

Laboratory Predictors of Risk for Developing Hepatocellular Carcinoma in Cirrhosis

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Abstract

Introduction: Hepatocellular carcinoma (HCC) is a significant cause of mortality and morbidity in patients with cirrhosis. Using laboratory tests to identify patients at high risk for developing HCC may improve outcome and reduce the cost of screening in this patient population.

Methods: In this retrospective study, we included all adult patients evaluated for liver transplantation between 1993 and 2012 at a single university-based transplant center. We used the hospital database and electronic medical records to obtain patients' demographics and laboratory data. ICD-9 code was used to determine patients who developed HCC.

Results: A total of 3284 Patients were included in the study. Patients were predominantly white (86%), male (56%), with mixed etiology of cirrhosis (26% viral, 17% EtOH, 52% other), had a mean age of 54 years (SD 11.4), MELD of 10 (SD 5.1), albumin 3.7 (SD 0.7), creatinine 1.0 (SD 0.7), INR 1.3 (SD 0.3), bilirubin 1.9 (SD 2.8) and platelet count of 149,000 (SD 94,000). After a follow up of 2.7 years (SD 2.7), 5% developed HCC, 24% died, and 10% underwent liver transplantation. In univariate analyses, the following parameters were predictive of HCC risk: age (HR 1.018, 95% CI 1.004-1.032, p 0.01), male gender (HR 3.268, 95% CI 2.265-4.715, p <0.0001), INR (HR 1.896, 95% CI 1.418-2.534, p <0.0001), albumin (HR 0.453, 95% CI 0.367-0.561, p <0.001), platelet count (HR 0.991, 95% CI 0.989-0.994, p <0.0001), bilirubin (HR 1.062, 95% CI 1.010-1.117, p 0.02) and MELD (HR 1.060, 95% CI 1.030-1.091, p <0.0001). Creatinine was not predictive. In multivariate analysis, only age, male gender, platelet count, and albumin were predictive of HCC. Patients with platelet count <100,000 and albumin <3 had the highest annual actuarial risk for HCC of 6.2%.

Conclusion: older males with low platelet count and albumin level are at higher risk for developing HCC. The interval of HCC screening for males with platelet count <100,000 and albumin < 3 g/dl may need to be shortened. Further prospective studies are warranted to determine the optimal screening interval.

Keywords: Hepatocellular Carcinoma; HCC; Liver; Cirrhosis; Platelet; Albumin; MELD; INR; Bilirubin; Creatinine;

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy worldwide accounting for around 600,000 deaths every year and its incidence has been rising in many countries [1]. In men, it is the fifth most frequently diagnosed cancer worldwide, and the second leading cause of cancer-related death. In women, it is the seventh most common cancer and the sixth cause of cancer-related death. Despite of significant advances in diagnosis and management, HCC continues to carry a very high case fatality rate [2, 3].

The majority of HCC cases (>80%) are reported in sub-Saharan Africa and Eastern Asia where the incidence rate exceeds 20 per 100,000 individuals. Southern European countries have lower incidence levels of 10-20 per 100,000, while North America, South America, and Northern Europe have the lowest incidence rate of less than 5 per 100,000 individuals. Over the past few years the incidence of HCC has been decreasing in Japan, Hong Kong, Shanghai, and Singapore while it has been increasing in USA and Canada [4, 5]. The pathogenesis of HCC is complex with the interaction between various factors including intercellular microenvironment, inflammation, oxidative stress, and hypoxia in addition to intracellular and nuclear changes that interact together leading to tumor initiation, progression, and metastasis [6, 7]. In recent years, it has become clear that innate immunity plays a role in development of HCC through the production of pro-inflammatory cytokines and chemokines [6].

Chronic inflammation and fibrosis associated with chronic liver disease and cirrhosis is the primary trigger of carcinogenesis in the liver [8]. Current guidelines recommend screening high-risk patients with ultrasound (US) examination and serum alpha-fetoprotein (AFP) at 6-month intervals. If a suspicious lesion is identified, further testing with 4-phase CT or dynamic contrast-enhanced MRI should be performed. Liver biopsy can be performed to confirm the diagnosis [9]. Initial screening with US

alone or in combination with AFP is likely to be the most cost-effective strategy [10].

A randomized controlled trial of surveillance with a 6-monthly combination of US and AFP versus no surveillance showed a survival benefit to surveillance. This study was conducted in China and enrolled 18,816 patients. Even though the adherence to surveillance was suboptimal in this study, patients in the surveillance arm had a 37% reduction in HCC related mortality [11].

