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An HCC Aggressiveness Index and Blood GTP, Bilirubin and Platelet Levels

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Abstract

Four HCC characteristics typically inform tumor behavior: maximum tumor size, number of nodules, portal vein thrombosis and serum AFP level. The sum of these parameters was recently published as an HCC Aggressiveness Index. We aimed to validate this index retrospectively in a larger and independent HCC cohort of 2706 Italian HCC patients, and to evaluate a possible relationship between the index and liver function parameters. The scores in the HCC Aggressiveness Index were again found to significantly relate to patient survival. Furthermore, in a multiple logistic regression model of the Aggressiveness Index score categories, there were significant differences in several liver parameter terciles amongst the score categories, suggesting a relationship of liver function to tumor aggressiveness. It was concluded that a prognostically significant Tumor Aggressiveness Index was validated and was found to be related to levels of some common liver function parameters.

Keywords: HCC; Aggressiveness index; Survival; Liver function

Abbreviations

HCC: Hepatocellular Carcinoma; AFP: Alpha-Fetoprotein; GGTP: Gamma Glutamyl Transpeptidase; ALKP: Alkaline Phosphatase; AST: Aspartate Aminotransferase; PVT: Portal Vein Thrombosis; MTD: Maximum Tumor Diameter; CT: Computerized Axial Tomography Scans.

Introduction

Four clinical HCC characteristics are generally considered in assessing an individual patient's tumor-related management and prognosis. They are: maximum tumor diameter (MTD), number of tumor nodules, portal vein invasion (PVT) and blood alpha-fetoprotein (AFP) levels (as well as presence or absence of metastasis, as with most solid tumor types). A recent 'HCC Aggressiveness' scoring system was recently described, which incorporated all 4 of these parameters and related them to survival [1]. These 4 tumor characteristics also were related to liver function parameters, given that many tumors, including HCC, arise on the basis of a chronically inflamed organ [2]. Furthermore, HCC patients with elevated bilirubin levels have also been shown to have larger tumors and increased incidence of PVT [3]. A recently validated index of systemic inflammation, using the levels of only 2 liver-derived proteins, C-reactive protein (CRP) and albumin has been shown in multiple studies to be an independent prognosticator of survival [4-6]. The platelet to lymphocyte ratio is another recently described, independent inflammatory prognostic index for HCC [7]. In this paper, we validated the new Aggressiveness Index on a separate and much larger HCC patient cohort in relation to survival and we examined liver function parameters in relation to the index. We found values for the index also related significantly to survival, as well as to several liver function parameters.

Methods

Patients and data collection

Data collection: We retrospectively analyzed prospectively-collected data in the Italian Liver Cancer (ITA.LI.CA) study group database of 2706 HCC patients accrued till 2008 at 11 centers [3] who had full baseline tumor parameter data, including CT scan information on

maximum tumor diameter (MTD), number of tumor nodules and presence of PVT and plasma AFP levels; blood counts (hemoglobin, white cells, platelets, prothrombin time); routine blood liver function tests, (total bilirubin, AST, ALKP, GGTP, albumin); demographics and survival information. ITA.LI.CA database management conforms to Italian legislation on privacy and this study conforms to the ethical guidelines of the Declaration of Helsinki. Approval for this retrospective study on de-identified patients was obtained by the Institutional Review Board of participating centers.

Aggressiveness index: To construct an index that was the expression of tumor aggressiveness, we have considered the following four parameters: Maximum Tumor dimension (MTD), alpha-fetoprotein (AFP), Portal Vein Thrombosis (PVT), and the number of tumor nodules.

The Index score of Aggression was obtained as follows: MTD (in tertiles): MTD < 4.5; $4.5 \leq$ MTD ≤ 9.6; MTD > 9.6; scores 1, 2, 3 respectively. AFP (cut-off): AFP < 100; $100 \leq$ AFP ≤ 1000; AFP > 1000 ng/ml; scores 1, 2, 3 respectively. PVT (No/Yes): PVT (No); PVT (Yes); scores 1, 3 respectively. Tumor Nodule (number): Nodules ≤ 3; Nodules > 3; scores 1, 3 respectively. Therefore the Aggressiveness Index was obtained as the sum of the scores for these 4 parameters (Table 1).

	Score		
	1	2	3
MTD (in tertiles) (cm)	<4.5	4.5 - 9.6	>9.6
AFP (cut-off) (ng/mL)	<100	100 - 1000	>1000
PVT (No/Yes)	No	--	Yes
Tumor Nodules (number)	≤3	--	>3

MTD: Maximum Tumor Diameter, AFP: Alpha-fetoprotein, PVT: Portal Vein Thrombosis. The Aggressiveness Index is the sum of the scores for MTD + AFP + PVT + # Nodules.

