

Review

Tumor Markers for Hepatocellular Carcinoma: Simple and Significant Predictors of Outcome in Patients with HCC

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Key Words

Alpha-fetoprotein · Des-gamma-carboxy prothrombin · Hepatocellular carcinoma ·
Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein · Prognosis

Abstract

Background: The effectiveness of tumor markers in evaluating outcomes of patients with hepatocellular carcinoma (HCC) remains to be clarified. **Summary:** The usefulness of the HCC tumor markers, alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP) was reviewed. Elevations in these tumor markers at the time of HCC diagnosis correlate with disease progression as assessed by both imaging studies and pathologic examinations. The combination of these three tumor markers results in good predictive ability for patient survival after diagnosis. In addition, combination at the time of HCC diagnosis of these three tumor markers (as a measure of tumor progression) and serum albumin and bilirubin levels (as indicators of remnant liver function) can be used for HCC staging and further predicts prognosis in patients with HCC. **Key Message:** The prognosis of patients with HCC can be well discriminated based solely on serum markers. Staging of HCC with serum markers is objective; if stored serum samples are available, HCC stages can be standardized across different countries and time periods.

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Introduction

Measuring levels of tumor biomarkers for hepatocellular carcinoma (HCC) is an important tool for disease management. Alpha-fetoprotein (AFP), *Lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP) have been established as HCC-specific tumor markers [1–14]. Although tumor marker levels are not included in the diagnostic criteria for HCC or in the screening recommendations in the guidelines of the American Association for the Study of Liver Diseases or the European Association for the Study of the Liver [15, 16], they provide valuable supportive information for diagnosing HCC. Furthermore, a recent study found that the combination of these three tumor markers was useful for diagnosing HCC; this combination had very high sensitivity and specificity for diagnosing HCC without the use of imaging studies [17].

The levels of AFP, AFP-L3, and DCP usually increase as HCC progresses, i.e., with increases in the size and number of HCC lesions and progression to portal vein invasion [18–22]. In addition, some studies have reported that an increase in tumor marker levels suggests a high degree of HCC malignancy regardless of morphological progression [22, 23]. Consequently, an increase in the levels of these tumor markers portends an unfavorable prognosis after initial diagnosis.

In this review, we evaluated three tumor markers of HCC, namely AFP, AFP-L3, and DCP, as indicators of tumor progression and predictors of patient outcome. In addition, we review attempts to predict prognosis solely based on serum markers.

Limitations in Estimating HCC Progression by Imaging Studies and Liver Function with the Child-Pugh Classification

The progression of HCC is usually evaluated morphologically, based on the size and number of tumors and the presence of portal vein invasion [24–26]. Such evaluations are mainly based on imaging studies prior to treatment; however, estimating tumor progression using imaging studies has several shortcomings. For example, the detectability of liver tumors by ultrasonography (US), a routine, basic imaging tool for HCC surveillance, depends on the skill of the sonographer. In addition, the detectability of liver tumors strongly depends on the resolution of the imaging modality and the quality of the equipment used for US, computed tomography (CT), or magnetic resonance imaging (MRI). Recent advances in imaging equipment such as those used in US, multidetector-row CT [27, 28], and MRI have improved the detection of hepatic nodules, including small, early-stage HCC tumors. Moreover, developments in contrast media have further enhanced the ability to detect and characterize hepatic nodules including HCC [29–36]. Thus, the number of HCCs detected will increase with advances in imaging technology, which will have the effect of upstaging HCC. Furthermore, advances in imaging techniques also improve the imaging evaluation of pathologic features of HCC including vascular invasion and macroscopic type [37–39]. This can also result in upstaging of HCC progression.

Discrepancies between findings on imaging and pathologic results are often found in patients who undergo hepatic resection. According to an annual survey of HCC by the Liver Cancer Study Group of Japan, the prevalence of HCC with portal vein invasion was 13.1% based on imaging studies and 26.0% based on pathologic analysis [40]. Using imaging studies, it is often difficult to detect microvascular invasion of HCC or minute satellite nodules; however, these entities are often found in pathologic analysis after resection, thereby indicating discrepancies in the staging of HCC progression.

