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Serum Mannose Binding Lectin As A Diagnostic And Disease Severity Biomarker In Hepatitis C Virus Related Cirrhosis AndHepatocellular Carcinoma: A Cross Sectional Study

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Abstract

Background: Early detection of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus (HCV) infection remains a clinical challenge. Mannose-binding lectin (MBL), a key component of innate immunity, has been implicated in liver inflammation and fibrosis and may serve as a potential biomarker associated with the presence and severity of HCC.

Methods: This cross-sectional study included 143 patients: 59 with HCV-related cirrhosis and 84 with HCV-induced HCC. HCC patients were stratified according to tumor burden and metastatic status, while HCV patients were classified using Child–Pugh and albumin–bilirubin (ALBI) scores. Serum MBL levels were measured using ELISA and analyzed in relation to disease stage, tumor characteristics, and diagnostic performance.

Results: Serum MBL levels increased significantly across disease stages, from HCV to advanced HCC ($p < 0.001$), with significant differences across all pairwise comparisons. MBL correlated with worsening liver function, showing higher levels in Child–Pugh C and ALBI III ($p < 0.001$). It also increased with tumor size and was markedly elevated in cases with vascular invasion and distant metastasis. MBL demonstrated excellent diagnostic performance for HCC (AUC = 0.994), with 100% sensitivity and 96.6% specificity.

Conclusion: MBL is a promising biomarker that shows a discriminatory performance in diagnosis and detection of advanced disease features of HCC in HCV-related cirrhosis, reflecting tumor burden and hepatic functional deterioration.

INTRODUCTION

The majority of initial liver cancers worldwide are hepatocellular carcinomas (HCC), comprising 75 to 85% of cases. Globally, HCC is the fifth most commonly diagnosed cancer and the fourth leading cause of cancer related deaths worldwide. According to the Egyptian statistics, HCC is responsible for 33.63% and 13.54% of all cancers among men and women, respectively [(1)]. According to CDC and WHO screening, most global HCC incidence is linked to infection by hepatitis B (HBV); (53%) or hepatitis C virus (HCV); (25%) [(2)], however there are other etiologies of HCC as alcohol drinking, Metabolic Associated Fatty Liver Disease (MAFLD) and exposure to aflatoxin B1 with epidemiology being different among countries [(3)].

HBV and HCV account for around 60% of the global burden of cirrhosis according to the most recent statistics [(4)]. Egypt used to have the highest rate of HCV infection globally, before the availability of Direct Acting Antivirals (DAAs) [(5)]. Concerning the natural history of the infection, about 20–30% of people infected with HCV are going to have cirrhosis, and from 1 to 7% of those people per year will get HCC. Complex molecular pathways involving viral and host components contribute to hepatocarcinogenesis in the context of viral hepatitis. Factors related to the virus include the HCV

genotype, viral load, and the presence of HBV infection [6]. The host's genetic history plays a crucial role in the development of HCC [7].

The liver secretes mannose-binding lectin (MBL), an essential part of the human innate immune system that acts as an acute phase reactant. It is a calcium-dependent C-type lectin with several lectin domains that binds to carbohydrate molecules produced on the surface of many different microbes. As a result of this binding process, the complement system cascade and macrophages are activated. It plays a crucial role in controlling the secretion of proinflammatory cytokines by monocytes in response to microbial infection, such as Tumor Necrosis Factor-α (TNF- α), Interleukin-6 (IL-6), and IL-1β.

Therefore, MBL has the potential to affect the degree of inflammation or the rate at which the illness advances [8]. Three predominant lectins- MBL, ficolin-2 and ficolin-3- are expressed in the liver [9]. The role of these lectins in disease progression of HCV infection has been well investigated revealed that liver fibrosis is linked mainly to MBL polymorphisms. Also, MBL and ficolin 2 were reported to have elevated levels close to the time of HCC diagnosis ([10].

Fibrosis of the liver and development of HCC are both connected to changes in innate immune signalling brought on by long-term HCV infection. When persistent inflammation activates liver stellate cells, they differentiate into myofibroblasts, which make collagen. This process starts fibrosis and the development of extracellular matrix [6]. Stellate cell activation may be triggered in part by the activity of MBL-associated serine proteases (MASPs). An upregulation of MASPs is linked to the progression of liver fibrosis [11]. In advanced stages of infection, the antiviral immune response may be severely hindered because chronic HCV infection also impairs the liver's ability to produce these lectins [12].

Researchers have looked at how MBL polymorphisms affect the course of HCV infection and found that functionally relevant MBL-2 promoter polymorphisms contribute to the development of HCC associated with HCV [8] [13][14]. However, studies comparing serum MBL protein expression in individuals with different disease progressions are scarce [10][15].

The clinical importance of identifying HCV-infected patients at risk of developing HCC motivated the researchers to conduct this study to determine whether serum MBL is elevated in Egyptian patients with chronic HCV infection before HCC diagnosis and whether it can be used clinically as a biomarker associated with the presence and severity of HCC. Additionally, the association between serum MBL levels and HCC progression to advanced stages of vascular invasion and distant metastasis has been also investigated.

METHODOLOGY

Study design:

This cross-sectional analytical study included 143 adult patients submitted to the endemic and infectious diseases department, Mansoura University. The research was conducted over a period of 10 months (from March 2025 to January 2026). Sample size was calculated using the following formula (16).

$$n = \left[\frac{Z_{\propto/2}}{\mathrm{E}}\right]^2 * \frac{S_n(1 - \mathrm{S}_n)}{(\mathrm{P})}$$

The above equation is expressed as follows:

where n is the sample size and Zα/2 equal 1.96. (The value that is considered crucial when dividing the 95% core region of a Z distribution by the 5% tail region)

P = 68%, which is the study's prevalence divided by the percentage of HCC (An article published in 2017 by Jie et al).

With a sensitivity of 89.1 percent, S_n = H An article published in 2017 by Jie et al.

For a margin of error of 10%, the formula E = M/WI = 10% is appropriate.

After adding 10% drop-out percentage, the sample size was 122 **(one hundred and twenty-two) patients.** In the final dataset, we were able to collect complete data from **143 patients**, therefore, the final sample size included 143 patients who were entered into the analysis.

Study subjects who were included in this cohort for the HCV related cirrhosis group those who were **HCV positive**, diagnosed by HCV Ab, HCV RNA PCR and **Cirrhotic**, diagnosed by real time abdominal ultrasonography or fibro scan. And for the HCC group, those who were diagnosed to have **pure HCC** laboratory by elevated alpha-fetoprotein (AFP) > 200 ng/ml and by imaging techniques as triphasic pelviabdominal CT scan or dynamic MRI. According to the guidelines (*EASL 2024*), HCC is diagnosed by the combination of hypervascularity in the late arterial phase (arterial phase hyperenhancement) and washout on portal venous and/or delayed phases. Exclusion criteria of the patients involved serious illness prohibits or makes it impossible to take part in the inquiry, HBV infection, any other etiology of liver cirrhosis other than viral hepatitis, presence of primary tumors other than HCC or primary liver tumors other than HCC as iCCA and combined type (cHCC-CCA) and the diagnosis of any inflammatory autoimmune diseases that cause elevated serum MBL level as Systemic Lupus Erythematosus.

