RESEARCH PAPER

Liver damage parameters and prognosis in patients with hepatocellular carcinoma

Abstract

Background: Patients with small HCCs typically have low AFP levels and there is a need for other biomarkers in this setting.

Objectives: To assess the usefulness of liver inflammation parameters in prognosis.

Methods: A retrospective study of prospectively collected data on 265 non-surgical HCC patients was analyzed, using Kaplan-Meier and Cox regression analysis.

Results: Patient survival was significantly different in subgroups after dichotomization according to serum levels of AST, GGT, albumin or ALKP, whether they had small (<5cm) or large (>5cm) HCCs. The main difference between patients with better or worse survival was the serum bilirubin level. Tumor aggressiveness parameter differences (PVT, AFP, multifocality) were also found, predominantly in patients with larger tumors. The survival differences after inflammation parameter dichotomization were mainly found in patients with low AFP levels.

Conclusions: Serum inflammation parameter levels were associated with differences in survival for patients with large or small tumors, but mainly in the absence of high serum AFP levels. Patients with smaller tumors may die of liver damage, but those with larger tumors likely die of both liver and tumor factors. These inflammation parameters may thus be useful in patients with low AFP levels, for whom there are few useful serum biomarkers.

Keywords: HCC, biomarkers, inflammation, phenotype, survival

Abbreviations: HCC: Hepatocellular Carcinoma; WBC, White Blood Count; TBil: Total Bilirubin; AST: Aspartate Amino Transferase; ALT: Alanine Aminotransferase; ALKP: Alkaline Phosphatase; GGT, Gamma Glutamyl Transferase; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; NLR, Neutrophil Lymphocyte Ratio; MTD, Maximum Tumor Diameter; PVT, Portal Vein Thrombosis; AFP, Alpha-Fetoprotein; CAT scan, Computed Axial Tomography; ROC, Receiver Operating Characteristic.

Introduction

Hepatocellular Carcinoma (HCC) typically arises on a liver that has been chronically diseased over multiple years, most often by cirrhosis, which is typically caused by chronic hepatitis B or C, alcoholism, obesity or plant mycotoxins [1, 2]. Thus, HCC patients usually have 2 diseases, namely, chronic liver damage and the HCC, and both factors contribute to patient morbidity and mortality [2, 3]. Cirrhosis-associated liver microenvironmental inflammation factors have become increasingly appreciated as contributing to hepatocarcinogenesis and HCC biology, likely contributing to HCC growth and invasion. We recently found that we could identify 2 phenotypes of very large HCCs, based upon the serum levels of Aspartate Amino Transferase (AST) [3]. In the current work we extend this line of enquiry to determine if AST levels might also reflect survival and HCC biology or aggressiveness factors [4,5] in smaller HCCs and whether other clinical markers of liver damage are also useful in this regard. We report that serum levels of AST, Gamma Glutamyl Transferase (GGT), Albumin And Alkaline Phosphatase (ALKP) each reflect differences in prognosis. The cause may be tumor factors in patients with large HCCs, but likely liver factors in patients with small HCCs.

Methods

Clinical

An HCC database of 265 prospectively-accrued

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non-surgical HCC patients was studied. Patients had known survival data and baseline tumor parameters data on CT scan-based measurements of HCC Maximum Tumor Diameter (MTD), number of tumor nodules, presence or absence of macroscopic Portal Vein Tumor Thrombosis (PVT) as well as Serum Alpha-Fetoprotein (AFP) levels. They also had complete blood counts and routine serum liver function tests, (total bilirubin, GGTP, ALKP, albumin and transaminase levels) plus CRP and ESR. Diagnosis was made either via tumor biopsy or according to international guidelines. Database management conformed to legislation on privacy and this study conforms to the ethical guidelines of the Declaration of Helsinki and approval for this retrospective study on de-identified HCC patients was obtained from Cukurova University and Inonu University Institutional Review Board (approval #2021/2572).