In a study by Cabibbo et al., the median survival of patients with untreated HCC was 9.8 (range 6.4-13), 6.1 (range 4.9-7.3), and 3.7 months (range 1.5-6) for Child-Pugh class A, B and C respectively ($P < 0.05$ for comparison between stages) [12]. Detection of HCC in early stages results in survival improvement while HCC detected after the onset of symptoms carries a very poor prognosis (5-year survival of 0-10%). In contrast, early diagnosis of HCC offers the potential for cure with studies showing the 5-year survival to exceed 50% [13]. Determining patients with higher risk for developing HCC may result in improving screening protocols and in lowering the overall cost. Thus, it was the aim of this study to determine demographic and laboratory factors associated with increased risk for developing HCC.

Methods

The single-center retrospective study that was conducted at our tertiary care transplant center after approval by the internal review board. All adult patients evaluated for liver transplantation at our Center between 1993 and 2012 were included in the study. Paediatric patients and those with a pre-existing diagnosis of HCC were excluded from the study. The clinical data repository at our center was used to gather patients' demographic and laboratory values at the first encounter at the Transplant Center. It included: age, gender, etiology of liver disease, serum bilirubin, serum creatinine, the international normalized ratio (INR), serum albumin, Model for End-Stage Liver Disease score (MELD), and platelet count. The primary endpoint of the study was determined as the development of HCC. Patients who died or underwent liver transplantation were censored in survival analyses. The association between demographics, etiology of liver disease, MELD, MELD components, platelet count, and albumin with risk for developing HCC was investigated. All statistical analyses were performed with SPSS (19.0, Chicago, IL). Categorical variables were compared using chi-square analysis or Fisher exact test where appropriate. Continuous variables were analyzed using the Wilcoxon sign-rank test or independent sample t-test as appropriate. Unadjusted survival was calculated using Kaplan-Meier estimates. Multivariate survival models were constructed using logistic regression and proportional hazards modelling with an endpoint of death on the waiting list and censoring at the time of liver transplantation using the method of maximum likelihood estimates. Type one error differences at the 0.05 level or less were considered statistically significant. All statistical tests were two-sided.

Results

A total of 3284 patients were included in the study. Patients were predominantly white, male, had a mean age of 54 years, with mean MELD of 10, albumin 3.7 and platelet count of 149,000. After a mean follow up of 2.7 years, 5% of the patients developed HCC, 24% died, and 10% underwent liver transplantation (Table 1). In univariate analyses, the following parameters were predictive of HCC risk: age (HR 1.018, 95% CI 1.004-1.032, $p < 0.01$), male gender (HR 3.268, 95% CI 2.265-4.715, $p < 0.0001$), INR (HR 1.896, 95% CI 1.418-2.534, $p < 0.0001$), albumin (HR 0.453, 95% CI 0.367-0.561, $p < 0.001$), platelet count (HR 0.991, 95% CI 0.989-0.994, $p < 0.0001$), Bilirubin (HR 1.062, 95% CI 1.010-1.117, $p < 0.02$) and MELD (HR 1.060, 95% CI 1.030-1.091, $p < 0.0001$). Creatinine was not predictive of HCC risk. Older age and male gender were associated with increased risk for HCC. Worse liver function- as indicated by increased bilirubin, INR, and MELD- and decreased albumin, were associated with higher risk for HCC. Patients with lower platelet count had an increased incidence of HCC.

Table 1

	HCC+	HCC-	p
#	165	3118	
Age (years)	55.2 (SD 9.5)	54 (SD 11.5)	0.007
Male	77.6%	55.2%	<0.0001
White	82.4%	85.8%	0.2
LOS (years)	2.3 (SD 2.5)	2.7 (SD 2.7)	0.07
Platelet	111.5 (SD 62.6)	151.1 (SD 95)	<0.0001
Creatinine	0.9 (SD 0.3)	1.0 (SD 0.7)	0.02
INR	1.3 (SD 0.3)	1.3 (SD 0.4)	0.05
Bilirubin	1.9 (SD 1.7)	1.9 (SD 2.8)	0.04
Albumin	3.5 (SD 0.6)	3.7 (SD 0.7)	0.02
MELD	10.2 (SD 4.3)	10.0 (SD 5.2)	0.02
Etiology:			
ETOH	13.3%	17%	<0.0001
Viral	37.6%	25.1% 4.9%	
ETOH/Viral	12.7%	53%	
Other	36.4%		

In multivariate analysis, only age (HR 1.031, 95% CI 1.016-1.046, $p < 0.0001$), male gender (HR 3.013, 95% CI 2.042-4.444, $p < 0.0001$), platelet count (HR 0.993, 95% CI 0.991-0.996, $p < 0.0001$), and albumin (HR 0.511, 95% CI 0.399-0.654, $p < 0.0001$) were predictive of risk for HCC. MELD and MELD components failed to reach statistical significance in predicting the risk for developing HCC.