Study subjects were classified into 2 groups: 59 patients with HCV infection and 84 patients with HCV-induced HCC; 40 for the non-metastatic HCC and 44 for the metastatic HCC. HCV patients were classified based on Child-Pugh classification into child B &C and based on ALBI score into grade I, II &III.

HCC patients were classified based on tumor size and *Barcelona Clinic Liver Cancer classification (BCLC)* in accordance with the tumor's extent and the prevalence of extrahepatic spread, into 4 groups: Tumor

dimension of 3 cm or less, tumor dimension: 3–5 cm, tumor dimension of 5 cm or greater and the last group for any tumor dimension with the presence of extrahepatic distant metastasis or vascular invasion.

Tumoral vascular invasion is defined by the extension of tumor cells into the hepatic and/or portal vein branches. HCC patients with vascular spread were classified according to the *Liver Study Group of Japan (LSGJ)* into 4 grades: Vp1; defined by the presence of a portal vein tumor thrombus (PVTT) distal to, but not in, the second-order branches of the portal vein; Vp2; defined by the presence of PVTT in the second-order branches of the portal vein; Vp3; defined by the presence of a PVTT in the first-order branches of the portal vein; and Vp4; defined by the presence of a PVTT in the main trunk of the portal vein or a contralateral portal vein branch or both. Vp2 and Vp3 were considered representatives of microvascular invasion andp4 for the macrovascular invasion. **Extrahepatic distant metastasis** is defined by the extension of the tumor cells to any of the reported sites of distant metastasis of HCC as the lungs, bones, adrenal glands, and brain, diagnosed by imaging techniques as dynamic MRI, Triphasic PA CT or Positron Emission Tomography (PET) scan.

Child Pugh Score

Parameter	**1 point**	**2 points**	**3 points**
Total bilirubin (mg/dL)	< 2	2–3	> 3
Serum albumin (g/dL)	> 3.5	2.8–3.5	< 2.8
INR *(or Prothrombin time)*	< 1.7	1.7–2.3	> 2.3
Ascites	None	Mild (controlled)	Moderate–severe (refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

	Total Score	Prognosis
A	5–6	Well-compensated disease
B	7–9	Significant functional compromise
C	10–15	Decompensated disease

ALBI grading

Inputs	**Serum Albumin (g/L) & Total Bilirubin (µmol/L).**
Formula	ALBI Score = (log10(Bilirubin) × 0.66) + (Albumin × −0.085).
Grading	**Grade 1**: Score ≤ -2.60 (Best Prognosis).
	Grade 2: Score > -2.60 to ≤ -1.39 (Intermediate).
	Grade 3: Score ≥ -1.39 (Worst Prognosis).

The study was approved by the Ethics Committee of Faculty of Medicine, Port said University. Written, informed consent was obtained from each patient included in this study.

All patient subjects included in this investigation were subjected to thorough history taking and previous reports of triphasic pelviabdominal CT scan and tumor markers, as serum AFP were revised. Complete clinical examination, Imaging including real time abdominal ultrasonography and Laboratory investigations were done for each patient.

For laboratory investigations, ten ml of blood were collected as following: two ml in EDTA tube for Complete blood count (CBC), two ml in citrate tube for Prothrombin Time (PT) and International Normalized Ratio (INR), three ml in plain tube for biochemical tests [Liver enzymes (serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), serum albumin and total bilirubin] and three ml in plain tube for serum MBL assay.

Serum MBL assay

Three ml of blood was collected in plain tube and transferred to the laboratory for MBL binding assay. The serum was centrifuged at a speed of 2000–3000 rpm for 20 minutes after being coagulated at ambient temperature for 10–20 minutes. Supernatant was collected and stored at -80°C. The assay was done through sandwich-Elisa technique using Human MBL2 ELSIA kit manufactured by Cloud-Clone Corp., Cat. No: E-03060hu; size: 96T, made in USA.

Statistical analysis

Data were analyzed using SPSS (version 26, IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using the Shapiro–Wilk test. As the data were not normally distributed, they were expressed as median and (interquartile range). Group comparisons for continuous variables were performed using the Kruskal–Wallis test, followed by post hoc pairwise Mann–Whitney tests with Bonferroni correction for adjusting for multiple testing. Categorical variables were summarized as counts and percentages. Comparisons between groups were carried out using the Chi-square test or Fisher's exact test where appropriate. When significant differences were found, pairwise column proportion comparisons were done with z-tests. Missing data were evaluated using Little's MCAR test, which indicated data were missing completely at random (p = 0.715). Therefore, the main analysis was

performed using pairwise deletion. All statistical tests were two-tailed, and a *p*-value of < 0.05 was considered statistically significant.

RESULTS

For serum MBL assay, this standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.

Study subjects were classified as mentioned before into 2 groups: 59 patients with HCV infection and 84 patients with HCV-induced HCC, subcategorized based on BCLC classification into 2 major groups; 40 HCC without extrahepatic metastasis (Local HCC) and 44 HCC with extrahepatic vascular and distant metastasis with a total of 143 participants.

The demographic and clinical history of a study population were investigated in those three groups as shown in **Table (1)**. The age distribution is similar across groups, with a mean age around 64 years. Males constitute the majority in all groups, especially in the third group (77.3%), while females are less represented overall (36.4%). Most participants reside in rural areas (64.3). Hypertension is prevalent in nearly 70%, and diabetes mellitus (DM) affects about 39.2% of the patients. Heart failure and chronic kidney disease (CKD) were found in only 4.2% and 6.3% of the patients respectively.

Table 1
Demographic and clinical characteristics across the three study groups

Dependent: Major Groups		HCV	HCC (local)	HCC (vascular/distant spread)	Total
Total N (%)		59 (41.3)	40 (28.0)	44 (30.8)	143
Age	Mean (SD)	64.7 (8.5)	63.9 (7.8)	64.3 (7.3)	64.4 (7.9)
Sex	male	33 (55.9)	24 (60.0)	34 (77.3)	91 (63.6)
	female	26 (44.1)	16 (40.0)	10 (22.7)	52 (36.4)
Residence	rural	40 (67.8)	22 (55.0)	30 (68.2)	92 (64.3)
	urban	19 (32.2)	18 (45.0)	14 (31.8)	51 (35.7)
Hypertension	No	45 (76.3)	26 (65.0)	29 (65.9)	100 (69.9)
	Yes	14 (23.7)	14 (35.0)	15 (34.1)	43 (30.1)
DM	No	35 (59.3)	26 (65.0)	26 (59.1)	87 (60.8)
	Yes	24 (40.7)	14 (35.0)	18 (40.9)	56 (39.2)
Heart failure	No	58 (98.3)	38 (95.0)	41 (93.2)	137 (95.8)
	Yes	1 (1.7)	2 (5.0)	3 (6.8)	6 (4.2)
CKD	No	53 (89.8)	40 (100.0)	41 (93.2)	134 (93.7)
	Yes	6 (10.2)	0 (0.0)	3 (6.8)	9 (6.3)

The clinical presentations observed among the study groups are outlined in **Table (2)**. Scleral jaundice was present in over half of the participants (52.4%), with the highest prevalence in the first group (66.1%). Ascites was also common, affecting 46.2% of the total sample, being most frequent in the first group (64.4%). The frequency in the second and the third groups was 37.5% and 29.5% respectively. Hepatic encephalopathy showed varied distribution, with most patients (76.9%) having no signs, with group 2 showing more prevalence of no encephalopathy (95%) than group 1 (64.4%) while the remaining cases were spread across different grades, notably grade II (9.1%) and grade I (4.9%). Gastrointestinal bleeding was reported in 25.2% of participants, with the first group showing the highest rate (40.7%).