■ Statistical

Normality of the quantitative data was examined by the Shapiro-Wilk test. Quantitative data were summarized by median, minimum and maximum values, while the Mann-Whitney U test was used for group comparisons. Distribution of qualitative data were presented by count and percentage. Pearson's chi-square, continuity-corrected chi-square or Fisher's exact tests were used for comparisons. Survival analyses were performed by Kaplan-Meier analysis and comparisons were made by Log-rank test. Hazard ratio estimations were obtained by Cox regression analysis. Significance level was considered as 0.05 in all analyses.

Results

■ Survival according to dichotomized liver damage parameters.

We had previously found that dichotomization of patients with very large HCCs according to Serum Aspartate Amino Transferase (AST) levels resulted in 2 different HCC phenotypes with quite different survival [3]. We therefore examined in the current study, whether this might apply to patients with various HCC sizes. We found that when we examined small (<5cm) or large (>5cm) HCCs, dichotomization according to AST levels, as previously determined by ROC, the resulting groups had significantly different survivals, as measured by Kaplan-Meier and univariate Cox regression analysis, with highest survivals being in patients having lower AST levels (Table 1A). The survival differences were between 3-fold (small HCC patients) and 4-fold (large HCC patients). These transaminase-based survival differences also occurred in patients with small HCCs.

We extended this approach to several other liver inflammation parameters, namely serum levels of Gamma Glutamyl Transferase (GGT), Albumin And Alkaline Phosphatase (ALKP) (Tables 1B, 1C, 1D). Dichotomization according to each of the 4 parameters, resulted in HCC patients having significantly different survival, whether the patients had small or large HCCs. We also examined serum CRP and ESR levels, as well as PLR and NLR but found no significant differences between groups (data not shown).

■ HCC aggressiveness characteristics and bilirubin levels in dichotomized HCC patients.

HCC patients are generally thought to have their survival limited by either liver failure or tumor growth, or both [1,2]. The inflammation parameter-dichotomized patients were then compared according to their tumor aggressiveness factors (Maximum Tumor Diameter or MTD, tumor number, presence or absence of macroscopic portal vein invasion by tumor or PVT, serum levels of Alpha-Fetoprotein or AFP), or according to median serum bilirubin levels as a measure of liver failure (Table 2).

Results for AST dichotomization (Table 2A) show that serum bilirubin levels were significantly elevated for patients with high AST levels, regardless of having small (<5cm) or large size (>5cm) HCCs. Patients with high AST and large size HCCs also had significantly more PVT (p=0.012) and tumor multifocality (p=0.021), significantly higher serum AFP levels (p=0.032) and higher MTDs, though not significantly (p=0.075). However, there were no significant differences in tumor characteristics in the patients having small HCCs.

Similar results were found after GGT dichotomization. Serum bilirubin levels were higher in high GGT patients, regardless of HCC size, but not significantly (Table 2B). PVT was significantly different in the patients with larger size HCCs, (p=0.03) as were the AFP levels (p<0.001). Again, there were no significant differences in any tumor characteristic for small HCCs, similar to the AST findings. After albumin dichotomization, serum AFP levels were significantly different in patients with both small and large size tumors, but tumor

TABLE 1. HCC patient survival (months) in relation to liver inflammation parameters.

Table 1A. AST dichotomisation

		Kaplan-Meier Analysis			Univariate Cox regression	
MTD	AST IU/mL	Survival time	Survival time	Log-Rank	HR	HR
		Mean±SE	Median±SE	p-value	(95% CI)	p-value
<5 cm	≤50 (n=49)	49.57±7.43	26±4.26	0.043	reference	
	>50 (n=44)	31.63±6.62	8±4.51		1.650 (1.000-2.723)	0.05
≥5 cm	≤50 (n=78)	38.30±4.90	21±3.81	<0.001	reference	
	>50 (n=94)	11.74±2.22	5±0.72		2.852 (2.006-4.055)	<0.001