The patients were stratified into two groups: those with a platelet count of $<100,000/\mu\text{l}$ and those with platelet count $\geq 100,000/\mu\text{l}$. The two groups were compared to define any differences. Patients with platelet count $<100,000/\mu\text{l}$ were older, white, males with higher MELD scores, and had a higher incidence

of HCC (Table 2). Cox regression survival analysis showed that patients with platelet count $<100,000/\mu\text{l}$ were at significantly increased risk to develop HCC than those patients with platelet count $\geq 100,000/\mu\text{l}$ (HR 2.5, 95% CI 1.82-3.38, $p < 0.0001$). (Figure 1) There was no difference in a time interval to the diagnosis of cancer between the two groups. Similarly, patients were stratified into two groups: those with serum albumin level <3 g/dl vs. patients with an albumin level of ≥ 3 . The lower albumin level was associated with significant increase in risk for HCC (HR 2.4, 95% CI 1.64-3.49, $p < 0.0001$) in Cox regression model. (Figure 2) In multivariate survival analysis including platelet count $<100,000$ and albumin <3 , both factors were predictive of risk for HCC (HR 2.4, 95% CI 1.73-3.21, $p < 0.0001$ and HR 2.2, 95% CI 1.48-3.15, $p < 0.0001$ respectively). The patients were further classified into four groups based on platelet count cut-off of $100,000/\mu\text{l}$ and albumin level cut-off of 3 grams/dl. Group A (platelet $\geq 100,000$ and albumin ≥ 3), B (platelet $<100,000$ and albumin ≥ 3), C (platelet $\geq 100,000$ and albumin <3), and D (platelet $<100,000$ and albumin <3). Group A had the highest cancer-free survival while group D had the lowest cancer-free survival. The survival curves of group B and C were similar. (Figure 3) The actuarial risk for developing HCC was 1% per year for patients with platelet count $\geq 100,000$ and albumin ≥ 3 . The highest risk was 6.2% per year for those with platelet $<100,000$ and albumin <3 . The remaining two groups had an intermediate risk of 2.6% per year.

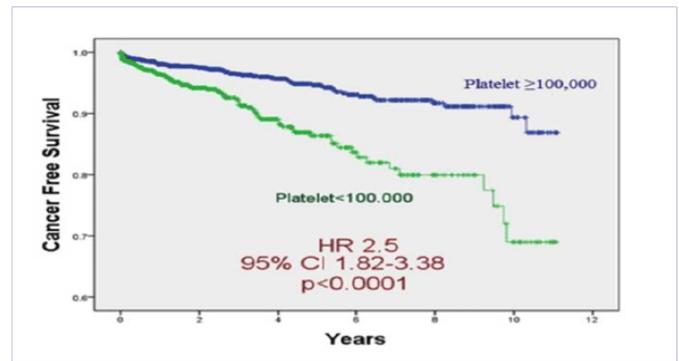


Figure 1: This Kaplan-Meier survival curve demonstrates the higher HCC free rate among patients with serum albumin level ≥ 3 grams/dl in comparison to those with albumin level <3 grams/dl

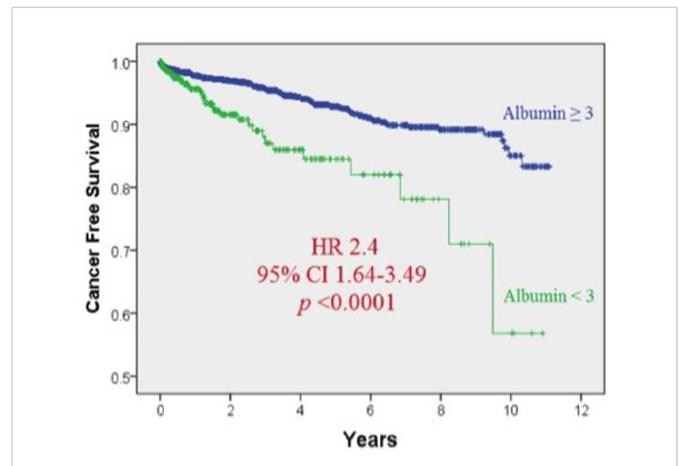


Figure 2: This Kaplan-Meier survival curve demonstrates the higher HCC free rate among patients with serum albumin level ≥ 3 grams/dl in comparison to those with albumin level <3 grams/dl.