Table 2
Clinical presentations across the study groups

Dependent: Groups		HCV	HCC (local)	HCC (vascular/ distant spread)	Total
Total N (%)		59 (41.3)	40 (28.0)	44 (30.8)	143
Scleral jaundice	No	20 (33.9)	24 (60.0)	24 (54.5)	68 (47.6)
	Yes	39 (66.1)	16 (40.0)	20 (45.5)	75 (52.4)
Ascites	No	21 (35.6)	25 (62.5)	31 (70.5)	77 (53.8)
	Yes	38 (64.4)	15 (37.5)	13 (29.5)	66 (46.2)
Encephalopathy	no	38 (64.4)	38 (95.0)	34 (77.3)	110 (76.9)
	I/ I-II	5 (8.5)	0 (0.0)	7 (15.9)	7 (4.9)
	II	10 (16.9)	1 (2.5)	3 (6.8)	13 (9.1)
	II-III/III	6 (10.2)	1 (2.5)	0 (0.0)	4 (2.8)
Gastrointestinal bleeding	No	35 (59.3)	34 (85.0)	38 (86.4)	107 (74.8)
	Yes	24 (40.7)	6 (15.0)	6 (13.6)	36 (25.2)

Table (3) displays a detailed analysis of the radiological features -documented by real time abdominal ultrasonography- across the groups. Cirrhosis is more prevalent in group 1 (86.4%) compared to group 2 (62.5%), while ascites shows a complex distribution with varying severity across groups. also, portal vein dilatation is obviously higher in the first group (96.6%) compared to the other groups. The prevalence of Porto-systemic vessels varies between groups, being highest in Group 1 (50.8%) compared to Groups 2 (20.0%) and 3 (18.2%). Similarly, splenomegaly was reported to be higher in Group 1 (96.6%) than in Groups 2 (77.5%) and 3 (72.7%) Dysplasia, fibrosis and steatosis show nearly similar prevalence among different groups.

Table 3
Radiological findings across the study groups

Parameters		HCV	HCC (local)	HCC (vascular/ distant spread)	Total
Total N (%)		59 (41.3)	40 (28.0)	44 (30.8)	143
Cirrhosis	No	8 (13.6)	15 (37.5)	12 (27.3)	35 (24.5)
	Yes	51 (86.4)	25 (62.5)	32 (72.7)	108 (75.5)
Dysplasia	No	55 (93.2)	38 (95.0)	42 (95.5)	135 (94.4)
	Yes	4 (6.8)	2 (5.0)	2 (4.5)	8 (5.6)
Fibrosis	No	53 (89.8)	32 (80.0)	36 (81.8)	121 (84.6)
	Yes	6 (10.2)	8 (20.0)	8 (18.2)	22 (15.4)
Steatosis	No	59 (100.0)	39 (97.5)	44 (100.0)	142 (99.3)
	Yes	0 (0.0)	1 (2.5)	0 (0.0)	1 (0.7)
ascites	no	10 (16.9)	16 (40.0)	17 (38.6)	43 (30.1)
	mild	5 (8.5)	4 (10.0)	3 (6.8)	12 (8.4)
	moderate	10 (16.9)	8 (20.0)	13 (29.5)	31 (21.7)
	Severe/gross	16 (27.1)	5 (12.5)	7 (15.9)	26 (18.2)
	Massive	18 (30.5)	7 (17.5)	4 (9.1)	29 (20.3)
Portal vein dilatation	No	2 (3.4)	11 (27.5)	12 (27.3)	25 (17.5)
	Yes	57 (96.6)	29 (72.5)	32 (72.7)	118 (82.5)
Porto systemic vessels	No	29 (49.2)	32 (80.0)	36 (81.8)	97 (67.8)
	Yes	30 (50.8)	8 (20.0)	8 (18.2)	46 (32.2)
splenomegaly	No	2 (3.4)	9 (22.5)	12 (27.3)	23 (16.1)
	Yes	57 (96.6)	31 (77.5)	32 (72.7)	120 (83.9)

Several laboratory parameters were documented across the study groups and shown in **Table (4)**. Albumin increases from the HCV group to the advanced HCC group, with median values rising from 2.9 g/dL in HCV patients to 3.5 g/dL in those with vascular/distant spread. Conversely, INR decreases across the same trajectory (1.4 to 1.2). Total bilirubin and direct bilirubin medians are highest in the HCV group (1.8 mg/dL and 1.4 mg/dL, respectively) and comparatively lower in both local and advanced HCC groups. Median ALT and AST values increase stepwise from the HCV group to the advanced HCC group, with ALT rising from 26.0 U/L to 47.0 U/L and AST increasing from 35.0 U/L to 61.0 U/L. The IQRs

broaden noticeably in the advanced HCC group, indicating greater dispersion and heterogeneity of hepatocellular injury in the context of more aggressive tumor biology. Hemoglobin and creatinine distributions are similar across the three cohorts with overlapping medians and IQRs. Platelet counts, however, show a progressive increase from the HCV cohort (median 103.5 ×10^3/μL) to the advanced HCC cohort (median 151.0 ×10^3/μL).

Table 4
Laboratory parameters across the study groups

Laboratory parameters **Median (IQR)**	**HCV** **N = 59**	**HCC** **(local)** **N = 40**	**HCC** **(Vascular** **/Distant spread)** **N = 44**
Hemoglobin	10.9 (9.2–12.3)	11.4 (10.3–12.5)	11.1 (9.6–11.9)
platelets	103.5 (65.2–161.5)	116.0 (84.0–196.0)	151.0 (112.0–228.0)
INR	1.4 (1.2–1.6)	1.3 (1.1–1.4)	1.2 (1.1–1.4)
Total Bilirubin	1.8 (1.1–2.8)	1.5 (1.0–2.1)	1.4 (0.7–2.5)
Direct Bilirubin	1.4 (0.8–1.9)	0.7 (0.5–1.2)	0.6 (0.3–1.6)
Albumin	2.9 (2.6–3.3)	3.3 (2.8–3.8)	3.5 (3.0–3.9)
ALT	26.0 (19.0–50.0)	36.0 (21.0–52.8)	47.0 (25.2–83.0)
AST	35.0 (22.0–71.0)	49.0 (31.0–68.0)	61.0 (31.0–103.2)
Creatinine	1.2 (0.9–1.5)	1.0 (0.7–1.2)	1.0 (0.8–1.2)

As outlined in **Table (5)**, Statistically significant differences between groups are evident for AFP (p = 0. 001) and MBL (p < 0.001), illustrating that these markers vary considerably between the groups which could be indicative of HCC. For instance, AFP levels are dramatically elevated in groups 2 and 3 compared to group 1 (p_{2} < .001, p_{3} = 0.004). MBL showed highly significant differences between all three groups (p < 0.001 for all pairwise comparisons), these levels are shown collectively in **Figure (1)**.