Table 1: Aggressiveness Index is the sum of parameter scores.

Thus: MTD (in tertiles): MTD < 4.5; $4.5 \leq$ MTD ≤ 9.6; MTD > 9.6; scores 1, 2, 3 respectively. AFP (cut-off): AFP < 100; $100 \leq$ AFP ≤ 1000; AFP > 1000 ng/ml; scores 1, 2, 3 respectively. PVT (No/Yes): PVT (No); PVT (Yes); scores 1, 3 respectively. Tumor Nodule (number): Nodules ≤ 3; Nodules > 3; scores 1, 3 respectively. The Aggressiveness Index score was divided into three categories for Cox analysis (Table 2): a, score= 4; b, 4 < score ≤ 7; and c, score ≥ 8.

Statistical analysis: Mean and SD for continuous variables were used as indices of centrality and dispersion of the distribution. For non-normally distributed values we was necessary used a non-parametric methods, the Wilcoxon rank-sum (Mann-Whitney) test, for continuous variables, to test the comparisons between the Aggressiveness Index categories of liver function parameters.

The Cox proportional hazards model was applied to evaluate the predictive factors as categories of Aggressiveness Index score associated with overall survival. The results were presented as Hazard Ratio (HR) with 95% C.I. Unconditional multiple logistic regression model was used to evaluate the Odds-Ratio of the Aggressiveness Index score (≥ 8) on the terciles of each serum variable, Gamma Glutamyl Transpeptidase (GGTP), Alkaline Phosphatase (ALKP), and Aspartate Aminotransferase (AST), total bilirubin and platelets as terciles; while albumin levels were dichotomized. All variables were included together in the model. The results were presented as Odds-Ratio (OR) with 95% C.I.

In all models, Cox regression and Logistic regression, the HR and the OR respectively, represent the risk for one-unit variation of the predictor variable considered as dummy variables. Patient survival between the three categories of Aggressiveness Index score was estimated with the Kaplan-Meier method and comparison of survival was made with the Breslow (generalized Wilcoxon) test. The Breslow test was used, as opposed to the log rank test, due to the large proportion of patients who died early.

When testing the hypothesis of significant association, p-value was < 0.05, two tailed for all analyses. Statistical analysis was performed with State Corp 2007 State Statistical Software: release 10. College Station, TX: StataCorp LP.

Result

Aggressiveness index and survival

An Aggressiveness Index was constructed as described previously [1] and as shown in Table 1, was the sum of the scores for MTD, tumor multimodality, PVT and AFP values. Using the designated cutoffs for point systems, patients were divided into 3 groups a, b and c (Table 1), using a score from 4 (lowest score, with 1 point per parameter) to 12 (highest score, with 3 points per parameter). The Kaplan-Meier survival graph (Figure 1).
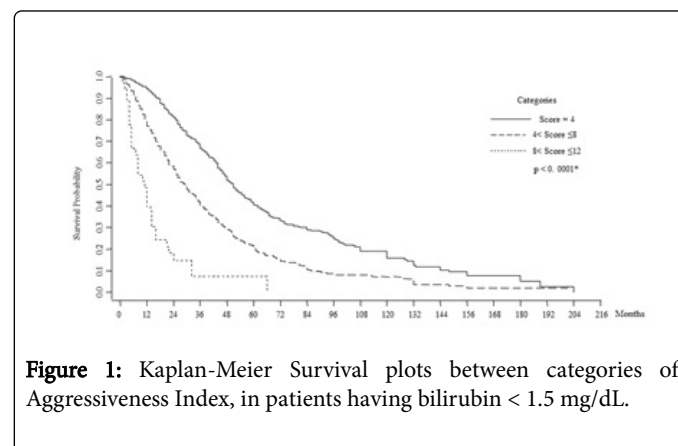
Figure 1: Kaplan-Meier Survival plots between categories of Aggressiveness Index, in patients having bilirubin < 1.5 mg/dL.

Showed statistically significant differences in survival between the 3 groups. The percent survival probabilities for the 3 groups, a, b and c were: 94%, 77% and 39% at 1 year, and 81%, 57% and 14% at 2 years, and 67%, 39% and 7% at 3 years, for the 3 groups, from the lowest to the highest scores.

