Identification of 2 large-size HCC phenotypes, with and without associated inflammation

Abstract

Background: Large HCCs can often be associated with low levels of cirrhosis. However, inflammation is also regarded as a driver of HCC growth.

Objectives: To compare patients with large >5 cm HCCs having high versus low serum inflammation parameters.

Materials and methods: A Turkish patient HCC dataset with known survivals was retrospectively analyzed after dichotomization according to several clinical inflammation markers.

Results: Amongst several parameters examined, only AST levels were significantly associated with elevated AFP levels and increased percent PVT and tumor multifocality. The dichotomization of the cohort according to high or low AST levels resulted in 2 subcohorts with a 5-fold difference in median survival. The 2 AST-dichotomised cohorts comprised patients with similar large-size HCCs, but which were significantly different with respect to serum AFP levels, percent PVT, and percent tumor multifocality.

Conclusions: Two large-sized HCC phenotypes were identified. One had more aggressive HCC characteristics, higher inflammatory indices, and worse survival. The other had the opposite. Despite inflammation being important for the growth of some large tumors, others of a similar size likely have different growth mechanisms.

Keywords: HCC, large, inflammation, phenotype.

Abbreviations: HCC: Hepatocellular Carcinoma; WBC: White Blood Count; TBil: Total Bilirubin; AST: Aspartate Aminotransferase; 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; EpCAM: Epithelial Cellular Adhesion Molecule

Introduction

The clinical presentation and survival of patients with Hepatocellular Carcinoma (HCC) depend in large part on the combination of both tumor factors (maximum tumor diameter or MTD, presence or absence of portal venous invasion or PVT, number of tumors or multifocality, serum levels of alpha-fetoprotein or AFP) and the tumor micro-environment or state of the underlying liver [1]. As with most tumors of solid organs in adult humans, HCC treatment options and prognosis is greatly influenced by tumor size at clinical presentation, with most options and best results occurring in patients with smaller tumors. Despite this, some patients with very large tumors have a good liver function and can be surgically treated [2], which has been ascribed in part to the growth of large tumors in the absence of cirrhosis or liver inflammation. To investigate large HCCs further, the current study focuses only on large MTD HCCs in relation to indices of inflammation and the associated clinical patient and tumor features. We found that dichotomization according to inflammatory parameters results in 2 identifiable large MTD patients and tumor phenotypes that also differed in their median survival.

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Methods

■ Clinical

An HCC database was studied, that comprised 149 prospectively-accrued non-transplant HCC patients who had both survival data and baseline tumor parameter data, including CT scan information on HCC Maximum Tumor Diameter (MTD), number of tumor nodules, and presence or absence of macroscopic Portal Vein Thrombosis (PVT), and serum Alpha-Fetoprotein (AFP) levels; complete blood counts; and routine serum liver function tests, (total bilirubin, GGTP, ALKP, albumin, transaminases) plus CRP. The 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 approval for this retrospective study on de-identified HCC patients was obtained from Cukurova University and Inonu University the Institutional Review Board (approval #2021/2572).

■ Statistical

The normality of the quantitative data was examined by the Shapiro-Wilk test. Median, first quartile (1stQ), and third quartile (3rdQ) were used as descriptive statistics. Comparisons were performed by the Mann-Whitney U test. Categorical variables were presented as counts and percentages, chi-square tests were applied for comparisons. Kaplan-Meier analysis and Log-rank test were used for survival analysis. The hazard ratio was estimated by univariate cox regression analysis. The significance level was considered as 0.05 in all analyses.

Results

■ Inflammation parameters and indices of HCC aggressiveness

A series of clinical serum parameters were examined, using previously-determined ROC cutoffs [3], in relation to 3 HCC aggressiveness indices, namely AFP, tumor multifocality, and PVT in large size MTD >5 cm HCC patients **(TABLE 1)**. Several of the parameters correlated

TABLE 1. Inflammation markers for PVT, multifocality, and AFP in patients (n=149) with HCCs >5 cm diameter.

