

A Combination of Blood Lymphocytes and AST Levels Distinguish Patients with Small Hepatocellular Carcinomas from Non Cancer Patients.

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Abstract

Purpose

HCC patients typically present at an advanced tumor stage, in which surgical therapies cannot be used. Screening ultrasound exams can increase the numbers of patients diagnosed with small tumors, but are often not used in patients at risk for HCC. We evaluated clinically-available and cheap potential blood tests as biomarkers for screening patients at risk for HCC.

Methods

A comparison was made of commonly used blood count and liver function parameters in a group of patients (n=101) with small HCCs (≤ 3 cm) or without HCC (n=275), who presented for liver transplantation in our institute.

Results

Significant differences were found for blood lymphocytes and AST levels. This 2-parameter combination was found to be significantly different between patients with small HCCs versus no HCC. Using the combination of lymphocytes and AST levels to dichotomize the HCC patients, only blood levels of alpha-fetoprotein amongst the tumor characteristics, were found to be significantly different amongst the 2 HCC groups, as well as levels of blood total bilirubin, ALKP and PLR ratio. The results were confirmed using a separate smaller cohort of non-transplanted small size HCC patients.

Conclusion

The combination of elevated blood levels of lymphocyte counts and AST levels holds promise for screening of patients with chronic liver disease who are at risk for HCC.

Introduction

Most hepatocellular carcinoma (HCC) patients are diagnosed at an advanced tumor stage, when surgical therapies cannot be used (1). In our experience and that of others, only a minority of cases can currently be treated by surgery (2), which is the main treatment that results in long survival. Although some larger HCCs can be treated by resection, survival post-surgery is significantly less than for smaller size HCCs (3). Thus, tumor size is prognostically important. Given the importance of small HCC size in HCC treatment outcomes, emphasis has been placed on screening of patients at risk, such as those with chronic hepatitis B or C. Nevertheless, surveillance programs can result in diagnosis of smaller HCCs with increased survival (4, 5). Thus, screening is important, considering the estimated global 905,677 new HCC cases annually, and 830,180 deaths (6). There is thus a need for simple, cheap and reliable tumor markers to be used at patients at risk of developing HCC, to diagnose smaller size HCCs, where surgical therapy is most effective. We report here a comparison of clinical laboratory parameters in patient with

small HCCs and in patients without known HCC, to evaluate differences that might have a possible future use in screening.

Methods

Clinical methods

Patients having live donor liver transplantation for HCC (n=101) or non-tumor (n=275) liver disease indications (predominantly chronic hepatitis B) at our Liver Transplantation Institute are the subjects of this study. The data were analyzed retrospectively. This study was approved by Inonu University Institutional Review Board (Approval no: 2021/2544). Patients had either a small HCC ≤ 3 cm or no tumor as judged by pre-transplant CAT scan evaluation. All patients had baseline radiological evaluation, complete blood counts and standard liver function tests.

Statistical methods

Normal distribution of the quantitative data was evaluated by Kolmogorov-Smirnov test. Quantitative data were summarized by median, minimum and maximum values. Mann-Whitney U test was used to compare two independent groups. Qualitative data was expressed as count and percentage. Pearson's chi-square, continuity corrected chi-square or Fisher's exact tests were used for comparisons where appropriate. Odds ratio (OR) estimations were obtained by both univariate and multivariate binary logistic regression analysis. Significance level was considered as 0.05 in all analyzes.

Results

Comparison of blood parameters for non HCC and HCC patients

A comparison was made of the common peripheral blood count and liver function test parameters between patients with small HCCs (n=101) versus patients with hepatitis or cirrhosis (n=275) who did not have an HCC diagnosis on pre-transplant evaluation. There were statistically significant differences between groups in terms of lymphocytes ($p < 0.001$) and AST ($p < 0.002$) levels (Table 1) using both univariate and multivariate logistic regression analyses.

Table 1

Univariate and multivariate binary logistic regression analysis of patients with small HCCs vs non-HCC patients.

