A simple 2-parameter blood test alert for the presence of small hepatocellular carcinomas

ClinicalPractice

Abstract

Objectives: The majority of HCCs present at an advanced stage in which potentially curative therapies cannot be used. Surveillance ultrasound has been found to increase the numbers of patients diagnosed with small tumors, but it is often not used. We aimed to try to identify widely-available and cheap potential serum markers for use in patients at risk for HCC.

Material and Methods: A comparison was made of the complete blood count and liver function tests in a group of patients (n=114) with proven small HCCs (≤ 2 cm) and patients without HCC (n=506), all of whom were treated by liver transplantation in our Liver Transplantation Institute.

Results: Significant differences were found for blood levels of WBC, lymphocytes, total bilirubin and transaminases. Several 2-parameter combinations were assessed, but only the combination of total bilirubin and lymphocytes was found to be significantly different between patients with small HCCs and no HCC. Multivariate regression analysis showed significance only for total bilirubin levels and lymphocyte counts. The results were confirmed using a separate small cohort of non-transplant patients.

Conclusion: The combination of elevated levels of total bilirubin and lymphocyte counts holds promise for identification of patients with chronic liver disease who are at risk for HCC.

Keywords: hepatocellular carcinoma, total bilirubin, lymphocyte, potential biomarkers, liver transplanty

Abbreviations: HCC: Hepatocellular Carcinoma, WBC: White Blood Count, T. Bil: Total Bilirubin, AST: Aspartate Amino Transferase, ALT: Alanine Aminotransferase, ALKP: Alkaline Phosphatase, GGT: Gamma Glutamyl Transferase, CAT: Computed Axial Tomography, AUC: Area Under the Curve, OR: Odds Ratio, ROC: Receiver Operating Characteristic

Introduction

The majority (>50%) of patients with newly-diagnosed Hepatocellular Carcinoma (HCC) present at an advanced stage, when potentially curative therapies cannot be used, with associated poor prognosis [1]. In practice, only a maximum of 20% of cases can currently be treated by surgery [2], which is the main effector of prolonged survival. Although larger size single HCCs can be treated by resection, the survival is significantly less than for smaller size HCCs [3]. Thus, size matters. Furthermore, survival tends to be poor, even in high-income countries [4], although there is wide subgroup variation. Given the importance of small HCC size in HCC outcomes, emphasis has been placed on surveillance screening of patients at risk, namely those with chronic hepatitis B or C or cirrhosis from any cause. However, compliance is often poor, with only 20% of newly diagnosed HCC patients being shown in some reports to have been diagnosed through screening [5]. Despite this, the use of surveillance can result in diagnosis of smaller HCCs and increased survival [6,7]. This

is particularly needed considering the estimated recent global 905,677 new HCC cases annually, and 830,180 deaths, with mortality to incidence ratio of 0.92 [8]. There is thus a need for simple, cheap and reliable tumor markers to be used for 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 patients with small HCCs versus chronic liver disease patients without known HCC, to evaluate differences that might have possible future use in screening.

Methods

■ Clinical methods

Patients who underwent liver donor liver transplantation for HCC (n=114) or non-tumor (n=506) chronic liver disease indications at our Liver Transplantation Institute are the subjects of this study. The data were collected prospectively but analyzed retrospectively. This study was approved by Inonu University Institutional Review Board (Approval no: 2021/2544) and

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the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Patients had either a small HCC<2 cm (a common application for radiofrequency ablation) 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 and the majority of patients with or without HCC has chronic HBV infection as their underlying disease.

■ 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 were 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 logistic regression analysis. To assess the discrimination ability of the parameters, sensitivity, specificity and AUC (95% C.I.) obtained by ROC analysis was used. The 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 with respect to the common peripheral blood count and liver function test parameters, between patients with small HCCs <2 cm (n=114) and patients with chronic hepatitis and/or cirrhosis (n=506) who did not have an HCC diagnosis based on pre-transplant CAT scan and evaluation. There were statistically significant differences between groups in terms of WBC (p=0.001), lymphocytes (p<0.001), eosinophils (p=0.01), basophils (p=0.149), neutrophils (p=0.043), and total bilirubin (p<0.001), AST and ALT (p<0.001 each) levels **(TABLE 1)**.