Table (5): Comparative analysis of AFP and MBL across the study groups

Laboratory parameters Median (IQR)	HCV N = 59	HCC (local) N = 40	HCC (Vascular /Distant spread) N = 44	χ^2	p	Pairwise comparisons
AFP	4.8 (2.6–32.2)	213.0 (24.4–1067.0)	246.0 (5.7–1994.5)	13.32	0.001	p[illegible] < .001, p[illegible] = 0.004, p[illegible] = 0.857
MBL	47.4 (25.0–59.7)	92.2 (89.9–190.0)	289.0 (284.5–519.0)	122.70	< 0.001	p[illegible] < .001, p[illegible] < .001, p[illegible] < .001

Kruskal–Wallis test was used with pairwise comparison using Mann–Whitney U tests with Bonferroni correction. **a**: *I vs II*, **b**: *I vs III*, **c**: *II vs III*

For detailed review of the statistically significant difference of serum MBL levels among the study groups, Median (IQR) level of serum MBL of the HCV category was 24.9 (21.9–28.2) in HCV Child B compared with 59.7 (52.3–63.0) in CHV Child C ($p < 0.001$). Regarding levels of serum MBL in the local HCC category, HCC tumor size <3cm showed Median (IQR) of 90.5 (80.9–91.5), range 17.3, HCC tumor size 3-5cm: 90.4 (88.4–92.8), range 19.9, while HCC tumor size >5cm: 191.0 (189.0–193.0), range 21.0 with significant differences between group 1–3 ($p < 0.001$), and 2–3 ($p < 0.001$), while groups 1,2 showed no significant difference (p:0.688).

For the HCC with extrahepatic vascular and distant metastasis, the group with vascular invasion had a median of 285.0 (IQR: 280.0–289.0; range: 100.0) while the group with distant metastasis showed a much higher distribution, with a median of 519.0 (IQR: 502.0–520.5; range: 333.0) ($p < 0.001$). The whole comparitive analysis of serum MBL levels among the different study groups is collectively shown in **Figure (2).**

Considering ALBI grading of the HCV group, **Table (6)** shows that MBL levels rise with worsening ALBI grade, with median (IQR) values of 19.8 (16.2–24.3) in ALBI I, 28.2 (24.5–56.1) in ALBI II, and 54.5 (50.8–61.1) in ALBI III. Group differences are significant ($\chi^2 = 17.12$, $p < 0.001$); pairwise tests show no significant difference between ALBI I vs II ($p = 0.127$), but significant differences for I vs III ($p = 0.001$) and II vs III ($p = 0.006$) **Figure (3)**.

Table (6): Comparative analysis of serum MBL in HCV group with ALBI grading

Laboratory parameters	ALBI grade I (N = 4)	ALBI grade II (N = 32)	ALBI grade III (N = 23)	χ^2	p	Pairwise comparisons
MBL median (IQR)	19.8 (16.2–24.3)	28.2 (24.5–56.1)	54.5 (50.8–61.1)	17.12	**< 0.001**	$p = 0.127^a$,**p = 0.001**[b], **p = 0.006**[c]
a: *I vs II*, **b**: *I vs III*, **c**: *II vs III*						

Figure (3): Distribution of MBL Levels Across ALBI Grades of HCV study subjects

As shown in **Table (7)**, the validity of using MBL for diagnosing HCC (AUC (95% CI) was 0.994 (0.985-1.0) (Fig. 4**)**, (p < 0.001) with a cut-off point of (≥ 73.950) for diagnosis. Serum MBL testing for diagnosis of HCC has a sensitivity of 100% and specificity of 96.6%. Regarding using MBL for diagnosing extra hepatic spread of HCC (AUC (95% CI): 0.955 (0.914–0.997) (Fig. 5**)**, (p < 0.001), with a cut-off point of (≥ 355.5). For diagnosing extra hepatic spread of HCC, serum MBL testing has a sensitivity of 100% and specificity of 88.9%.

Table (7): Validity of MBL for diagnosing of HCC and Extrahepatic spread in cases of HCC

Using MBL	Cut off point	AUC	95% CI	P value	Sensitivity	Specificity
For diagnosing HCC	≥ 73.950	.994	0.985-1.0	< 0.001	100%	96.6%
For diagnosing extrahepatic spread	≥ 355.5	.955	0.914–0.997	< 0.001	100%	88.9%

MBL levels were documented to have progressive increase across ALBI grades as shown in **Table (8)**, with the lowest median in Grade I and the highest in Grade III. Grade II shows the widest variability, while Grade III exhibits several outliers, indicating heterogeneity among severe cases. The differences between groups are statistically significant ($\chi^2 = 17.12$, p < 0.001), with pairwise comparisons showing no significant difference between Grades I and II (p = 0.127), but significant differences between I vs III (p = 0.001) and II vs III (p = 0.006).

For identifying ALBI > I, an MBL cut-off ≥ 25.0 yields (AUC = 0.916) (Fig. 6**)** with a sensitivity of 80.0% and specificity of 100.0%. For discriminating ALBI III, an MBL cut-off ≥ 41.2 yields (AUC = 0.780) **(Fig. 7)** with a sensitivity of 87.0% and specificity of 72.2%, supporting the utility of MBL as a severity marker for developing of HCV induced HCC.

Table (8): Diagnostic Accuracy of MBL for ALBI Grade Classification

Using MBL	Cut off point	AUC	95% CI	P value	Sensitivity (%)	Specificity (%)
For classifying ALBI > I	≥ 25.0	0.916	0.825–1.000	0.006	80.0	100.0
For classifying ALBI III	≥ 41.2	0.780	0.659–0.901	< 0.001	87.0	72.2

MBL levels were found to be nearly identical between microvascular and macrovascular invasion groups **(Table 9)**, with medians around 285.0 in both. The boxplot shows minimal variability and a few outliers in each group. Statistical analysis (Mann–Whitney U test) confirmed no significant difference between the two patterns (Z = 63.5, p = 0.925), indicating that MBL didn't differentiate microvascular from macrovascular invasion and so, it can't be used as predictive factor for progression of vascular invasion of HCC.