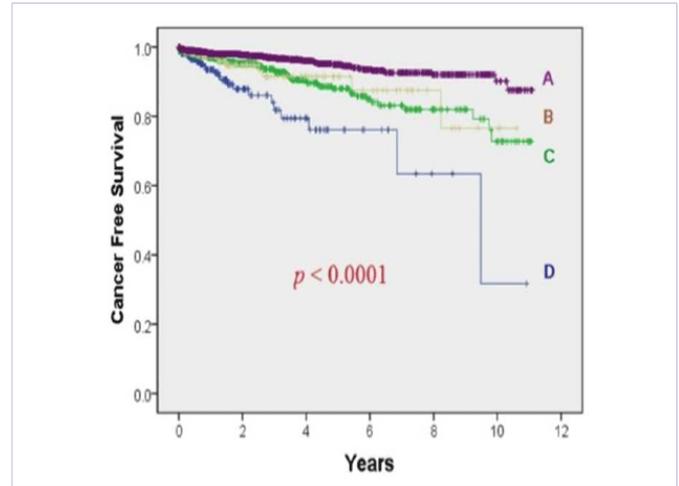


Figure 3: This Kaplan-Meier survival curve compares the HCC free survival of 4 groups of patients based on platelet count cut-off of $100,000/\mu\text{l}$ and albumin level cut-off of 3 grams/dl: A (high platelet and high albumin), B (low platelet and high albumin), C (high platelet and low albumin), and D (low platelet and low albumin). Group A had the highest cancer-free survival while group D had the lowest cancer-free survival. The survival curves of group B and C are very similar.

	Plt $<100,000$	Plt $\geq 100,000$	p
#	1210	2073	
Age (years)	53.8 (SD 10.5)	54.2 (SD 11.8)	<0.0001
Male	63.6%	52.1%	<0.0001
White	87.3%	84.70%	0.04
LOS (years)	2.3 (SD 2.4)	2.9 (SD 2.9)	<0.0001
Platelet	71.6 (SD 17.8)	195 (SD 90.6)	<0.0001
Creatinine	1.0 (SD 0.8)	1.0 (SD 0.7)	0.6
INR	1.4 (SD 0.4)	1.2 (SD 0.3)	<0.0001
Bilirubin	2.3 (SD 2.6)	1.7 (SD 2.8)	<0.0001
Albumin	3.5 (SD 0.6)	3.9 (SD 0.7)	<0.0001
MELD	11.3 (SD 5.3)	9.2 (SD 4.8)	<0.0001
Etiology:			
ETOH	15%	17.8%	
Viral	28.9%	23.9%	<0.0001
ETOH/Viral	7.7%	3.9%	
Other	48.4%	54.4%	
HCC	7.30%	3.70%	<0.0001
Time to HCC (years)	2.3 (SD 2.4)	2.4 (SD 2.5)	0.7

Discussion

Approximately 80 percent of HCC cases are due to underlying chronic viral hepatitis [14]. Epidemiological studies have shown that the majority of cases are diagnosed in older patients with underlying cirrhosis and mean age at presentation of 50 to 60 years [15]. Patients with chronic hepatitis B viral (HBV) infection are at increased risk for developing HCC even in the absence of cirrhosis, yet 70-90% of HCC cases diagnosed in patients with chronic HBV are in the setting of underlying cirrhosis [16, 17]. Chronic hepatitis C (HCV) infection has also been shown to be associated with increased risk for developing HCC [18, 19]. HCV accounts for over one-third of the cases of HCC in the USA (Davila) and around 90% of these patients have underlying cirrhosis or advanced fibrosis [20]. Over the past decade, the number of HCC cases related to NASH has been increasing [21]. In multivariate analysis, the investigators have identified four factors predictive for development of HCC; those include age, male gender, albumin and low platelet count. Studies have shown that men are at higher risk for developing HCC than women. The cause of this is not fully understood. Possible explanations include the higher incidence of liver diseases in men, higher incidence of environmental exposure to toxins, and the potential effect of androgens [2]. A study conducted on mice revealed a possible cause of gender disparity, chemical carcinogen diethylnitrosamine (DEN) caused a more significant increase in serum interleukin-6 (IL-6) concentration in males as compared to females. In females, estrogen inhibited the increases of IL-6 in response to DEN [22]. Our study showed that older males with low platelet count and low serum albumin are at higher risk for developing HCC. In a study by Chang et al., thrombocytopenia, older age, male gender, higher stage of hepatic fibrosis, genotype 1, and failure to eradicate HCV were shown to be associated with increased risk for developing HCC after interferon-based treatment [23]. In another study by Velazquez et al., a total of 463 patients aged 40 to 65 years with liver cirrhosis in Child-Pugh class A or B were evaluated to determine predictive factors of HCC. In their multivariate analysis, the independent predictive factors for the development of HCC included: age ≥ 55 years, anti-HCV positivity, prothrombin activity $\leq 75\%$, and platelet count $< 75,000/\text{mm}^3$ [24]. Thrombocytopenia is a common complication of cirrhosis affecting up to 76% of this patient Population. Several studies have shown an association between low platelet count and development of HCC [25, 26]. The etiology of thrombocytopenia associated with cirrhosis is multifactorial [27]. It has been for many years considered to be simply a result of portal hypertension and splenomegaly. Recent studies have suggested a direct role of cirrhosis and loss of liver mass, due to parenchymal extinction caused by microthrombosis, in lowering the production of thrombocytes via decreased levels of circulating thrombopoietin (TPO) [28, 29]. Thrombocytopenia can be seen in cirrhosis patients with normal spleen size [30]. Also, the reduction of portal pressure using portosystemic shunting does not frequently correct thrombocytopenia [31, 32]. A study by El-Sayed et al. confirmed that lower platelet counts and TPO levels correlated with increased severity of liver disease graded by Child-Turcotte-Pugh (CTP) scoring system [33]. Furthermore,