Table 1B. GGT dichotomization

		Kaplan-Meier Analysis			Univariate Cox regression	
MTD	GGT IU/mL	Survival time	Survival time	Log-Rank	HR (95% CI)	HR p-value
<5 cm	≤50 (n=45)	59.60±8.68	26±16.44	0.003	reference	
	>50 (n=47)	25.88±5.08	12±5.90		2.129 (1.264-3.586)	0.004
≥5 cm	≤50 (n=47)	42.13±6.58	24±5.32	<0.001	reference	
	>50 (n=126)	15.54±2.38	6±1.15		2.509 (1.667-3.776)	<0.001

Table 1C. Albumin dichotomization

		Kaplan-Meier Analysis			Univariate Cox regression	
MTD	Albumin g/dL	Survival time Mean±SE	Survival time Median±SE	Log-Rank p-value	HR (95% CI)	HR p-value
<5 cm	≥3.5 (n=41)	54.18±8.00	29±5.60	0.005	reference	
	<3.5 (n=52)	36.08±5.98	12±2.85		1.929 (1.199-3.104)	0.007
≥5 cm	≥3.5 (n=61)	37.57±5.71	29±5.60	<0.001	reference	
	<3.5 (n=111)	16.35±2.09	12±2.85		2.049 (1.442-2.911)	<0.001

Table 1D . ALKP dichotomization

		Kaplan-Meier Analysis			Univariate Cox regression	
MTD	ALKP IU/mL	Survival time Mean±SE	Survival time Median±SE	Log-Rank p-value	HR (95% CI)	HR p-value
<5 cm	≤150 (n=61)	45.34±6.17	25±2.63	0.019	reference	
	>150 (n=37)	29.03±7.63	6±2.63		1.799 (1.079-2.998)	0.024
≥5 cm	≤150 (n=73)	28.77±4.41	12±2.92	0.011	reference	
	>150 (n=94)	18.35±3.08	7±1.02		1.529 (1.084-2.157)	0.016

Abbreviations: MTD, Maximum Tumor Diameter; HR, Hazard Ratio; AST, Aspartate Transaminase; ALKP, Alkaline Phosphatase; GGT, Gamma Glutamyl Transferase.

characteristics in patients with large HCCs (only) were significantly different for multifocality (p=0.021), AFP levels (p,0.001) and MTD (p=0.028), but not for PVT (p=0.285). Patients with large tumors after ALKP dichotomization showed significant differences in serum bilirubin levels (p<0.001), but no significant differences in other tumor characteristics (Table 2D).

In summary, patients with large tumors had elevated bilirubin levels after each parameter dichotomization. They also had differences in tumor characteristics, except after ALKP dichotomization. For the patients with smaller tumors, there were little differences in tumor characteristics after dichotomization, but patients had significantly different serum bilirubin levels after AST and albumin dichotomization, but not after GGT or ALKP dichotomization.

■ Effects on survival of parameter dichotomization in different AFP groups.

It is thought that the majority of HCC patients do not have elevated serum AFP levels, especially in patients with small size tumors [6]. This is one of the reasons that the results of surveillance screening using AFP has been disappointing [7]. To assess the prognostic usefulness of the liver parameters, especially in the important small HCC group in which treatment can often be so effective, we therefore examined the effects of parameter dichotomization on survival, separately in patients with small or large size HCCs, each grouped according to presence of low or high serum AFP levels (Table 3). We found that in patients with low serum

TABLE 2. Tumor characteristics according to parameter dichotomization.

Table 2A. Tumor characteristics according to serum AST dichotomization.