Table (9): Comparison of MBL Levels in Microvascular vs. Macrovascular Invasion

Laboratory parameters	Microvascular invasion (N = 10)	Macrovascular invasion (N = 13)	Z	p
MBL median (IQR)	285.0 (280.0–289.0)	285.0 (281.0–288.5)	63.5	0.925

MBL levels were nearly identical between microvascular and macrovascular invasion groups, with medians of 285.0 (IQR: 280.0–289.0) and 285.0 (IQR: 281.0–288.5), respectively. The Mann–Whitney U test showed no statistically significant difference (Z = 63.5, p = 0.925), indicating that MBL does not discriminate between these two invasion patterns.

DISCUSSION

This cohort aimed to evaluate serum MBL's effectiveness as a biomarker aligned with the existence of HCC in cirrhotic individuals with chronic HCV infection and its performance in recognition of advanced disease endpoints as malignant vascular invasion and extrahepatic distant metastasis. A total number of 143 patients were investigated in this study being classified into two groups, HCV cirrhotic patients and HCV induced HCC. The demographic characteristics of the study groups showed a mean age of approximately 64 years. Males predominated across all groups, and the majority of participants (64.3%) were from rural areas. This is consistent with what has been reported in **EL-Ghitany EM**, that male gender and residence in rural areas represent risk factors for HCV infection in Egypt[17].

Regarding the distribution of clinical presentation across the study groups, ascites predominates the presentation with a percentage of 46.2% of the total sample. A similar conclusion was reached by **Abd-Elrazek, M. M., et al.**, reporting that about 39.2% of viral hepatitis Egyptian patients in Nile Delta often presented with ascites as a sign of decompensated cirrhosis[18].

Ascites was reported in 33.5% of our HCC patients. A similar proportion was reported by **Abd-Elrazek, M. M.**, **et al.**, that about 26% of HCC patients had rapidly developing ascites[18]. In the same study, GI bleeding estimated 22.8% of the clinical presentations compared to a 40.7% prevalence in our cohort. Hepatic encephalopathy had a varied distribution among study subjects in our study, being clinically noted in about 7% of the patients in the form of asterixis and inverted sleep rhythm. **Abd-Elrazek, M. M.**, **et al.**, documented a comparable percentage of 3.7% of patients showing clinical manifestations of hepatic encephalopathy[18].

Considering the radiological findings documented by real time abdominal ultrasonography, our study reported liver cirrhotic echo pattern in 86.4%, splenomegaly in 96.6% and the presence of dilated portal vein in 96.6% of the HCV group of study population. Our results were broadly in line with those of **Abd-Elrazek, M. M.**, **et al.**, as they stated that common radiological features of viral hepatitis patients were splenomegaly, liver cirrhosis and dilated portal veins (49%, 40.9% and 24.7% respectively) [18].

The laboratory profile across the study groups demonstrates a pattern that, while partially expected in the spectrum of chronic liver disease to malignancy, also reveals several paradoxical trends that merit careful interpretation in the context of tumor biology and underlying hepatic reserve. Albumin levels showed a progressive increase from the chronic HCV group to advanced HCC. This finding contrasts with the classical paradigm of worsening hepatic synthetic dysfunction along disease progression. A plausible explanation is a selection and stage migration effect, whereby patients with advanced HCC in this cohort may have relatively preserved liver function (e.g., non-cirrhotic or Child–Pugh A predominance), allowing tumor progression to occur in a biologically permissive hepatic environment. This observation aligns with reports indicating that tumor burden and liver function may evolve somewhat independently, particularly in HCV-related hepatocarcinogenesis[19].

Similarly, the observed decline in INR from the HCV to advanced HCC groups further supports relatively preserved synthetic capacity in patients with more advanced tumor stages. This again diverges from traditional expectations and suggests that coagulation parameters in HCC cohorts may reflect baseline liver function rather than tumor extent per se, a finding described in several contemporary series where advanced tumors arise in compensated cirrhosis or even non-cirrhotic livers.

In contrast, bilirubin levels were highest in the HCV group and lower in both local and metastatic HCC groups. This pattern may indicate that the HCV cohort included a higher proportion of patients with active necro-inflammatory disease or decompensated cirrhosis, whereas patients with HCC—particularly those eligible for inclusion—may represent a subset with better biliary excretory function. Comparable findings have been reported in studies where bilirubin is more reflective of cirrhotic decompensation than oncologic stage[20].

The progressive rise in transaminases (ALT and AST) from HCV through to metastatic HCC is biologically plausible and consistent with increasing hepatocellular injury. Importantly, the marked widening of IQRs in the advanced HCC group suggests significant heterogeneity in tumor-related liver

injury, likely reflecting variability in tumor size, vascular invasion, ischemia, and background liver disease. This dispersion is frequently noted in advanced malignancy and underscores the limited discriminatory value of transaminases as standalone markers in late-stage HCC.

Hematological parameters revealed stability in hemoglobin and creatinine across groups. However, platelet counts demonstrated a progressive increase from HCV to metastatic HCC. The relative increase in platelets in HCC patients may reflect less severe portal hypertension, selection bias toward operable or treatable cases, or a potential role of platelets in tumor progression, as supported by emerging evidence linking thrombocytosis to pro-tumorigenic and pro-angiogenic pathways [19]. Overall, these findings suggest that in this cohort, HCC progression is not strictly coupled with worsening liver function, and in some cases may preferentially occur in patients with relatively preserved hepatic reserve.

In our study, the statistical analysis demonstrates that both AFP and MBL differ significantly across the study groups, supporting their potential diagnostic and pathophysiological relevance in HCC. The marked elevation of AFP in HCC groups compared with the HCV cohort is consistent with its established role as a tumor biomarker stated in *AASLD 2023* practice guidance to be used for screening and early detection of HCC together with real time abdominal ultrasonography as biannual surveillance program[21].

MBL demonstrated highly significant differences not only between HCV and HCC groups but also across all pairwise comparisons, indicating a graded association with the disease status. The progressive increase in MBL levels from Child–Pugh B to Child–Pugh C within the HCV cohort suggests that MBL is closely linked to worsening hepatic dysfunction and systemic inflammatory activation. **Jalal PJ.**, **et al**., documented similar differences in serum MBL levels between HCV cirrhotic and HCV induced HCC patients being consistently elevated for about 3 years prior to the diagnosis of HCC[10].

These findings were also consistent with those reported by **Li, J. Et al.**, as plasma levels of MBL (P = 0.014) were significantly higher in HCC patients than in the healthy controls. This study estimated an optimal cut-off value for MBL to be 1422.14 ng/mL, with sensitivities of 89.1%[15]. Our study's findings were slightly different reporting the validity of using MBL for recognition of HCC among the HCV cirrhotic patients at a cut-off point of (≥ 73.950 ng/mL), with a sensitivity of 100% and specificity of 96.6%.

As an alternative objective tool for assessing hepatic functional reserve in patients with HCC, ALBI score is recommended to be used for stratifying liver impairment into three progressive categories (grades I to III). It also enables discrimination of prognostic subgroups across different *BCLC* stages and Child Pugh classes, thereby enhancing its utility as a clinically relevant predictor of outcomes[22].