A Cox proportional hazard model for death was then calculated (Table 2), with score (a) being the reference category. The hazard ratios (HRs) for groups a, b and c were: 1, 1.94 and 6.64, respectively, with p-values of <0.001 for a vs. b, a vs. c and b vs. c.

		HR*	se(HR)	p	95% C.I.	Comparisons of HRs	p
Aggressiveness Index							
Score = 4 [Ref. category]	(a)	1	--	--	--	(a) vs (b)	<0.001
4 < Score ≤ 8	(b)	1.94	0.14	<0.001	1.68 to 2.25	(a) vs (c)	<0.001
8 < Score ≤ 12	(c)	6.64	1.27	<0.001	4.56 to 9.66	(b) vs (c)	<0.001

Table 2: Cox proportional hazard model in HCC patients for death, on Aggressiveness Index Score categories in the total cohort.

Aggressiveness index and liver function parameters

The relationship between the Aggressiveness Index and liver function parameters was then examined. Firstly, the mean liver function parameters for the worst Index group (score ≥ 8) were compared to the other 2 groups combined (score < 8) (Table 3).

	Aggressiveness Index Score		
(M ± SD)	Score < 8	Score ≥ 8	p°
GGTP (IU/mL)	77.0 ± 103.3	96.7 ± 87.5	<0.0001
ALKP (IU/mL)	285.2 ± 1266.4	312.8 ± 656.0	<0.0001
AST (IU/L)	44.4 ± 48.1	56.3 ± 56.5	0.0005
Total Bilirubin (mg/dL)	1.7 ± 2.1	2.9 ± 4.2	<0.0001
Albumin (g/dL)	3.6 ± 0.63	3.2 ± 0.62	<0.0001
Platelets (x10^9/L)	116.0 ± 62.0	136.4 ± 81.9	0.0018

° Wilcoxon rank-sum (Mann-Whitney) test. Abbreviations: Plt, Platelet count; Hb, Haemoglobin; GGTP, Gamma glutamyl transpeptidae; ALKP, Alkaline phosphatase; AST, Aspartate Aminotransaminase.

Table 3: Comparisons in HCC patients between Aggressiveness Index Score of Platelets, GGTP, ALKP, AST, Total Bilirubin and Albumin, in the total cohort.

The mean values were all statistically higher for group the worst Index group than for the lower group in the Index, with respect to GGTP, ALKP, AST, total bilirubin, and platelets, only albumin had a slightly lower value (an index of liver fibrosis) [8,9]. Secondly, a multiple logistic regression model was calculated for the Aggressiveness Index on the liver parameters, each divided into terciles (Table 4).

	OR	se(OR)	p	95% C.I.	p§
GGTP (IU/mL)					0.001
≤33.0 [Ref. category]	1				
>33.0/ ≤73.2	1.98	0.44	0.002	1.29 to 3.06	
>73.2	2.09	0.47	0.001	1.34 to 3.26	
ALKP (IU/mL)					0.07
≤186 [Ref. category]	1				
>186/ ≤232	0.84	0.18	0.42	0.54 to 1.29	
>232	1.41	0.28	0.08	0.96 to 2.08	
AST (IU/L)					0.05
≤26.0 [Ref. category]	1				

>26.0/ ≤44.8	1.07	0.23	0.77	0.70 to 1.63	
>44.8	1.46	0.3	0.06	0.98 to 2.19	
Total Bilirubin (mg/dL)					<0.001
<1.0 [Ref. category]	1				
≥1.0/ ≤2.0	1.52	0.33	0.06	0.99 to 2.33	
>2.0	2.36	0.58	0.001	1.45 to 3.82	
Albumin (g/dL)					<0.001
<3.5 [Ref. category]	1				
≥3.5	0.46	0.08	<0.001	0.32 to 0.65	
Platelets (×10^9/L)					0.001
<100 [Ref. category]	1				
≥100/ ≤300	1.55	0.27	0.01	1.11 to 2.18	
>300	4.98	2.3	0.001	2.01 to 12.30	
§ p-value for trend. Abbreviations: OR: Odds-Ratio, GGTP: Gamma Glutamyl Transpeptidase, ALKP: Alkaline Phosphatase, AST: Aspartate Aminotransferase.					

Table 4: Multiple logistic regression model of Aggressiveness Index score (≤7/ ≥8), on variables as terciles included together in the model in the total cohort.

The highest Odds Ratios (ORs) were for GGTP, total bilirubin and platelets, and each parameter had a significant p-value for trend of the terciles. Albumin was dichotomized at <3.5 or >3.5 g/dl, as in the Glasgow score and showed a significant protective effect.