			AST≤50 IU/mL	AST>50 IU/mL	p
MTD<5 cm	PVT (%)	No	87.7	83.7	0.674
		Yes	12.3	16.3	
	Tumor foci (%)	1	61.7	72.2	0.238
		>1	38.3	27.8	
	AFP [median (min.-max.)]		16 (1.03-1000)	32.05 (1-1000)	0.172
	MTD [median (min.-max.)]		3 (1-4)	3 (1-4)	0.707
	T.Bil [median (min.-max.)]		1.1 (0.11-7.4)	1.6 (0.4-21.33)	<0.001
MTD≥5 cm	PVT (%)	No	73.7	54	0.012
		Yes	26.3	46	
	Tumor foci (%)	1	69.2	51.4	0.021
		>1	30.8	48.6	
	AFP [median (min.-max.)]		14.78 (1.84-1000)	47.8 (1.3-1000)	0.032
	MTD [median (min.-max.)]		7 (5-24)	9 (5-21)	0.075
	T.Bil [median (min.-max.)]		0.91 (0.3-65)	1.8 (0.24-28.33)	<0.001

Table 2B. Tumor characteristics according to serum GGT dichotomization.

			GGT≤50 IU/mL	GGT>50 IU/mL	p
MTD<5 cm	PVT (%)	No	91.7	81.7	0.186
		Yes	8.3	18.3	
	Tumor foci (%)	1	62.7	70.4	0.444
		>1	37.3	29.6	
	AFP [median (min.-max.)]		16 (1.29-870)	38.1 (1-1000)	0.224
	MTD [median (min.-max.)]		3 (1-4)	3 (1-4)	0.652
	T.Bil. [median (min.-max.)]		1.2 (0.31-41.33)	1.35 (0.11-26.02)	0.518
MTD≥5 cm	PVT (%)	No	77.3	57.4	0.03
		Yes	22.7	42.6	
	Tumor foci (%)	1	59.6	58	0.983
		>1	40.4	42	
	AFP [median (min.-max.)]		8.39 (1.47-1000)	54.1 (1.3-1000)	<0.001
	MTD [median (min.-max.)]		7 (5-19)	8 (5-24)	0.255
	T.Bil. [median (min.-max.)]		1.09 (0.33-27.51)	1.31 (0.24-35)	0.058

Table 2C. Tumor characteristics according to serum albumin dichotomization.

			ALB≥3.5 g/dL	ALB<3.5 g/dL	p
MTD<5 cm	PVT (%)	No	87.8	82.6	0.35
		Yes	12.2	17.4	
	Tumor foci (%)	1	79.2	73	0.337
		>1	20.8	27	
	AFP [median (min.-max.)]		9 (1.2-1000)	20.28 (1-1000)	0.05
	MTD [median (min.-max.)]		3 (1-4)	3 (1-4)	0.613
	T.Bil. [median (min.-max.)]		1.03 (0.11-10.7)	1.47 (0.4-41.3)	<0.001
MTD≥5 cm	PVT (%)	No	69.2	63.1	0.285
		Yes	30.8	36.9	
	Tumor foci (%)	1	78.3	65	0.021
		>1	21.7	35	
	AFP [median (min.-max.)]		8.71 (1.12-1000)	34.6 (0.5-1000)	<0.001
	MTD [median (min.-max.)]		7 (5-21)	8 (5-30)	0.028
	T.Bil. [median (min.-max.)]		0.83 (0.3-28.33)	1.57 (0.24-35)	<0.001

AFP levels, regardless of HCC size, abnormal parameter levels were associated with worse survival than for patients with normal parameter levels. This was seen after dichotomization for AST, GGT, albumin or ALKP (Tables 3A, 3B, 3C and 3D). Interestingly, survival was not so different amongst patients with small or large size HCCs who had low parameter levels, although patients with abnormal parameter levels had uniformly bad survival regardless of their AFP grouping. In patients with elevated AFP levels, parameter dichotomization resulted in non-significant survival differences for patients with either small or large HCCs, except for AST