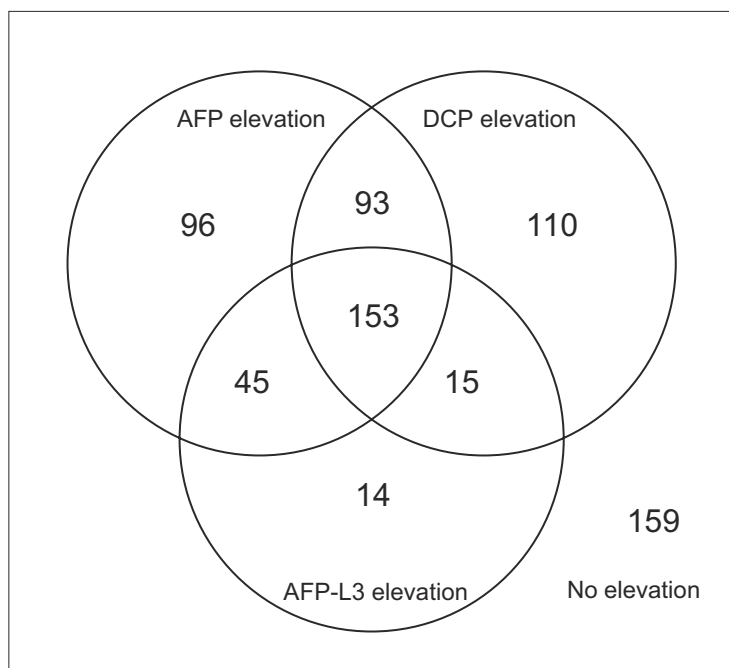


Fig. 1. Patterns of tumor marker elevation in 685 patients with hepatocellular carcinoma [43]. The tumor marker cut-off points were AFP 20 ng/dL, AFP-L3 10%, and DCP 40 mAU/mL.

Liver function in patients with HCC is usually estimated using the Child-Pugh score [41]. This score is based on patient serum albumin and bilirubin levels, prothrombin time, and the presence and controllability of ascites and hepatic encephalopathy. However, the presence and controllability of ascites and hepatic encephalopathy are often subjective. The presence of ascites varies from symptomatic ascites and ascites detected by physicians on physical examination to mild ascites only detectable by US. In addition, the controllability of ascites depends on the dose and the type of medications used. Moreover, the severity of hepatic encephalopathy may range from coma to subclinical encephalopathy.

Association between Tumor Marker Levels and HCC Progression

Several studies have reported an association between elevated tumor markers, especially AFP-L3 and DCP, and HCC progression [19, 20, 42]. Elevated AFP-L3 has been associated with microsatellite lesions and hypervascularity of HCC tumor [18, 19]. In contrast, elevated DCP has been associated with a higher prevalence of portal vein invasion [20]. Our previous study of HCC characteristics according to elevations in various tumor markers yielded similar results [43]. Among 685 patients in whom AFP, AFP-L3, and DCP were measured at diagnosis, we found one of three tumor markers elevated in 220 patients, whereas no tumor markers were elevated in 159 patients (fig. 1). When these patients were compared (table 1), patients with elevated AFP-L3 alone had a larger number of tumors, and patients with elevated DCP alone had a higher prevalence of portal vein thrombosis. In contrast, no differences were observed in HCC progression between patients with elevated AFP alone and those with no elevated tumor markers. When elevations of these three tumor markers were considered together (table 2), there were increases in the size of the largest tumor, the number of tumors,

Table 1. Morphological tumor progression based on imaging findings in patients with no elevations in tumor markers and those with elevation of only one tumor marker (n=379) [43]

Elevated tumor marker	None (n=159)	AFP alone (n=96)	AFP-L3 alone (n=14)	DCP alone (n=110)
Size of largest tumor (cm) ^a	2.24 ± 1.41	2.17 ± 1.20	3.99 ± 3.90	3.94 ± 3.00
Number of tumors ^b	1.42 ± 0.97	1.52 ± 0.83	2.21 ± 1.48	1.54 ± 1.05
Portal vein thrombosis ^c	3 (1.9%)	0	1 (7.1%)	13 (11.8%)

Cut-off points: AFP, 20 ng/mL; AFP-L3, 10%; DCP, 40 mAU/mL.

^aNone vs. AFP-L3 alone, p=0.0614; none vs. DCP alone, p<0.0001 (Mann-Whitney U test). ^bNone vs. AFP-L3 alone, p=0.0075 (Mann-Whitney U test). ^cNone vs. DCP alone, p=0.0018 (Chi-square test).