In our study, stratification of the HCV group based on ALBI score revealed a clear stepwise increase in serum MBL levels with worsening hepatic functional reserve. Median MBL values increased from 19.8 in ALBI I to 28.2 in ALBI II and markedly to 54.5 in ALBI III, with an overall highly significant difference across groups ($\chi^2 = 17.12$, $p < 0.001$). Pairwise comparisons demonstrated that this effect is primarily driven by the transition to advanced liver dysfunction, as differences between ALBI I and II were not

statistically significant, whereas both comparisons involving ALBI III (I vs III and II vs III) reached significance.

Our study reported that for identifying ALBI > I, an MBL cut-off ≥ 25.0 yields (AUC = 0.916) with a sensitivity of 80.0% and specificity of 100.0%. For discriminating ALBI III, an MBL cut-off ≥ 41.2 yields (AUC = 0.780) with a sensitivity of 87.0% and specificity of 72.2%, supporting the utility of MBL as a severity marker for developing of HCV induced HCC.

Importantly, the lack of significant separation between ALBI I and II limits the utility of MBL as a marker for early stratification of liver function, but its strong differentiation at ALBI III highlights potential value in identifying patients with advanced functional compromise and poorer prognosis. When compared with other studies, this supports the concept that biomarkers such as MBL may complement established scoring systems like ALBI by capturing immunological and inflammatory dimensions of disease severity that are not fully reflected by conventional biochemical parameters alone. This observation in confirmed in our cohort results revealing that differences in serum MBL levels among HCC subjects without extrahepatic metastasis were only significant between HCC groups 1–3 ($p < 0.001$), and 2–3 ($p < 0.001$), while groups 1,2 showed no significant difference (p:0.688). These groups were classified as lesion less than 3 cm (group 1), lesion from 3 to 5 cm (group 2), lesion more than 5 cm in dimension (group 3).

As a biomarker for discriminating advanced disease endpoints as malignant vascular invasion and extrahepatic distant metastasis, our study reported that vascular invasion group had a serum MBL median of 285.0 (IQR: 280.0–289.0; range: 100.0) while the group with distant metastasis showed a much higher distribution, with a median of 519.0 (IQR: 502.0–520.5; range: 333.0) ($p < 0.001$). The validity of using MBL for recognition of malignant vascular invasion and extrahepatic distant metastasis among the HCC patients was at a cut-off point of (≥ 355.5 ng/mL), with a sensitivity of 100% and specificity of 88.9%. **Li, J. Et al.**, reported similar findings that serum MBL levels were significantly elevated in patients with vascular invasion compared to those without ($p = 0.011$), suggesting an association between MBL and vascular invasion, a key feature linked to tumor progression and metastatic potential[15].

Comparing the results between microvascular and macrovascular invasion groups, our study reported that MBL levels were found to be nearly identical between both groups, with medians around 285.0 in both. Statistical analysis confirmed no significant difference between the two patterns, indicating that MBL didn't differentiate microvascular from macrovascular invasion. While our cross-sectional study shows MBL levels do not differentiate existing micro- from macrovascular invasion, further research should test whether longitudinal changes in MBL predict transition from micro- to macrovascular invasion, using serial sampling and time-to-event analysis in a prospective cohort stratified by liver disease etiology.

CONCLUSION

In summary, this study demonstrates that serum MBL is significantly associated with both the presence and advanced disease's severity of HCC in patients with HCV related cirrhosis. MBL levels increased consistently across the disease spectrum—from cirrhosis to advanced HCC—and showed strong correlations with markers of hepatic functional deterioration, including Child Pugh and ALBI grades. Notably, MBL levels were higher in patients with adverse tumor characteristics, such as larger tumor size, vascular invasion, and distant metastasis, suggesting a link with tumor aggressiveness.

In comparison to conventional biomarkers, MBL demonstrated excellent differentiating performance, highlighting its potential utility as a complementary tool for HCC assessment, particularly in cases where AFP may be insufficient. Furthermore, its association with both liver dysfunction and tumor biology underscores its dual role as a marker of disease severity. Collectively, these findings support the incorporation of MBL into multimodal assessment strategies for HCC risk stratification and monitoring.

Limitation:

This study is limited by its cross-sectional, single-center design and relatively small sample size, which restrict causal inference and generalizability. The cohort included only HCV-related cases, limiting applicability to other HCC etiologies. External validation was not performed, and the absence of longitudinal follow-up precludes assessment of MBL's prognostic value. Additionally, potential confounders affecting MBL levels were not fully controlled, and reliance on a single measurement may not reflect dynamic disease changes.

RECOMMENDATIONS

Future research should include large, multicenter prospective cohorts to validate the diagnostic and prognostic value of MBL across diverse populations and liver disease etiologies, with an emphasis on longitudinal assessment. Integrating MBL with established biomarkers and imaging may develop multimodal diagnostic algorithms with improved accuracy. Further research into underlying molecular mechanisms, as well as standardization of assays and evaluation of genetic variability, is needed to support clinical application.

Declarations

Author Contribution

Shaker Wagih Shaltout, Hatem Elalfy, Mamdouh Elnahas,and Rovan Elghnnam put the idea of the research, built the research team, designed the study, revised the manuscript, and approved the final version of manuscript. Mohamed Elegezy participated in designing the study, revised the manuscript, and approved the final version of manuscript. Gehad Mohsen participated in designing the study, analyzed the data, revised the manuscript, and approved the final version ofmanuscript. Nesrine Saad

Farrag participated in designing the study and statistical analysis, revised the manuscript, and approved the final version.