	PVT (%)			Tumor Focality (%)			AFP (IU/mL)	
	No	Yes	p	1	≥ 2	p	Median (min.-max)	p
AST ≤ 50	66.7	33.3	0.047	73.7	26.3	0.007	13.76 (1.84-1000)	0.022
AST>50	48.2	51.8		50	50		67.9 (1.3-1000)	
ALT ≤ 50	58.2	41.8	0.496	65.4	34.5	0.084	16 (1.5-1000)	0.207
ALT>50	52.5	47.5		51.5	48.5		54.5 (1.3-1000)	
ALKP ≤ 150	58.2	41.8	0.788	58.9	41.1	1	15 (1.47-1000)	0.149
ALKP>150	54.3	45.7		58	42		58 (1.30-1000)	
GGT ≤ 50	70.6	29.4	0.072	62.9	37.1	0.728	6.13 (1.47-1000)	<0.001
GGT>50	51	49		57.7	42.3		71.45 (1.3-1000)	
Albumin ≥ 3.5	61.5	38.5	0.573	76.3	23.7	0.103	8.43 (1.12-1000)	<0.001
Albumin<3.5	57.8	42.2		65	35		46 (0.5-1000)	
CRP ≤ 10	60	40	0.617	71	29	0.805	25.2 (0.5-1000)	0.823
CRP>10	63.8	36.2		68.1	31.9		18.02 (0.7-1000)	
ESR ≤ 15	64.3	35.7	0.685	60.7	39.3	0.898	12.7 (1.6-1000)	0.045
ESR>15	57.5	42.5		57	43		74.5 (1.3-1000)	
NLR ≤ 2.3	70.4	29.6	0.264	71.1	28.9	0.531	57 (1.3-1000)	0.3
NLR>2.3	55	45		62.7	37.3		19.51 (1.47-1000)	

Abbreviations and units: AFP: Alpha-Fetoprotein (IU/mL); PVT: Portal Vein Thrombosis; AST: Aspartate Amino Transferase (IU/mL); ALT: Alanine Aminotransferase (IU/mL); ALKP: Alkaline Phosphatase (IU/mL); GGT: Gamma Glutamyl Transferase (IU/mL); Albumin (g/dL); CRP: C-Reactive Protein (mg/dL); ESR: Erythrocyte Sedimentation Rate (mm/hour); NLR: Neutrophil to Lymphocyte Ratio

with 1 or more aggressiveness indices, but only AST was associated with differences in all 3 indices.

■ AST in relation to survival

The relationship of serum AST values to survival was next examined **(TABLE 2)**. A statistically significant survival difference was found when the 2 AST groups were compared, with low AST patients having 5-fold the median survival of high AST patients.

■ Tumor and clinical characteristics of AST cohorts

The clinical characteristics of the 2 AST patient cohorts were next compared **(TABLE 3)**. The MTD of the 2 groups was similar, by design **(TABLE 3)**. However, the AFP levels, percent of patients with both PVT and multifocality was significantly higher in the high AST group compared with the low AST group **(TABLE 3)**.

The clinical characteristics also displayed several differences **(TABLE 4)**. Patients in the high AST group had significantly higher levels of serum CRP, ALKP, GGT, ALT, and bilirubin, with significantly lower albumin levels and a higher percentage of patients with cirrhosis (81.2% *vs.* 60.3%) than patients in the low AST group.

■ Relationships of AST levels to tumor characteristics

An attempt was then made to examine semi-

TABLE 2. Kaplan-Meier and Cox survival analysis according to serum AST levels (IU/mL) in patients with large HCCs.

		Kaplan-Meier Analysis		Univariate Cox regression	
	Survival time	Survival time	Log-Rank	HR	HR
	Mean ± SE	Median ± SE	p-value	(95% CI)	p-value
AST ≤ 50 (n=61)	40.90 ± 5 .99	21 ± 4.69	<0.001	reference	
AST>50 (n=88)	9.70 ± 2.17	4 ± 0.50		3.27 (2.17-4.92)	<0.001

TABLE 3. Tumor and laboratory serum parameters of 2 groups of patients with large HCCs.