Table 2. Morphological tumor progression based on imaging findings according to the number of elevated tumor markers (n=685) [43]

Number of elevated tumor markers	0 (n=159)	1 (n=220)	2 (n=153)	3 (n=153)
Size of largest tumor (cm) ^a	2.24 ± 1.41	3.18 ± 2.61	3.72 ± 3.18	5.57 ± 3.69
Number of tumors ^b	1.42 ± 0.97	1.57 ± 1.00	2.09 ± 2.19	2.67 ± 2.59
Portal vein thrombosis ^c	3 (1.9%)	14 (6.4%)	24 (15.7%)	50 (32.7%)

Cut-off points: AFP, 20 ng/mL; AFP-L3, 10%; DCP, 40 mAU/mL.

^aNone vs. 1 marker, none vs. 2 markers, and 2 markers vs. 3 markers, p<0.0001 (Mann-Whitney U test). ^bNone vs. 2 markers, p=0.0105; 2 markers vs. 3 markers, p=0.0189 (Mann-Whitney U test). ^cNone vs. 1 marker, p=0.0677; 1 marker vs. 2 markers, p=0.0055; 2 markers vs. 3 markers, p=0.0009 (Chi-square test).

and the prevalence of portal vein thrombosis as the number of elevated tumor markers increased. Consequently, there was a correlation between the number of elevated tumor markers and the TNM tumor stage (as defined by the Liver Cancer Study of Japan [26], table 3). Thus, elevations of these tumor markers are associated with morphological progression of HCC as evaluated by imaging studies.

Elevations in tumor markers are also associated with pathologic characteristics of HCC [44]. Table 4 demonstrates the association between the number of elevated tumor markers and the size and number of HCC lesions, differentiation, growth type [40], and portal vein invasion based on pathologic examination of resected HCC specimens in 173 patients who underwent hepatectomy. In addition to increases in tumor size, there were increases in the prevalence of moderately or poorly differentiated HCC, HCC with infiltrative growth, and microscopic portal vein invasion as the number of elevated tumor markers increased, all of which indicate the progressive nature of HCC. Thus, these tumor markers reflect HCC progression in terms of pathologic features as well as findings on imaging studies.

Predicting Survival of Patients with HCC Based on Tumor Markers

Figure 2 shows the survival rates after HCC diagnosis according to elevations in each tumor marker. The survival rate in patients with any elevated tumor marker was significantly lower than that in patients without any elevated tumor markers (p<0.0001). Lower survival

Table 3. Association between TNM tumor stage^a and the number of elevated tumor markers (n=685) [43]

Number of elevated tumor markers	Stage I (n=182)	Stage II (n=261)	Stage III (n=147)	Stage IV (n=95)
None (n=159)	69 (43.4%)	68 (42.8%)	19 (11.9%)	3 (1.9%)
1 marker (n=220)	61 (27.7%)	101 (45.9%)	45 (20.5%)	13 (5.9%)
2 markers (n=153)	44 (28.8%)	51 (33.3%)	34 (22.2%)	24 (15.7%)
3 markers (n=153)	8 (5.2%)	41 (26.8%)	49 (32.0%)	55 (36.0%)

Cut-off points: AFP, 20 ng/mL; AFP-L3, 10%; DCP, 40 mAU/mL.

^aTNM stage as defined by Liver Cancer Study of Japan [26].

Table 4. Morphological tumor progression based on pathologic examination according to the number of elevated tumor markers (n=173) [44]

Number of elevated tumor markers	0 (n=47)	1 (n=57)	2 (n=38)	3 (n=31)
Size of largest tumor (cm) ^a	2.25 ± 1.09	2.96 ± 2.02	3.75 ± 2.88	4.87 ± 3.74
Multiple tumors	7 (14.9%)	7 (12.3%)	8 (21.1%)	7 (22.6%)
Moderate or poor differentiation ^b	21 (44.7%)	30 (52.6%)	29 (76.3%)	29 (93.5%)
Infiltrative growth type ^c	3 (6.4%)	4 (7.0%)	4 (10.5%)	11 (35.5%)
Portal vein invasion ^d	3 (6.4%)	5 (8.8%)	11 (28.9%)	21 (67.7%)

Cut-off points: AFP, 20 ng/mL; AFP-L3, 5%; DCP, 40 mAU/mL.

^ap<0.0001 (Jonckheere-Terpstra test), ^{b,d} p<0.0001, ^c p=0.0010 (Cochran-Armitage test).

