0.0002	1.33 (1.15–1.55)
Portal vein invasion†				
Absent		1		1
Present	<0.0001	5.24 (4.44–6.17)	<0.0001	2.51 (1.96–3.20)
Child-Pugh class‡				
A		1		1
B	<0.0001	2.83 (2.43–3.30)	0.0036	1.40 (1.12–1.75)
C	<0.0001	7.59 (6.05–9.44)	0.0003	1.95 (1.36–2.78)
Log ₁₀ AFP	<0.0001	1.49 (1.41–1.58)	<0.0001	2.99 (1.91–4.67)
Log ₁₀ DCP	<0.0001	1.63 (1.53–1.73)	0.0058	1.91 (1.21–3.02)
ALBI score	<0.0001	3.32 (2.07–3.71)	<0.0001	23.0 (10.7–49.1)
APRI	<0.0001	1.13 (1.09–1.17)	0.1103	0.43 (0.14–1.20)
FIB-4 index	<0.0001	1.07 (1.05–1.08)	0.0098	5.35 (1.50–19.1)

†Based on imaging studies. ‡Child–Pugh class A includes patients without cirrhosis. AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; APRI, aspartate aminotransferase to platelet ratio index; CI, confidence interval; DCP, des-gamma-carboxy prothrombin.

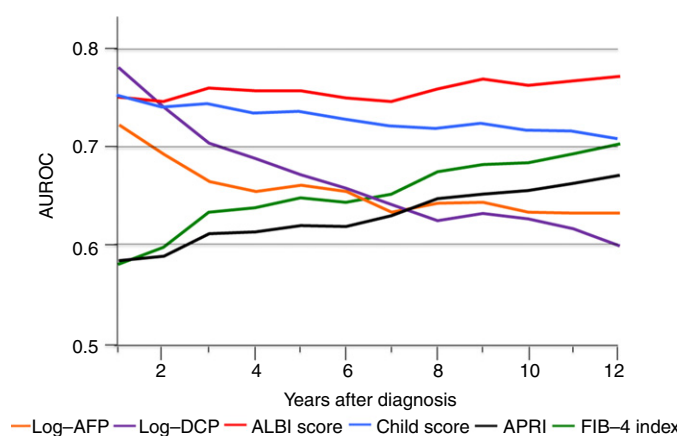


Fig. 2. Plots of annual area under the receiver-operating characteristics curve (AUROC) for serum alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) levels, albumin-bilirubin (ALBI) score, Child score, aspartate aminotransferase to platelet ratio index (APRI), and FIB-4 index at diagnosis for patient survival or death after diagnosis.

evaluated using laboratory values, such as ALB, T-Bil, or prothrombin time as well as symptoms such as ascites or encephalopathy, usually based on Child–Pugh classification. Degree of liver fibrosis is evaluated based on the histology of liver tissue adjacent to HCC in resected liver specimens. However, these factors are measured as categorical values such as stage, class, and grade, which are not appropriate for ROC analysis. In contrast, continuous values such as serum marker levels are suitable for seeing changes in prognostic impact over time using time-dependent ROC analysis and, therefore, we analyzed

these serum markers. The serum markers evaluated in the present study are indicators of HCC progression, liver function, and liver fibrosis.^(7,8,14) The present study showed the distinct correlation between AFP or DCP and tumor progression, ALBI score and liver function by Child–Pugh class, and APRI or FIB-4 index and liver fibrosis. AFP and DCP are established biomarkers for HCC and elevation of these markers reportedly reflects the progression of HCC from several aspects including size, number, and portal vein invasion,^(7,14) although some advanced HCC lack elevation of these markers. ALBI score is a recently proposed measure for liver function,⁽⁹⁾ and its reliability as a prognostic liver function marker have been reported in HCC with several degrees of progression.^(10,11,28,29) APRI and FIB-4 index are also established laboratory indicators for the degree of liver fibrosis. These laboratory values can easily be obtained from serum tests and can be measured repeatedly. Therefore, the serial changes of these markers can be monitored in the course of HCC.

The present study clearly showed the prognostic significance of all markers studied on survival rate in patients with HCC. We found significant differences in survival rate based on AFP, DCP, APRI, FIB-4 index, and ALBI grade. Analysis for survival rate typically does not include time points after the start of the observation period. Indeed, comparisons of survival rate using the Kaplan–Meier method and the log–rank test showed statistically significant differences in all comparisons (all, $P < 0.0001$). However, there were distinctive differences in the shape of the survival curves when patients were categorized based on various markers. This indicated that the time point after diagnosis when prognostic factors have strongest impact on outcome varies. In particular, the shapes of the Kaplan–Meier curves were different for tumor markers and liver fibrosis markers. Whereas HCC tumor markers had a strong impact on short-term survival of

patients with HCC, liver fibrosis markers had a stronger impact on long-term survival and recurrence. Tumor markers reflect tumor progression, which may have less impact in patients who survive more than 5 years, usually because of curative treatment. Therefore, the prognostic impact of tumor markers decreases in the long term. In contrast, liver fibrosis may indicate the potential for HCC development,^(5,15) and the increased impact of liver fibrosis in the long term after the initial diagnosis of HCC may indicate the effect of this potential for new HCC development (i.e. multicentric occurrence).