■ Logistical regression models

A logistical regression model was then calculated for the parameters of **TABLE 1** that had statistically significant differences using both univariate and multivariate analysis. Only total bilirubin levels and lymphocyte counts reached a p-value of <0.002, each with a high OR of 2.34 and 1.46, respectively, in the univariate analysis, and of 2.335 and 1.49 respectively in the multivariate analysis **(TABLE 2)**.

■ Two-parameter model

To assess the discriminatory ability of the parameters, sensitivity, specificity and also AUC (95% C.I.) obtained by ROC analysis was used, as shown in **TABLE 3**. AUC p-values of 0.001 or less were found for total bilirubin, white blood count, lymphocytes, AST and ALT. Using the cutoffs identified from **TABLE 3** for total bilirubin and lymphocyte counts, which were the only parameters that were significant in the multivariate analysis in **TABLE 2**, we then built a 2-parameter model for evaluating differences between non-HCC and small (<2 cm) HCC patients **(TABLE 4)**, p<0.001.

TABLE 1. Non-HCC patients versus patients with small HCC (≤ 2cm): blood count and liver function parameters.			
	Non-HCC (n=506) Median (Min-Max)	**HCCs (≤ 2 cm) (n=114) Median (Min-Max)**	**p**
WBC (× 10⁹/L)	4.8 (0.31-66)	5.7 (1.9-19.7)	0.001
Basophil (× 10⁹/L)	0.02 (0-1)	0.03 (0-0.13)	0.049
Eosinophil (× 10⁹/L)	0.1 (0-3.02)	0.11 (0-6.4)	0.01
Lymphocyte(× 10⁹/L)	1.02 (0.1-4.33)	1.3 (0.4-7.7)	<0.001
Neutrophil (× 10⁹/L)	2.9 (0.4-35.41)	3.06 (0.7-14.3)	0.043
Platelets (× 10⁹/L)	91 (6-744)	96 (19-394)	0.918
T.Bil (mg/dL)	1.51 (0.23-2.59)	1.94 (0.3-33.8)	<0.001
Albumin (g/dL)	2.9 (1-4.5)	2.75 (1.6-4.8)	0.745
AST (IU/L)	44 (10-7456)	57 (21-589)	<0.001
ALT (IU/L)	31 (6-4578)	39 (11-675)	<0.001
ALKP (IU/L)	116 (25-826)	114 (53-799)	0.464
GGT (IU/L)	66 (9-2511)	58.5 (13-412)	0.487
Abbreviations:WBC: White Blood Count; T.Bil: Total Bilirubin; AST: Aspartate Amino Transferase; ALT: Alanine Aminotransferase; ALKP: Alkaline Phosphatase; GGT: Gamma Glutamyl Transferase			

TABLE 2. Univariate and multivariate logistic regression analysis of patients with small HCCs (<2 cm) vs. Non-HCC patients.

	Univariate		Multivariate (Forward selection)	
	OR (95% CI)	p	OR (95% CI)	p
WBC (× 10⁹/L)	1.027 (0.986-1.070)	0.205		
Basophil (× 10⁹/L)	1.282 (0.055-30.077)	0.877		
Eosinophil (× 10⁹/L)	1.596 (0.897-2.843)	0.112		
Lymphocyte(× 10⁹/L)	1.468 (1.153-1.868)	0.002	1.499 (1.108-2.027)	0.009
Neutrophil (× 10⁹/L)	1.020 (0.956-1.088)	0.551		
T.Bil (mg/dL)	2.340 (1.842-2.971)	<0.001	2.335 (1.831-2.979)	<0.001
AST (IU/L)	1.000 (1.000-1.001)	0.737		
ALT (IU/L)	1.000 (0.999-1.001)	0.642		

Abbreviations: OR: Odds Ratio; WBC: White Blood Count; TBil: Total Bilirubin; AST: Aspartate Amino Transferase; ALT: Alanine Aminotransferase

TABLE 3. Sensitivity and specificity of single parameters and AUC for small HCC (≤ 2cm).