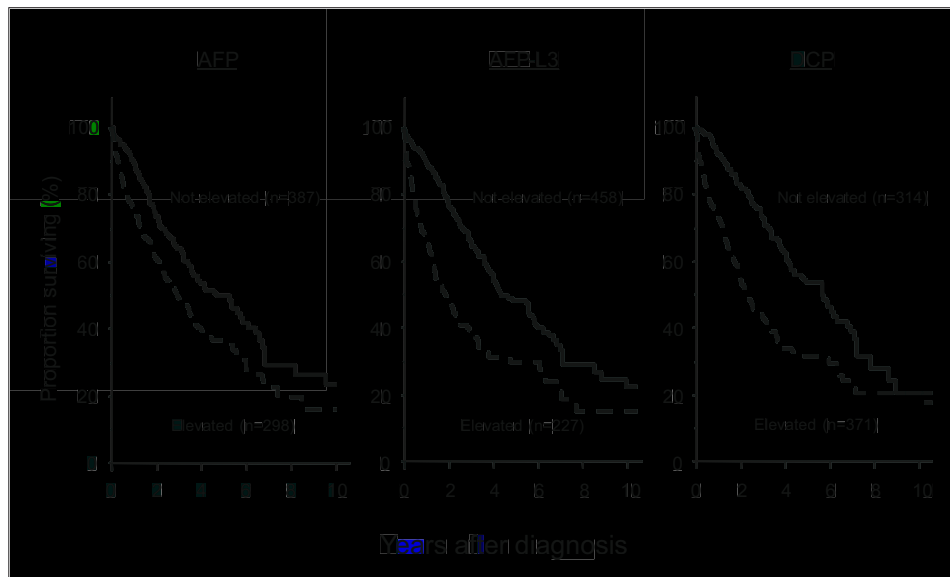


Fig. 2. Patient survival according to the presence of tumor marker elevation. p<0.0001 for all comparisons.

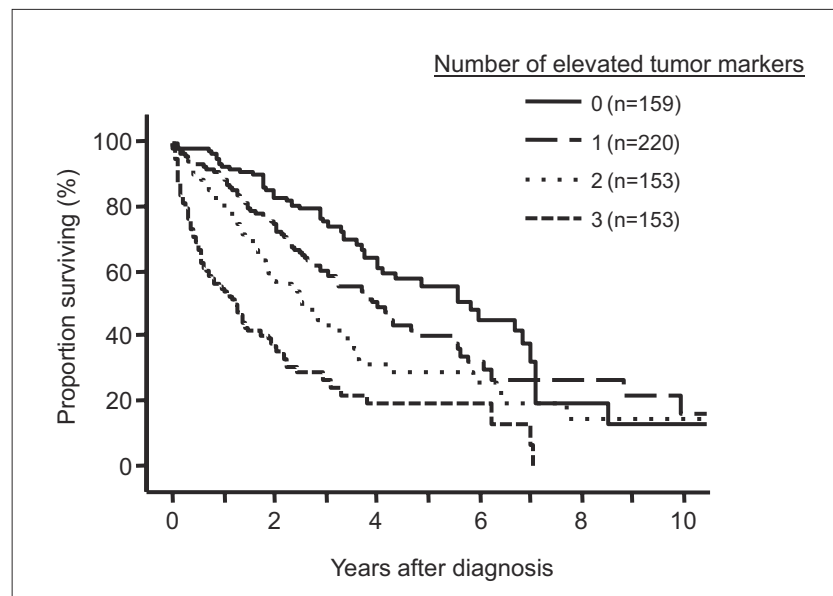


Fig. 3. Patient survival according to the number of the elevated tumor markers [43]. 0 vs. 1, $p=0.0181$; 1 vs. 2, $p=0.0141$; 2 vs. 3, $p<0.0001$.

rates in patients with elevated AFP-L3 or DCP at diagnosis may result from the progressive nature of HCC in these patients. Indeed, previous studies reported that AFP-L3 elevation is associated with a higher rate of recurrence [45] and a lower survival rate [45, 46]. In addition, DCP elevation has been associated with higher recurrence and lower survival rates [47, 48].

Despite the weak association between HCC progression and AFP elevation, the survival rate is lower in patients with AFP elevation. Previous studies have found a higher incidence of HCC in patients with elevated AFP [49, 50]. Therefore, AFP elevation may reflect the potential risk of HCC development in the background liver more strongly than it reflects HCC progression.

Elevations of combinations of these three tumor markers further discriminate the survival of patients with HCC (fig. 3). According to our previous analysis, the number of elevated tumor markers is associated with patient survival after diagnosis independent of remnant liver function (i.e., Child-Pugh class) and treatment modalities used [43].