A.Tumor parameters	AST ≤ 50 IU/mL	AST>50 IU/mL	p
MTD (median)	10	9	0.938
AFP (median)	13.76	67.9	0.022
PVT+ve (%)	33.3	51.8	0.047
Tumor #>2 (%)	26.3	50	0.007

Abbreviations: AFP: Alpha-Fetoprotein; MTD: Maximum Tumor Diameter; PVT: Portal Vein Thrombosis.

TABLE 4. Median laboratory serum parameter values*.

Lab parameters	AST ≤ 50 IU/mL	AST>50 IU/mL	p
CRP	3.85	6.95	0.019
ESR	21	35	0.069
ALKP	143	196.5	<0.001
GGT	68	140	<0.001
ALT	26	71	<0.001
T. Bilirubin	0.9	1.71	<0.001
Albumin	3.25	2.8	0.001
Cholesterol	167.5	146.5	0.023
HDL	35.2	30.02	0.016
Platelets	232 (153-300)	189.5 (119-336)	0.471
Cirrhosis %	60.30%	81.20%	0.011

Abbreviations and units: as in Tables 1 and 2. *Significant parameters from Table 2.

quantitatively, the relationships between serum AST levels and the 3 HCC aggressiveness indices of serum AFP levels, percent PVT, and percent multifocality **(FIGURE 1)**. There was a significant relationship between AST levels and serum AFP levels ($p<0.001$) and percent multifocality ($p<0.015$) and approaching significance in relation to percent PVT ($p<0.068$).

Discussion

Tumor size is one of several important prognostic factors for HCC [1] and the larger MTD HCCs can often occur in noncirrhotic livers [2,4]. It was previously shown that characteristic differences of tumor biology relate to tumor size, with larger tumors having increased PVT and AFP levels, but lower bilirubin and higher albumin and platelet levels, than smaller tumors, suggesting differences in both HCC biology as well as microenvironment [5]. Massive HCCs also tend to have higher levels of AFP, ALKP, GGTP, and platelets [6]. Large tumor size usually has a negative prognosis for most tumor types, but not necessarily for large single HCCs [7-11]. Large HCCs in patients with relatively normal liver function and thus less cirrhosis might have different biology or different drivers of their tumor growth (predominantly tumor factors such as oncogenes and other tumor growth regulators) compared with smaller or large size HCCs that arise on the basis of cirrhosis. Those HCCs might have both tumor drivers and cirrhosis-associated inflammatory microenvironmental tumor growth factors. Patients with high ALKP, GGTP, and AFP all have been shown to have in common, significantly higher mean bilirubin levels than patients with low levels of these parameters [5]. Both AFP and **FIGURE 1c** have been shown to reflect different HCC cell lineages. Some of these lineages have features of stem or progenitor cells, possibly with different HCC developmental pathways, and have unique molecular signatures and tumor biology [12,13]. Although AFP is known to be a strong negative prognostic factor, exactly how high AFP HCC cells behave differently and thus cause lower survival rates, is still unclear.

The tumor microenvironment is increasingly recognized as contributing to HCC growth biology [14,15]. Amongst the several tumor microenvironmental processes, inflammation has gained much recent attention and there is evidence for a role of inflammation in many cancers, including HCC [16,17]. Inflammation-based prognostic scores have been shown to be associated with survival [18-21], possibly via the effects of inflammation on various parameters of tumor aggressiveness [22]. Inflammation is also thought to induce a microenvironment that is involved in DNA damage, tumor growth, and angiogenesis. It seems to be a bidirectional process, in which inflammation can be seen as a response to growing tumor cells; yet is also involved in their growth and invasiveness [16,23,24].

In this work, therefore, investigated whether inflammation is necessarily associated with the development of large size HCC or whether, in view of the absence of cirrhosis in a proportion of large MTD patients, other pathways might possibly also be involved. We found that although several of the 8 inflammation parameters that were examined, correlated with one or more