	Univariate		Multivariate	
	OR (95% C.I.)	p	OR (95% C.I.)	p
WBC \leq 10	reference		reference	
WBC $>$ 10	1.175 (0.588-2.350)	0.648	1.041 (0.513-2.114)	0.911
Lymphs $<$ 1	reference		reference	
Lymphs \geq 1	1.886 (1.312-2.711)	0.001	1.683 (1.140-2.484)	0.009
Eosinophil \leq 01	reference		reference	
Eosinophil $>$ 01	1.406 (0.995-1.986)	0.053	1.210 (0.835-1.752)	0.314
AST \leq 40	reference		reference	
AST $>$ 40	1.807 (1.253-2.607)	0.002	1.564 (0.982-2.491)	0.050
ALT \leq 40	reference		reference	
ALT $>$ 40	1.592 (1.121-2.261)	0.009	1.188 (0.770-1.831)	0.436
T.Bil \leq 1	reference		reference	
T.Bil $>$ 1	1.251 (0.835-1.873)	0.278	1.063 (0.690-1.638)	0.781
Abbreviations: WBC, white blood count ($\times 10^9/L$); Lymphs, lymphocytes ($\times 10^9/L$); T. bil, total bilirubin (mg/dL); AST, aspartate amino transferase; (IU/mL) ALT, alanine aminotransferase (IU/mL); Eosinophils ($\times 10^9/L$).				

Two-parameter models

We next built a 2-parameter model, based upon the only 2 parameters that were individually significantly different in Table 1, namely blood lymphocyte and AST levels. The median values of lymphocytes and AST for the non HCC patients were used for the dichotomization (Table 2a). The combination of low total bilirubin and low lymphocytes versus high total bilirubin and high lymphocytes showed significant differences for non HCC versus small HCC patients. The low lymphocyte and AST combination was found 18.8% of small HCC patients. But the high lymphocyte and AST combination was found in 81.2% of small HCC patients. Non HCC patient percentages were 42.9% for the low parameter combination and 57.1% for the high parameter combination. This 2-parameter pair had a sensitivity of 0.812 and a specificity of 0.429 for small HCCs (Table 2b).

Table 2
 b. Sensitivity and specificity of the 2-parameter pair for small HCCs.

Small HCC criterion	Sensitivity	Specificity	AUC	p-value for AUC
Lymphs \geq 1 & AST $>$ 40	0.812	0.429	0.620 (0.559-0.682)	<0.001
Abbreviations: Lymphs, lymphocytes ($\times 10^9/L$); AST, aspartate amino transferase (IU/mL).				

HCC tumor and blood characteristics using the 2-parameter model.

Table 2a showed that 18.8% of the tumor patients had low values in the 2-parameter model. To examine for difference in the patients with HCCs who had high versus low values, the HCC patients with high or low 2-parameter values were next compared. Of the 4 HCC aggressiveness characteristics (maximum tumor diameter, multifocality, portal vein invasion or alpha-fetoprotein levels), only blood alpha-fetoprotein levels were significantly different between the 2 dichotomized small HCC groups (Table 3). Interestingly, there were significant differences in the blood levels of white blood count, total bilirubin, ALT, ALKP and PLR ratio (Table 4). This 2-parameter dichotomization suggests the presence of 2 small HCC phenotypes. The survival of these transplanted patients with small HCCs was examined, after dichotomization according to presence of low or high levels of blood lymphocytes and AST. However, the mean survival for HCC patients was not significantly different between the low or high 2-parameter levels, 3172 versus 4001 days ($p=0.055$).

Table 3
Comparison of HCC parameters within the 2-parameter pairs.

Small HCC: tumor parameters					
	Lymphs<1 & AST≤40		Lymphs≥1 & AST>40		
	n	Median (min.-max.)	n	Median (min.-max.)	p
MTD size	19	2 (0.2-3)	82	1.5 (1-3)	0.262
# nodules	19	1 (1-5)	82	1 (1-21)	0.740
AFP	19	5 (0.4-436)	82	13.4 (0.7-2324)	0.002
	n	%	n	%	
Tumor # ≤ 2	14	73.7	58	70.7	1.000
Tumor # > 2	5	26.3	24	29.3	
PVT (none)	16	84.2	63	76.8	0.758
PVT (micro)	3	15.8	19	23.2	

Abbreviations: Lymphs, lymphocytes (x10⁹/L); AST, aspartate amino transferase (IU/mL); MTD, maximum tumor diameter; AFP, alpha-fetoprotein (IU/mL); PVT, portal vein invasion by tumor.