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Figures

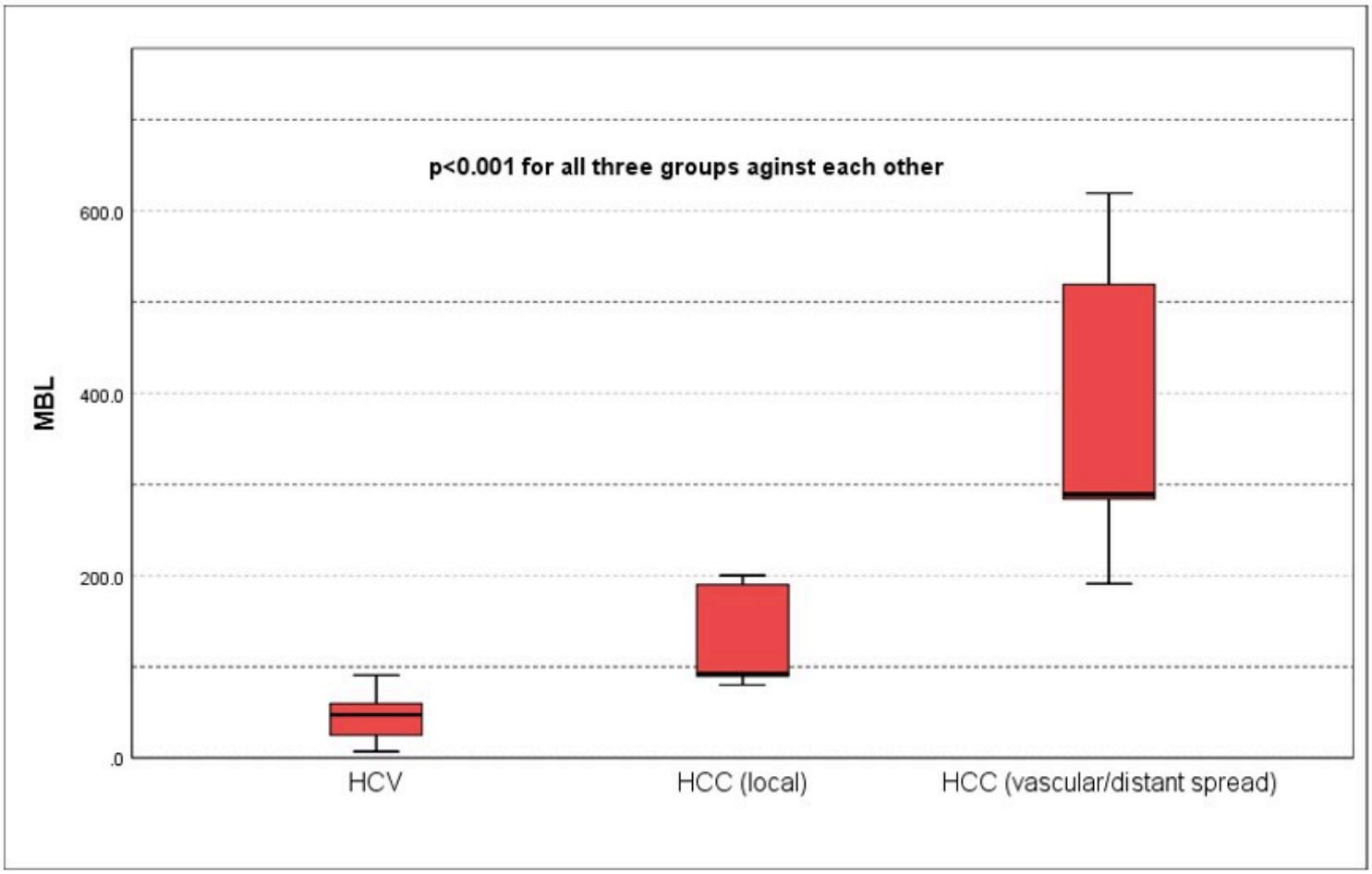


Figure 1

MBL levels across the three groups (p<0.001 for all pairwise comparisons)