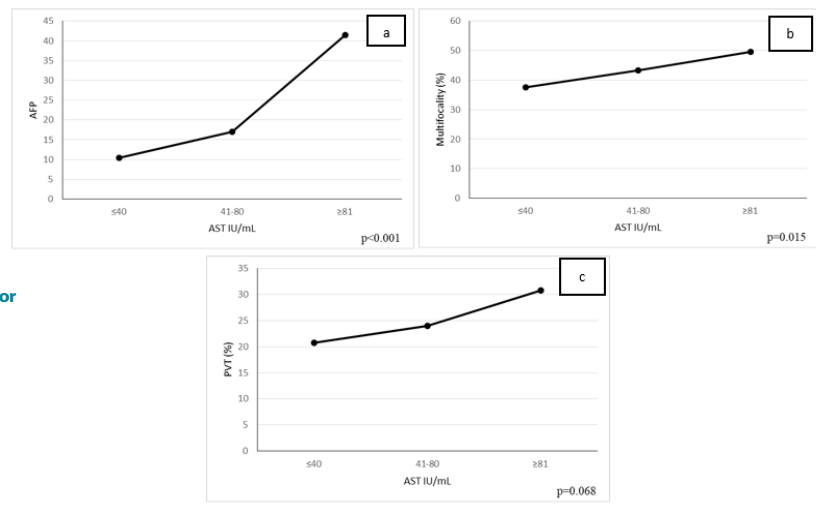

FIGURE 1. AST concentration (IU/mL) in relation to tumor parameter values. a) Serum AFP levels (IU/mL). b) PVT (%). c) Tumor Multifocality (%).

HCC aggressiveness characteristics, only AST correlated with all 3 of the aggressiveness characteristics that we studied, namely AFP levels, tumor focally, and PVT. MTD was not studied as large MTD patients were selected for the study. Despite allowing a large range of MTD (>5 cm HCCs), the 2 groups of patients with either high or low serum AST levels were fairly closely matched for MTD. The 2 groups of patients were significantly different with respect to the tumor characteristics of AFP levels and percent of patients with PVT and multifocality. There were also striking general differences. Liver enzymes were different between the 2 groups, as expected from the AST selection, with bilirubin levels being higher and albumin levels being lower in the high compared to the low AST group. Percent cirrhosis was also different, being higher in the high compared to the low AST group (81.2 *vs.* 60.3%). Unsurprisingly, there were semi-quantitative differences between AST levels and serum AFP, percent of patients with PVT, and percent with tumor multifocality (FIGURE 1). The 5-fold difference in median survival between the groups was striking.

Even though there were significant differences in percent cirrhosis between the 2 AST groups, at least 60% of the patients had cirrhosis, with the majority of our Turkish patients having hepatitis B-based HCC. We have previously noticed that albumin decreases as AFP increases since, in development, there is an increase in albumin as AFP (an oncofetal form of albumin) decreases.

Total bilirubin has previously been shown to increase with an increase in the size of large HCCs. This has been explained as due to parenchymal encroachment and destruction by the growing HCC. However, in this study, the HCCs in the 2 groups were of a similar size, so this explanation would seem to be inadequate, with the elevated bilirubin being in the high AST/inflammation group.

Conclusion

Inflammation has been shown to be associated with HCC growth, multifocality and portal venous invasion by tumor and several inflammation-associated effectors have been suggested to be involved in these processes. However, the non-inflammation associated HCCs, as determined here by low serum AST levels (and associated low ALT, ALKP, and GGT) presumably have some different mechanisms of growth increase and tumor invasion. Presumably, these involve endogenous factors such as tumor oncogenes, vascularity factors and endogenous growth factors, and possibly immune mechanisms. The notable finding of such a large survival difference between the 2 groups of patients with large size HCCs suggests possible surgical implications. In this view, patients with large MTD HCCs with low serum levels of AST (or ALT and ALKP and GGT) may be predicted to have better survival post-liver transplant or resection, than patients with similar large MTD but an elevation of these serum parameters.

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-a checklist of items.

Author contributions

Brian I Carr-concept, ideas, and writing; HGB-biostatistics; Volkan Ince, Hikmet Akkiz, Umit Karaogullarından, Burak Isik, Sezai Yilmaz-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.

References

Carr BI, Guerra V. HCC and its microenvironment. *Hepatogastroenterology*. 60, 1433-1437 (2013).

Okuda K, Nakashima T, Kojiro M, et al. Hepatocellular carcinoma without cirrhosis in Japanese patients. *Gastroenterology*. 97, 140-146 (1989).