In previous studies, evaluation of prognostic factors was based on simple comparisons of overall survival or recurrence rates according to the target factor, and the change in their prognostic impact over time was not taken into consideration. However, divergent patterns in survival curves were not the same for all factors. This indicates that each prognostic factor has a time period when it has the most significant impact on survival. In the future, this point should be taken into consideration when evaluating the clinical significance of prognostic markers, not only in cases of HCC but also in other cancers.

These differences between prognostic factors were clearly shown in the time-dependent ROC analysis. Whereas AUROC for the prediction of patient survival or death for tumor markers decreased over time, AUROC for liver fibrosis markers increased. In contrast, the AUROC for ALBI score, a measure of liver function, was constantly high throughout the study period, indicating that liver function has a strong impact on the prognosis of patients with HCC after diagnosis both in the short term and long term. In comparison to ALBI score, the prognostic value of Child score decreased with the increase in the year after diagnosis. This will be because of the inclusion of patients with high Child scores that were classified into Child–Pugh classes B or C (i.e. decompensated cirrhosis) whose survival rates are low as a result of the deteriorated liver function.

The prognostic impact of tumor markers was modest in patients with HCC within Milano criteria who underwent curative therapy, because curative treatment could overcome tumor factors. Indeed, we found no difference in HCC recurrence rates when patients were categorized on tumor markers. In contrast, the impact of liver fibrosis markers became evident 2 to 3 years after curative treatment. A previous study also reported that the degree of liver fibrosis was an important factor associated with HCC recurrence in patients long after curative hepatic resection.^(5,15) The high potential for hepatocarcinogenesis in patients with high liver fibrosis markers may contribute to the high recurrence rate in this patient subpopulation by the high rate of multicentric recurrence.

There are several limitations to the present study. We assessed serum markers that reportedly reflect tumor progression, liver function, and liver fibrosis, because it is difficult to analyze changes in prognostic impact over time using time-dependent ROC analysis with categorical values such as tumor stage, Child–Pugh class, or liver fibrosis grade. However, tumor marker elevations do not always accurately represent tumor progression. We used the ALBI score as an indicator of liver function. Although we found a significant correlation between

Child–Pugh class and ALBI score and several recent studies have reported that ALBI score is a good indicator of liver function,^(9–11,28–31) further verification of ALBI score as a measure of liver function is required. When patients were categorized based on tumor markers or liver fibrosis markers, the cut-offs were fixed as the median for each value. Although defining cut-off values for these markers using the maximum Youden's index in ROC analysis would be preferable, it was difficult because cut-off values with the maximum Youden's index changed over time. In addition, whereas survival rates were compared with categorization of patients using cut-offs of each prognostic marker, prognostic values of these markers were compared as continuous variables (AUROC) when analyzing time-dependent ROC. Finally, these prognostic markers can change serially after the diagnosis of HCC as a result of HCC treatment or antiviral therapy for hepatitis virus in patients with HBV or HCV. Therefore, prognostic significance of the changes of these markers should be investigated in the future.

In conclusion, prognostic factors for HCC (i.e. tumor progression, liver function, and liver fibrosis) significantly influenced survival of patients with HCC. Serum markers reflect these factors as continuous variables. Time-dependent ROC analysis of these markers showed variations in the timing when factors had a strong impact on prognosis. Whereas tumor progression factors had a strong impact in the short term, liver fibrosis factors had a strong impact long after diagnosis. Liver function factors had a high prognostic impact throughout the study period. Clinicians should take this variation into consideration when evaluating the prognostic significance of each factor.

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Disclosure Statement

Hidegori Toyoda reports lecturer fees from AbbVie and Bristol-Myers Squibb and Takashi Kumada reports lecturer fees from Gilead Sciences and Bristol-Myers Squibb. Other authors declare no conflicts of interest.

Abbreviations

AFP	alpha-fetoprotein
ALB	albumin
ALBI	albumin-bilirubin
ALT	alanine aminotransferase
APRI	aspartate aminotransferase to platelet ratio index
AST	aspartate aminotransferase
AUROC	area under the ROC curve
BCLC	Barcelona Clinic Liver Cancer
DCP	des-gamma-carboxy prothrombin
HCC	hepatocellular carcinoma
RFA	radiofrequency ablation
ROC	receiver-operating characteristic
T-Bil	total bilirubin

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Survival rates of patients with hepatocellular carcinoma (HCC) within Milan criteria who underwent curative therapy ($n = 620$) based on (A) serum alpha-fetoprotein (AFP) level, (B) serum des-gamma-carboxy prothrombin (DCP) level, (C) aspartate aminotransferase to platelet ratio index (APRI), (D) FIB-4 index, and (E) albumin-bilirubin (ALBI) grade at diagnosis.

Fig. S2. Recurrence rates of patients with hepatocellular carcinoma (HCC) within Milan criteria who underwent curative therapy ($n = 620$) based on (A) serum alpha-fetoprotein (AFP) level, (B) serum des-gamma-carboxy prothrombin (DCP) level, (C) aspartate aminotransferase to platelet ratio index (APRI), and (D) FIB-4 index at diagnosis.