Koruk et al. demonstrated a similar correlation between the severity of liver disease grade by CTP classification and serum levels of TPO [34]. In a recent study, we have shown that cirrhotic patients with lower platelet counts have increased mortality rate while awaiting liver transplantation [35]. These data indicate that the severity of thrombocytopenia correlates with the severity of liver fibrosis. In a study by Sato et al., low albumin level has been shown to be associated with increased risk for developing HCC after eradication of HCV infection [36]. Similarly, low albumin level was associated with increased risk for HCC in cirrhosis patients with no HBV or HCV infection [37]. A study conducted in Switzerland suggested that albumin suppresses cell proliferation, hence suppressing the proliferation of hepatocellular carcinoma [38].

Despite the advances in diagnosis and management of HCC a retrospective study from Germany compared the outcome of 385 cases of HCC diagnosed between 1998 and 2003 and 681 cases diagnosed between 2004 and 2009 and found no difference in overall survival between the two time periods. The authors attributed this to the more advanced stage of HCC and increasing age at the time of diagnosis in the second period [39]. Other studies have shown an increased number of HCC cases and improved outcome with current treatment protocol [40]. Thus, diagnosis of HCC at earlier stages via screening protocols may potentially reduce morbidity and mortality.

Based on the findings of our study older cirrhotic patients with lower platelet count and serum albumin level might be at increased risk for developing HCC than other patients. This subgroup of patients may benefit from more frequent interval screening examinations. A cut-off platelet count of $100,000/\mu\text{l}$ showed a 2.5-fold increase in risk for HCC in patients with lower platelet count. A similar increase in HCC risk was noted in patients with albumin level < 3 grams/dl in comparison to patients with higher albumin level. The highest risk (annual actuarial risk of 6.2%) was seen in patients with a combination of platelet count $< 100,000$ and albumin < 3 . This subgroup of patients (especially males) may benefit from more intensive screening protocol (every 4 months US and AFP). The lowest annual actuarial risk (1%) was seen in patients with platelet count $\geq 100,000$ and albumin ≥ 3 . This group may be screened once per year. The remaining groups with either high platelet count or high albumin count may be screened every six months. Large-scale studies are required to evaluate these findings further. Despite the advances in diagnosis and management of HCC a retrospective study from Germany compared the outcome of 385 cases of HCC diagnosed between 1998 and 2003 and 681 cases diagnosed between 2004 and 2009 and found no difference in overall survival between the two time periods. The authors attributed this to the more advanced stage of HCC and increasing the age at the time of diagnosis in the second period [39]. Other studies have shown an increased number of HCC cases and improved outcome with current treatment protocols [40]. Thus, diagnosis of HCC at earlier stages via screening protocols may potentially reduce morbidity and mortality.

Conclusion

Older cirrhotic males with low platelet count and low serum albumin are at higher risk for developing HCC. The interval of HCC screening may warrant further prospective studies with shortened (every four months) for males with a platelet count of less than 100,000/ μ l and albumin of fewer than 3 grams/dl. Patients with high platelet count and high albumin level may be screened less intensely.

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