	Small HCC criteria	Sensitivity (%)	Specificity (%)	AUC (95% CI)	AUC p-value
T.Bil	>2.59	38.6	100	0.652 (0.613-0.690)	<0.001
Lymphocytes	>0.86	81.6	39.7	0.606 (0.566-0.645)	<0.001
WBC	>3.77	86.8	30.8	0.601 (0.562-0.640)	<0.001
Basophils	>0.03	46.5	65.8	0.558 (0.518-0.597)	0.047
Eosinophils	>0.24	29	83	0.577 (0.537-0.616)	0.007
Neutrophils	>2.55	73.7	39.1	0.561 (0.520-0.600)	0.035
AST	>46	67.3	53.6	0.628 (0.588-0.666)	<0.001
ALT	>23	85.1	35	0.617 (0.577-0.655)	<0.001

Abbreviations: AUC: Area Under the ROC Curve; others, as in Table 2

TABLE 4. Comparison of 2-parameter combination in Inonu patients with small HCCs (≤ 2 cm) vs. non-HCC patients.

Two parameter combination	Non-HCC	Small HCCs	p	OR (95% CI)
T.Bil ≤ 2.59 and Lymphocytes ≤ 0.86	201 (95.3)	10 (4.7)	<0.001	NA
T.Bil > 2.59 and Lymphocytes > 0.86	0 (0)	33 (100)		

Abbreviations: ORL Odds Ratio, Lymphocytes: × 109/L; T.Bil: Total Bilirubin (mg/dL)

◼ Validation of findings using a non-transplant HCC dataset

We evaluated a completely separate small HCC dataset from a non-transplant cohort **(TABLE 5)** to confirm our findings. Using the same 2-parameter model of total bilirubin levels and lymphocyte counts, we confirmed that there was a statistically significant difference between patients with small HCCs who had low 2-parameter values, p<0.001, with 100% of the patients who had high values for the 2-parameter pair being the small HCC patients, although with admittedly small numbers of HCC patients (n=22). The Positive Predictive Value (PPV) and the Negative Predictive Value (NPV) of the 2-parameter model for the main

test group were 100% and 95.3%, for the validation cohort 100% and 99.5%, respectively **(TABLE 5)**.

The analysis was then repeated for sensitivity and specificity of the 2-parameter pair and AUC for patients with small HCCs (≤ 2cm) in both the Inonu transplant patient test group and in the non-transplant confirmation group of HCCs. For the main test group, we found that the sensitivity was 76.7% and specificity was 100%, with AUC of 0.884 and AUC p-value of <0.001 **(TABLE 6)**. Similarly, values for the non-transplant confirmation group were: sensitivity of 95.5%, specificity of 100%, AUC 0.977, AUC p-value of <0.001.

TABLE 5. Confirmation of findings using non-transplant HCC patients with HCCs ≤ 2 cm.

Two parameter combination	Non-HCC	Small HCCs	p	OR (95% CI)
T.Bil ≤ 2.59 and Lymphocytes ≤ 0.86	201 (99.5)	1 (0.5)	<0.001	NA
T.Bil >2.59 and Lymphocytes >0.86	0 (0)	21 (100)		
Abbreviations: Lymphocytes: × 10⁹/L; T.Bil: Total Bilirbin (mg/dL)				

TABLE 6. Sensitivity and specificity of two parameter pair and AUC for small HCC (≤ 2cm).