Staging of HCC Patients Based Solely on Serum Markers

Since liver function, in addition to tumor extension, is an important factor affecting the prognosis of patients with HCC, several prognostic staging systems for HCC that incorporate parameters indicating both tumor progression and remnant liver function have been proposed [51–56]. Only a few staging systems include tumor markers as factors [51, 53]; however, as shown in this review, tumor markers are associated with survival in HCC patients.

As described above, Child-Pugh classification is not perfectly objective as an estimate of liver function. In a previous study, Tateishi et al. proposed a new staging system for HCC in which only serum albumin and bilirubin values are used as indicators of remnant liver function [56]; this approach allows for objective and standardized evaluation of remnant liver function. A more recent study also reported that the combination of serum bilirubin and

Table 5. Calculation of the BALAD score

Contribution to BALAD score	0	1	2	3
Bilirubin-albumin score	A	B	C	
Number of elevated tumor markers	0	1	2	3

The BALAD score is the sum of the remnant liver function score (bilirubin-albumin score) and tumor progression score (number of elevated tumor markers), e.g., a bilirubin-albumin score of B contributes 1 point, so a patient with two elevated tumor markers and a bilirubin-albumin score of B has a BALAD score of 2 + 1 = 3.

albumin shows better discriminatory ability than Child-Pugh class for prognosis in patients with HCC [57]. The question therefore arises, to what extent does the combination of serum values of AFP, AFP-L3, and DCP (as tumor progression indicators) and bilirubin and albumin (as remnant liver function indicators) constitute an objective prognostic staging system for patients with HCC?

A staging system for HCC that is based solely on serum markers, not involving imaging or pathologic or clinical evaluations, was developed and its ability to discriminate patient survival was evaluated [58]. Among factors reflecting remnant liver function, serum albumin levels of above 3.5 g/dL, 2.8–3.5 g/dL, or below 2.8 g/dL were scored as 0, 1, or 2, respectively. Serum total bilirubin levels of below 1.0 mg/dL, 1.0–2.0 mg/dL, or above 2.0 mg/dL were scored as 0, 1, or 2, respectively. Liver function was then categorized by the sum of these two scores as A (0 or 1), B (2 or 3), or C (4). As a tumor progression factor, we simply used the number of elevated tumor markers. The HCC staging score based on these laboratory data was calculated as the sum of the tumor progression factor and liver function factor, as shown in table 5. We referred to this as the BALAD score, based on the first letter of each of the five serum markers (Bilirubin, Albumin, *Lens culinaris* agglutinin A-reactive fraction of AFP, AFP, and DCP). The BALAD score discriminates patient survival after diagnosis well, and is comparable to staging systems based on imaging studies and Child-Pugh class [58]. In addition, a recent study showed that this score could predict the prognosis of patients with HCC in different countries despite differences in HCC etiology [59].

Advantages and Disadvantages of Evaluating Patients with HCC Based on Serum Markers Alone

The advantage of this staging system based solely on serum markers is its objectivity. The results are numerical and are not influenced by the quality of imaging technology, operator skill, or subjective evaluation. Since this staging can be based on one serum sample and uses one standard, HCC stage can be standardized across countries and time periods (if stored serum samples are available). In contrast, a staging system based on serum markers alone is not applicable to diagnosis, treatment planning, or treatment itself because of the lack of imaging information; imaging studies are mandatory for these purposes. This staging system is not intended to be a treatment allocation system: it is solely for staging HCC to predict patient outcomes. In addition, this serum marker-based staging system is not applicable for patients being administered drugs that can influence the levels of serum markers or in the presence of disorders that can influence the levels of these serum markers.

Perspective

Although biomarkers are not widely accepted as important clinical tools, they contribute valuable information for the management of patients with HCC, with regards to surveillance, diagnosis, evaluation of treatment efficacy, and prediction of outcomes. Their usefulness does not vary by country or HCC etiology. In addition to the HCC tumor markers reviewed here (AFP, AFP-L3, and DCP), many serum biomarkers, including glypican-3, insulin-like growth factor, osteopontin, golgi protein-73, and squamous cellular carcinoma antigen, have been investigated as candidates for HCC tumor markers [60–66]. These novel potential HCC tumor markers, alone or in combination with other markers, will further contribute to the management of patients with HCC. In addition, several serum markers or indices of liver fibrosis have been reported as risk factors of HCC development or prognostic factors in HCC [67–69]. These markers of liver fibrosis may further improve the prediction of outcome in patients with HCC.

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