Table 2D. Tumor characteristics according to serum ALKP dichotomization.					
			ALKP≤150 IU/mL	ALKP>150 IU/mL	p
MTD<5 cm	PVT (%)	No	83.8	86.7	0.811
		Yes	16.2	13.3	
	Tumor foci (%)	1	68.7	67.7	1
		>1	31.3	32.3	
	AFP [median (min.-max.)]		15.45 (1-1000)	53.85 (1.03-1000)	0.059
	MTD [median (min.-max.)]		3 (1-4)	3 (1-4)	0.737
	T.Bil. [median (min.-max.)]		1.2 (0.11-23.2)	1.42 (0.4-21.33)	0.205
MTD≥5 cm	PVT (%)	No	64.3	61.4	0.7
		Yes	35.7	38.6	
	Tumor foci (%)	1	44 (58.7)	62 (57.4)	0.865
		>1	31 (41.3)	46 (42.6)	
	AFP [median (min.-max.)]		16.5 (1.47-1000)	54.4 (1.3-1000)	0.177
	MTD [median (min.-max.)]		7 (5-24)	9 (5-21)	0.137
	T.Bil. [median (min.-max.)]		1.06 (0.3-27.51)	1.5 (0.24-35)	<0.001

Abbreviations: MTD, Maximum Tumor Diameter (cm); PVT, Portal Vein Macroscopic Invasion; AFP, Alpha-Fetoprotein (IU/mL); HR, Hazard Ratio; AST, Aspartate Transaminase; ALKP, Alkaline Phosphatase; GGT, Gamma Glutamyl Transferase; ALB, Albumin; T. Bil., Total Bilirubin (mg/dL).

TABLE 3. Survival (months) of patients with small or large HCCs, having high or low serum AFP levels.

Table 3A. Patient survival with small or large HCCs, having low or high serum AFP and dichotomized by serum AST.							
			Kaplan-Meier Analysis			Univariate Cox regression	
AFP IU/mL	MTD	AST IU/mL	Survival time Mean±SE	Survival time Mean±SE	Log-Rank p-value	HR (95% CI)	HR p-value
<100	<5 cm	≤50 (n=35)	55.98±9.02	29±10.29	0.051	Reference 1.699 (0.939-3.074)	0.054
		>50 (n=34)	33.13±7.58	12±5.63			
	≥5 cm	≤50 (n=59)	43.18±6.20	24±6.23	<0.001	Reference 2.941 (1.908-4.532)	<0.001
		>50 (n=56)	11.39±2.64	4±1.14			
≥100-1000	<5 cm	≤50 (n=12)	25.0±4.150	19±4.22	0.234	Reference 1.762 (0.667-4.655)	0.253
		>50 (n=10)	22.70±11.66	4±0.78			
	≥5 cm	≤50 (n=19)	24.34±3.99	21±3.50	0.001	Reference 2.563 (1.389-4.732)	0.003
		>50 (n=40)	11.16±3.21	5±0.88			

Table 3B. Patient survival (months) with small or large HCCs, having low or high serum AFP and dichotomized by serum GGT.							
			Kaplan-Meier Analysis			Univariate Cox regression	
AFP IU/mL	MTD	GGT IU/mL	Survival time Mean±SE	Survival time Mean±SE	Log-Rank p-value	HR (95% CI)	HR p-value
<100	<5 cm	≤50 (n=35)	55.41±10.16	46.5±4.60	0.01	reference 2.178 (1.171-4.052)	0.014
		>50 (n=31)	27.97±5.82	14±7.42			
	≥5 cm	≤50 (n=68)	46.98±7.70	28±7.26	<0.001	reference 2.742 (1.685-4.461)	<0.001
		>50 (n=77)	16.56±3.27	5±1.32			
≥100-1000	<5 cm	≤50 (n=10)	30.56±6.81	19±1.49	0.087	reference 2.258 (0.842-6.053)	0.106
		>50 (n=12)	17.13±8.20	4±1.60			
	≥5 cm	≤50 (n=10)	22.56±7.73	16±5.96	0.162	reference 1.674 (0.782-3.583)	0.185
		>50 (n=22)	12.92±2.58	6±1.08			