Carr BI, Bag HG, Ince V, et al. A simple 2-parameter blood test alert for the presence of small hepatocellular carcinomas. *Clin Pract (Lond)*. 18, 1804-1809 (2021).

Nzeako UC, Goodman ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and noncirrhotic livers. A clinic-histopathologic study of 804 North American patients. *Am J Clin Path*. 105, 65-75 (1996).

Carr BI, Guerra V, Donghia R, et al. Relationships between indices of tumor aggressiveness in hepatocellular carcinoma. *J Gastrointest Cancer*. 52, 1340-1349 (2021).

Carr BI, Guerra V. Features of massive hepatocellular carcinomas. *Eur J Gastroenterol Hepatol*. 26, 101-108 (2014).

Tsoulfas G, Mekras A, Agorastou P, et al. Surgical treatment for large hepatocellular carcinoma: does size matter? *ANZ J Surg*. 82, 510-517 (2012).

Shah SA, Wei AC, Cleary SP, et al. Prognosis and results after resection of very large (>or=10 cm) hepatocellular carcinoma. *J Gastrointest Surg*. 11, 589-595 (2007).

Liau KH, Ruo L, Shia J, et al. Outcome of partial hepatectomy for large (>10 cm) hepatocellular carcinoma. *Cancer*. 104, 1948-1955 (2005).

Yang LY, Fang F, Ou DP, et al. Solitary large hepatocellular carcinoma: a specific subtype of hepatocellular carcinoma with good outcome after hepatic resection. *Ann Surg*. 249, 118-123 (2009).

Lee NH, Chau GY, Lui WY, et al. Surgical treatment and outcome in patients with hepatocellular carcinoma greater than 10 cm diameter. *Br J Surg*. 85, 1654-1657 (1998). [Google Scholar]

Terris B, Cavard C, Perret C. EpCAM, a new marker for cancer stem cells in hepatocellular carcinoma. *J Hepatol*. 52, 280-281 (2010).

Yamashita T, Ji J, Budhu A, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology*. 136, 1012-1024 (2009).

Utsonomiya T, Shimada M, Imura S, et al. Molecular signatures of noncancerous liver tissue can predict the risk for late recurrence of hepatocellular carcinoma. *J Gastroenterol*. 45, 146-152 (2010).

Tsuchiya M, Parker JS, Kono H et al. Gene expression in nontumoral liver tissue and recurrence-free survival in hepatitis C virus-positive hepatocellular carcinoma. *Mol Cancer*. 9, 74 (2010).

Balkwill F, Mantovani A. Inflammation and cancer. Back to Virchow? *Lancet*. 357, 529-545 (2001).

Hernandez-Gea V, Toffanin S, Friedman SL, et al. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology*. 144, 512-527 (2013).

McMillan DC, Crozier JE, Canna K, et al. Evaluation of an inflammation-based prognostic score (mGPS) in patients undergoing resection for colon and rectal cancer. *Int J Colorectal Dis*. 22, 881-886 (2007).

Ishizuka M, Kubota K, Kita J, et al. Impact of an inflammation-based prognostic system on patients undergoing surgery for hepatocellular carcinoma: a retrospective study of 398 Japanese patients. *Am J Surg*. 203, 101-106 (2012).

Li MX, Bi XY, Li ZY, et al. Prognostic role of glasgow prognostic score in patients with hepatocellular carcinoma: a systematic review and meta-analysis. *Medicine*. 94, e2133 (2015).

Kinoshita A, Onoda H, Imai N, et al. Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. *Br J Cancer*. 107, 988-993 (2012).

Carr BI, Akkiz H, Guerra V, et al. C-reactive protein and hepatocellular carcinoma: analysis of its relationships to tumor factors. *Clin Pract (London)*. 15, 625-634 (2018).

Carr BI, Guerra V, Donghia R. Portal vein thrombosis and markers of inflammation in hepatocellular carcinoma. *J Gastrointest Cancer*. 51, 1141-1147 (2020).

Akkiz H, Carr BI, Bag HG. Serum levels of inflammatory markers CRP, ESR and albumin in relation to survival in patients with hepatocellular carcinoma. *Int J Clin Pract*. 75, e13593 (2021).