Table 4
Comparison of blood parameters within the 2-parameter pairs.

Small HCC: labs			
	Lymphs<1 & AST≤40 (n=19)	Lymphs≥1 & AST>40 (n=82)	p
T.bil	1.1(0.37-5.05)	2.15(0.3-33.83)	0.004
WBC	3.8(1.6-9.3)	5.9(2.1-19.7)	<0.001
Platelets	73(19-189)	86(20-394)	0.084
Albumin	2.7(2.2-3.9)	2.6(1.8-4.1)	0.222
ALT	17(11-57)	46(18-675)	<0.001
ALKP	84(60-199)	128(56-799)	0.005
GGT	56(11-412)	63.5(15-323)	0.148
PLR	100(38-472.5)	57.57(9.74-207.37)	<0.001

Abbreviations: WBC, white blood count; T. bil, total bilirubin; AST, aspartate amino transferase; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; GGT, gamma glutamyl transferase; PLR, platelet lymphocyte ratio.

Validation of findings using a non-transplant HCC dataset

We evaluated a different dataset of small HCC patients who were not transplanted to confirm our findings. Using the same 2-parameter model of blood lymphocyte counts and AST levels, we also showed a statistically significant difference between patients with small HCCs who had low 2-parameter values (2.6% of the small HCC patients), compared with patients with small HCCs having high 2-parameter values (97.4% of the small HCC patients), although with only 39 HCC patients (Table 5) .

Table 5
Confirmation table on non-transplant HCC patients.

	Lymphs<1 & AST≤40	Lymphs≥1 & AST>40	p
	n (%)	n (%)	
Non-HCC	118 (42.9)	157 (57.1)	<0.001
Small HCC	1 (2.6)	38 (97.4)	
Abbreviations: Lymphs, lymphocytes (x10 ⁹ /L); AST, aspartate amino transferase (IU/mL).			

Discussion

This report shows that the combination of elevated blood levels of elevated lymphocyte counts and AST values are associated with presence of small size (≤ 3 cm) HCCs, compared with non HCC chronic liver disease patients, both groups of whom were transplanted at our institute.

We validated the finding with a small set of non-transplant patients. Our plan is to test this 2-parameter combination on a future large HCC dataset, and then to consider evaluating the combination for use in the screening of chronic liver disease patients, who are thought to be at increased risk for subsequent HCC development.

The strength of this study is that these bloods parameters are routinely used blood tests in common clinical practice and are cheap. The weakness of the study is the fairly small HCC sample size of 101 HCC patients and the very small confirmation sample size. The reason for the latter is that adherence to screening guidelines is uncommon and most patients get diagnosed with quite advanced tumors, which were excluded from our study by design. Thus, we recognize that this is a preliminary finding and that confirmation from another, larger dataset is needed. If validated, this 2-parameter combination will need evaluation as a screening tool in a large group of prospectively followed patients with chronic liver disease, who have not yet developed HCC.

A likely reason these parameters might be useful is that HCC develops on the basis of a chronically inflamed liver (7). Elevated lymphocytes represent a peripheral blood inflammation parameter, and AST elevation is a liver damage marker. Inflammation has also been previously found to be more associated especially with smaller HCCs (8), as have other elevated liver function tests and white blood cell

constituents (9). The finding of readily available and cheap biomarkers to better diagnose the presence of HCC at small and treatable size, is one of the main objectives of current HCC research.

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; PLR, platelet lymphocyte ratio; CAT, computed axial tomography.

Declarations

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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Author contributions

BIC – concept, ideas and writing; HGB-biostatistics; VI, SA, VE, SU, BI, ZO, AT, 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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