Table 3C. Patient survival with small or large HCCs, having low or high serum AFP and dichotomized by serum Albumin.							
			Kaplan-Meier Analysis			Univariate Cox regression	
AFP IU/mL	MTD	ALB g/dL	Survival time Mean±SE	Survival time Mean±SE	Log-Rank p-value	HR (95% CI)	HR p-value
<100	<5 cm	≥3.5 (n=30)	64.07±9.79	36±22.32	0.001	Reference 2.486 (1.385-4.462)	
		<3.5 (n=64)	34.58±6.67	12±2.81			0.002
	≥5 cm	≥3.5 (n=51)	44.45±6.82	27±5.98	<0.001	Reference 2.455 (1.609-3.745)	
		<3.5 (n=97)	16.54±2.59	7±1.61			<0.001
≥100-1000	<5 cm	≥3.5 (n=11)	24.00±4.49	23±5.27	0.974	Reference 1.014 (0.430-2.389)	
		<3.5 (n=18)	35.99±10.97	15±6.78			0.975
	≥5 cm	≥3.5 (n=14)	11.21±1.93	11±2.76	0.875	Reference 0.952 (0.498-1.820)	
		<3.5 (n=54)	15.11±2.93	6±1.03			0.881

Table 3D. Patient survival with small or large HCCs, having low or high serum AFP and dichotomized by serum ALKP.

			Kaplan-Meier Analysis			Univariate Cox regression	
AFP IU/mL	MTD	ALKP IU/mL	Survival time Mean±SE	Survival time Mean±SE	Log-Rank p-value	HR (95% CI)	HR p-value
<100	<5 cm	≤150 (n=45)	50.54±7.33	25±6.90	0.025	reference 1.934 (1.061-3.526)	
		>150 (n=24)	28.29±8.33	8±4.40			0.031
	≥5 cm	≤150 (n=54)	33.79±5.87	16±5.87	0.017	reference 1.652 (1.072-2.545)	
		>150 (n=63)	20.46±4.20	8±1.64			0.023
≥100-1000	<5 cm	≤150 (n=13)	23.09±4.19	19±4.54	0.404	reference 1.496 (0.561-3.986)	
		>150 (n=9)	24.78±12.76	4±1.49			0.421
	≥5 cm	≤150 (n=22)	16.86±4.66	9±2.17	0.459	reference 1.230 (0.693-2.185)	
		>150 (n=35)	12.87±2.91	6±1.11			0.48

Abbreviations: HR, Hazard Ratio; MTD, Maximum Tumor Diameter; AFP, Alpha-Fetoprotein; AST, Aspartate Transaminase; ALKP, Alkaline Phosphatase; GGT, Gamma Glutamyl Transferase; ALB, Albumin.

dichotomization in large HCC patients, which yielded significant survival differences (Table 3A, bottom row). Thus, in presence of elevated AFP levels, parameter dichotomization still resulted in survival differences, but they were not significant.

Discussion

The main findings reported here were that serum levels of all 4 investigated liver damage parameters were predictive of survival in patients who had either small or large size HCCs (Table 1). The reasons for this include the fact that abnormal levels of each parameter (high AST, GGT, ALKP, or low albumin), but especially AST and albumin, were associated with increased total serum bilirubin levels, in patients with either small or large HCCs (Table 2). Higher levels of GGT and ALKP were also associated with increased bilirubin levels than lower parameter levels, but not significantly.