Small HCC criteria		Sensitivity (%)	Specificity (%)	AUC (95% CI)	AUC p-value
T.Bil >2.59 and Lymphocytes >0.86	Inonu patients	76.7	100	0.884 (0.807-0.960)	<0.001
	Confirmation patients	95.5	100	0.977 (0.927-1.000)	<0.001
Abbreviations: AUC: Area Under the ROC Curve; Lymphocytes: × 10⁹/L; T.Bil: Total Bilirbin (mg/dL)					

Discussion

The main findings in this report are that the combination of elevated blood levels of total bilirubin and elevated lymphocyte counts are associated with presence of small size (<2 cm) HCCs, compared with non HCC chronic liver disease patients, both groups of whom were transplanted at our institute.

Our aim was to try to identify potential markers amongst routinely used clinical tests, that could differentiate these 2 groups of patients with chronic liver disease, but only one of them having HCC. We validated the finding with a small set of non-transplant patients. Our hope is to test this combination on a future large HCC dataset, as a preliminary to evaluating this 2-parameter combination for possible use in the screening of chronic liver disease patients, who are at increased risk for subsequent HCC development. We considered a comparison of patients with HCC versus normal controls, but rejected that idea, as we believe the appropriate HCC controls to be patients with similar liver disease, but without HCC.

The strength of this study is that these blood parameters are routinely used in blood tests in common clinical practice and are cheap. The weakness of the study is the fairly small HCC sample size (n=114) of the HCC patients and the very small confirmation sample size (n=22). 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. When small HCCs get diagnosed, they are typically potential liver transplant candidates. Thus, we recognize that ours is a preliminary finding and that confirmation from another, independent dataset is needed. If validated, then 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.

We can only speculate on the possible reasons why these parameters might be useful. A likely reason is that HCC is one of those tumor types that develop in association with chronic hepatic inflammation [9]. Elevated lymphocytes represent a peripheral blood inflammation parameter, and bilirubin elevation is a liver damage marker (among other things). Interestingly, transaminases were also significantly increased in the small HCC group but did not contribute to the 2-parameter tests. Inflammation has also been previously found to be more associated with smaller, rather than larger HCCs [10], as have elevated serum bilirubin levels [11]. The finding of readily available and cheap serum biomarkers to better diagnose the presence of HCCs at a small and thus treatable size is one of the main unmet needs of HCC care.

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

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

Llovet JM, Montal R, Sia D, et al. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol.* 15, 599-616 (2018).

Pons F, Varela M, Llovet JM. Staging systems in hepatocellular carcinoma. *HPB (Oxford).* 7, 35-41 (2005).

Pelizzaro F, Penzo B, Peserico G, et al. Monofocal hepatocellular carcinoma: How much does size matter? *Liver Int.* 41, 396-407 (2021).

Rutherford MJ, Arnold M, Bardot A, et al. Comparison of liver cancer incidence and survival by subtypes across seven high-income countries. *Int J Cancer.* 149, 2020-2031 (2021).

Davila JA, Morgan RO, Richardson PA, et al. Use of surveillance for hepatocellular carcinoma among patients with cirrhosis in the United States. *Hepatology.* 52, 132-141 (2010).

Trevisani F, De Notariis S, Rapaccini G, et al. Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: effects on cancer stage and patient survival (Italian experience). *Am J Gastroenterol.* 97, 734-744 (2002).

Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol.* 130, 417-422 (2004).

Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 71, 209-249 (2021).

Carr BI. Biological aspects of HCC. In Liver Cancer in the middle East. *Springer Nature.* 3-12 (2021).

Pancoska P, Lu SN, Carr BI. Phenotypic Categorization and Profiles of Small and Large Hepatocellular Carcinomas. *J Gastrointest Dig Syst.* 12, 001 (2013).

Carr BI, Guerra V, De Giorgio M, et al. Small hepatocellular carcinomas and thrombocytopenia. *Oncology.* 83, 331-338 (2012).