Discussion

Tumor factors and liver factors have separately and together been acknowledged to be important for HCC prognosis and management decisions. As a result, elements of both sets of factors are included in most HCC classification and prognostication systems [10] with increasing refinements [11]. The underlying liver has been increasingly understood as a complex dynamic of cells, tissues, inflammatory cytokines and growth factors [12-18]. This has been most extensively studied with respect to the neo vascular endothelial cells that form the blood vessels that are needed to support the growing tumor mass, and are an important target for various multikinase inhibitors, which are used in cancer therapy. In addition, several cell types in the tumor microenvironment and their secreted products, are involved in the immune and inflammatory pathways [13,14]. Two general categories of inflammation can be discerned in cancer in general, including HCC. They are: (a), local, micro environmental inflammation [17,18], such as that associated with chronic hepatitis [19]; and (b), systemic inflammation, as measured by blood C-reactive protein and albumin levels in the Glasgow and similar inflammation-based prognostic scoring systems [4-6,20]. Some micro environmental cell types have multiple functions, such as platelets, which are involved in blood coagulation, metastasis [21], and hepatitis B immune modulation [22], HCC cell growth and migration [23] and the production of HCC growth stimulants [24].

Recently, there has been a paradigm shift, as it has become apparent that the liver microenvironment and liver disease factors are not only independent negative prognostic factors for HCC survival, but may actually be involved in HCC biology. It has long been accepted that growing HCC tumor masses can destroy or replay liver parenchyma and thus enhance liver damage. There is now evidence that the converse is also likely true and that liver micro environmental factors may influence HCC growth and invasion [12-19]. Molecular signatures of the underlying liver in HCC patients provide support for this idea [25-27], as well as recently reported clinical associations of changes in liver function with HCC biology [3].

The experiments reported here had 2 goals. Firstly, to confirm using a different and larger HCC cohort, that the recently-described HCC Aggressiveness Index could be reproduced, with significant survival differences for patients in its 3 score groups of a, b and c (Figure 1). To our knowledge, there has not previously been an HCC index that takes into account all 4 of the commonly-used modalities of HCC human biology, namely tumor size (MTD), tumor multifocality, and portal venous invasion by tumor (PVT) and blood AFP levels. It was expected that the worst category group (highest points) would have the worst survival, and vice versa. We found a decrease in survival with increase in score for each of years 1, 2 and 3 (Figure 1, Kaplan-Meier graph and its associated table). The Cox regression model (Table 2) showed an increasing Hazard Ratio (HR) for death in groups a, b and c, of 1 (reference), 1.94 and 6.64, respectively. The second goal was to examine any possible relationship between the Aggressiveness Index groups and parameters of liver function (Table 3 and multiple logistic regressions in Table 4). We found (Table 3) significantly higher liver parameter values for Aggressiveness group c (Score > 8) compared to Aggressiveness groups a plus b (Score < 8), with respect to GGTP, ALKP, AST and total bilirubin, as well as for platelets (a surrogate for liver cirrhosis [8,9]). The converse was found for albumin, as expected from its published protective association for survival [4,5]. A multiple logistic regression model for these same parameters as terciles, on the Aggressiveness Index score (Table 4), showed the highest Odds Ratios (OR) were for GGTP, total bilirubin and platelet counts (highest, OR).

The results presented here show an association between liver function parameters and increasing tumor aggressiveness, but do not show a causal relationship. Nevertheless, the association is quite remarkable. Given the newly available and effective therapies for hepatitis B and C (and possibly also anti-inflammatory agent, such as aspirin, NSAIDS, Cox inhibitors, in patients without bleeding disorder), it may be that their use in suppressing hepatic inflammation and/or improving liver function in HCC patients with inflamed livers, offer the possibility of decreasing HCC aggressiveness through improvement of the underlying liver function [28-32]. What might be the mechanisms by which liver function parameters could influence HCC aggressiveness Two possible general explanations might be considered. A first is that the liver function parameters are only passive measures that reflect the underlying hepato carcinogenic processes of cirrhosis. A second possibility is that the liver parameters actually have HCC-modifying functions. Thus, albumin has been shown to subdue HCC cell growth [33]; HCC-specific GGT isoforms are membrane glycoproteins that catalyze the first enzymatic step in the metabolism of glutathione and is important in maintaining intracellular cysteine and glutathione levels [34-36], ALKP might modulate cancer drug resistance [37] as might bilirubin [38,39]. Furthermore, platelet factors have HCC-stimulatory properties [24].

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