Abnormal inflammation parameter levels were not associated with increased tumor aggressiveness factors in patients with small HCCs (excepting AFP after albumin dichotomization). However, in large HCCs, abnormal inflammation parameters were associated with increased tumor aggressiveness factors. There seemed to be different tumor patterns according to which parameter was increased. Thus, elevated AST was associated with significantly increased PVT, focality and AFP. Elevated GGT was associated with increased PVT and AFP, but not focality. Decreased albumin was associated with significantly increased focality, AFP and MTD, but not PVT. However, elevated ALKP was not associated with any tumor aggressiveness parameter, excepting AFP levels (Tables 2). Interestingly, differences in tumor size (MTD) did not feature in any of the parameter dichotomizations apart from albumin levels, and yet each parameter dichotomization was associated with significantly different survival, as seen in Table 1. Some of these different HCC patterns might be explained by the different hepatic functions of these 4 parameters. AST is a liver enzyme that is released into the bloodstream when liver cells are damaged. Albumin, by contrast, reflects liver synthetic activity and decreases with loss of liver parenchyma, but also is decreased in HCC patients as a result of inflammation and cancer-associated nutritional deficiency. Increased serum GGT activity is a marker of hepatobiliary injury and especially cholestasis and is a membrane-bound glycoprotein that catalyzes glutamyl groups between peptides and functions in detoxification. It is also an HCC biomarker that is especially useful in HCC patients with low AFP levels [8-11]. ALKP is a hepatic hydrolase enzyme that is also released into the blood after hepatic damage and particularly after biliary tract obstruction.

Thus, for patients with small tumors, liver damage would seem to be a main factor in limiting survival, whereas in patients with larger tumors, both liver damage and tumor aggressiveness factors appear to both be important for survival. It has previously been reported that patients with cirrhosis and liver damage have smaller HCCs than patients with better liver function [12], which in turn might permit growth of larger HCCs in their parenchyma.

The influence of AFP in Table 3 is worthy of comment. The main significant differences in survival associated with inflammation parameter dichotomization were in patients with low AFP levels. Perhaps this reflects the importance of inflammation in the absence of AFP as a driving force, especially in the large size HCCs. Consistent with this interpretation are the generally low AFP levels in small size HCCs, resulting in several authors recommending against use of AFP as a surveillance tool [7]. Perhaps AFP is such an important driver of HCC growth when it is elevated, that its presence trumps other influences on HCC growth, such

as inflammatory ones. However, since less than half of HCC patients in various studies have elevated AFP levels, especially in the presence of small size HCCs, these 4 liver inflammation-associated parameters appear to most clearly be associated with survival differences in patients with low AFP levels (Table 3), where there are limited other biomarker choices.

The mechanisms for inflammation-mediated HCC growth have been much studied [13, 14]. These include persistent necro-inflammation and hepatocyte regeneration which results in increased hepatocyte mutation. Pathways involved in the associated growth mechanisms are thought to include inflammation-associated and stress-associated signaling, including mediators NFKB and STAT and suppression of immune surveillance.

Given the significance reported here of elevated inflammation parameters in low AFP patients with small tumors, these parameters might be attractive potential biomarkers in guiding prognostication and thus decision-making when small HCCs are diagnosed.

A weakness of this study is the relatively small cohort size, which limits statistical analysis of sub-cohorts. Another weakness is the absence of data on other clinical HCC biomarkers such as Des Gamma Carboxy Prothrombin (DCP), glypican-3, AFP-L3 and the new circulating tumor cell assays, all of which were unavailable to us. Furthermore, HCC aggressiveness parameter levels could be shown in association with levels of AST, GGT and albumin, but not for ALKP (Table 2), which is also thought to be an HCC prognosis marker [15, 16]. Thus, other tumor biological indices must exist to explain the survival differences, apart from MTD, AFP, PVT and focality, which were the HCC parameters measured in this study. Despite this, the current study points to the potential of inflammation and liver damage parameters as a guide to HCC biology and thus prognosis, especially in low alpha-fetoprotein patients with small HCCs.

■ Conflict of interest statement

The authors declare no conflict of interest. All authors have read and agree with the contents of this paper.

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Strobe statement

The authors have read the STROBE statement - checklist of items, and the manuscript was prepared according to the STROBE statement-checklist of items.

Author contributions

BIC - concept, ideas and writing; HGB-biostatistics; VI, HI, UK, VI, BI, SY- data collection and paper proofing.

Statement of ethics

This work complies with the guidelines of the World Medical Association, Declaration of Helsinki. This work was approved by our institution's IRB as documented in the methods section.

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