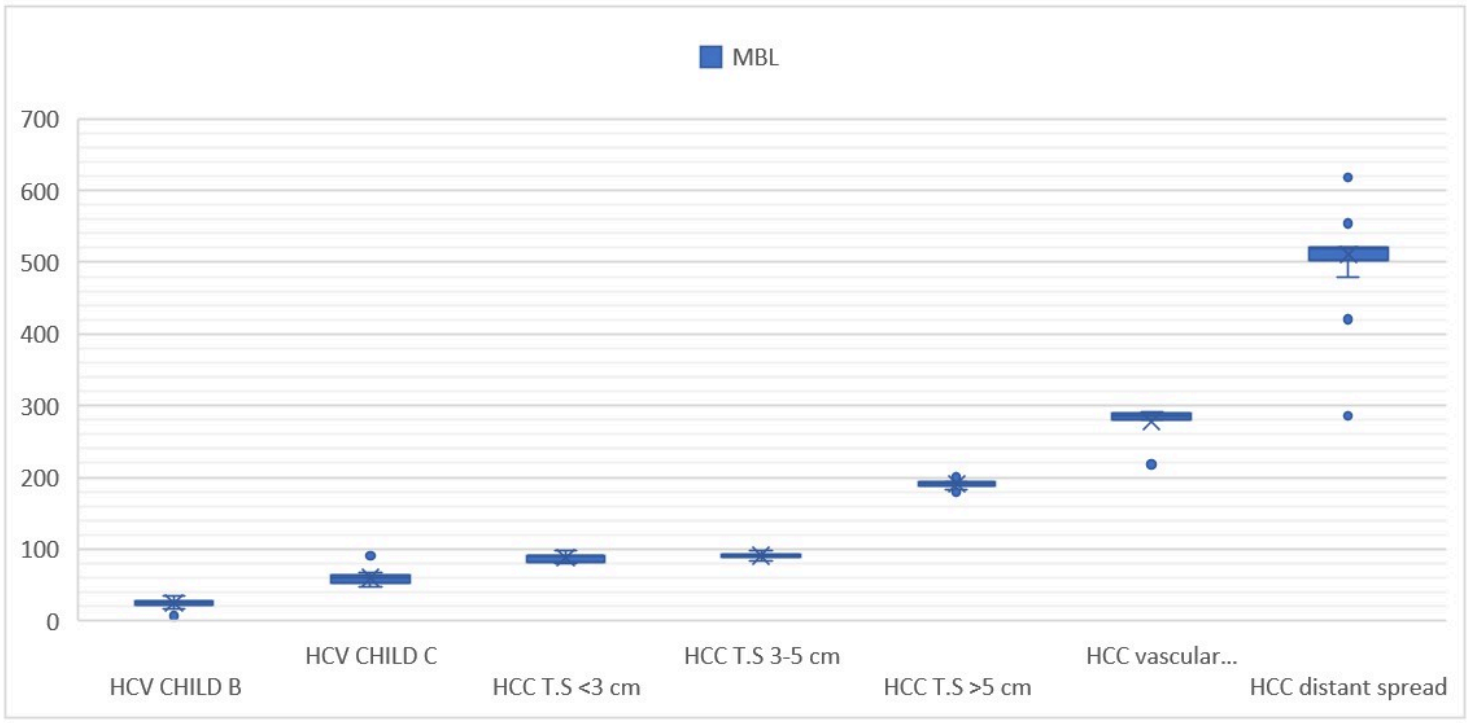


Figure 2

Comparative analysis of serum MBL levels among different study groups

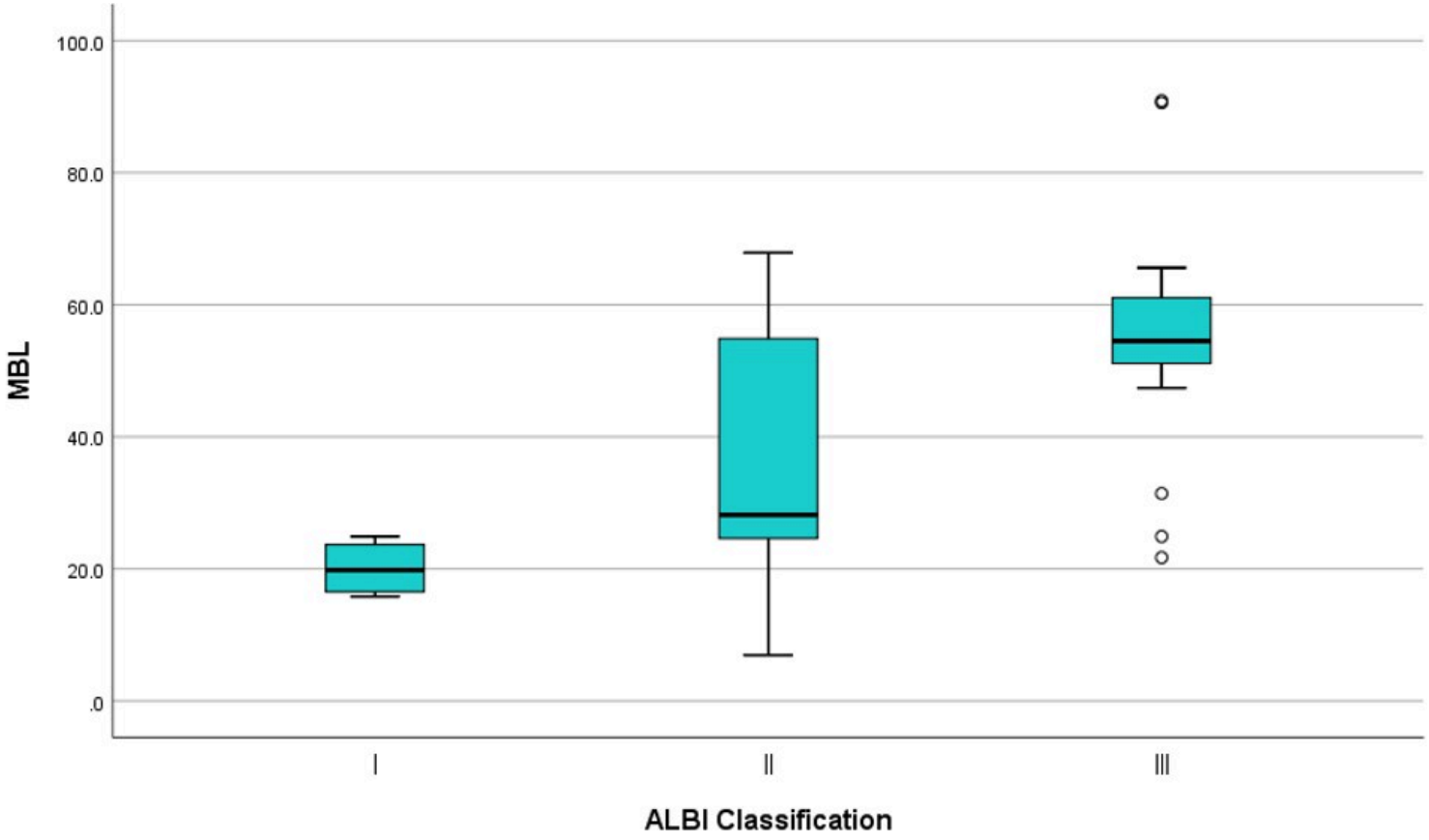


Figure 3

Distribution of MBL Levels Across ALBI Grades of HCV study subjects

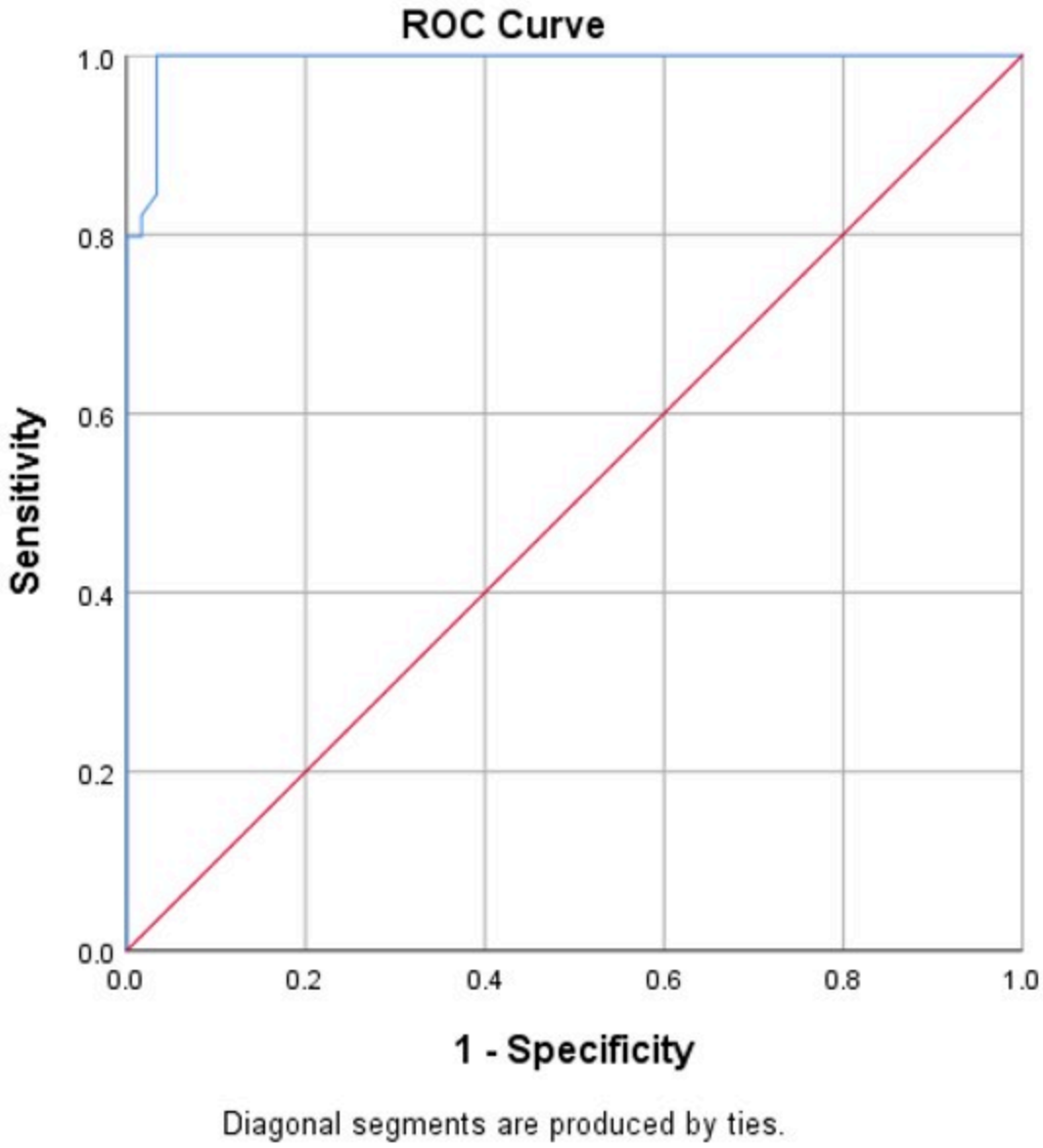


Figure 4

ROC curve showing AUC for using MBL for diagnosing HCC (AUC (95% CI): 0.994 (0.985-1.0), p <0.001.

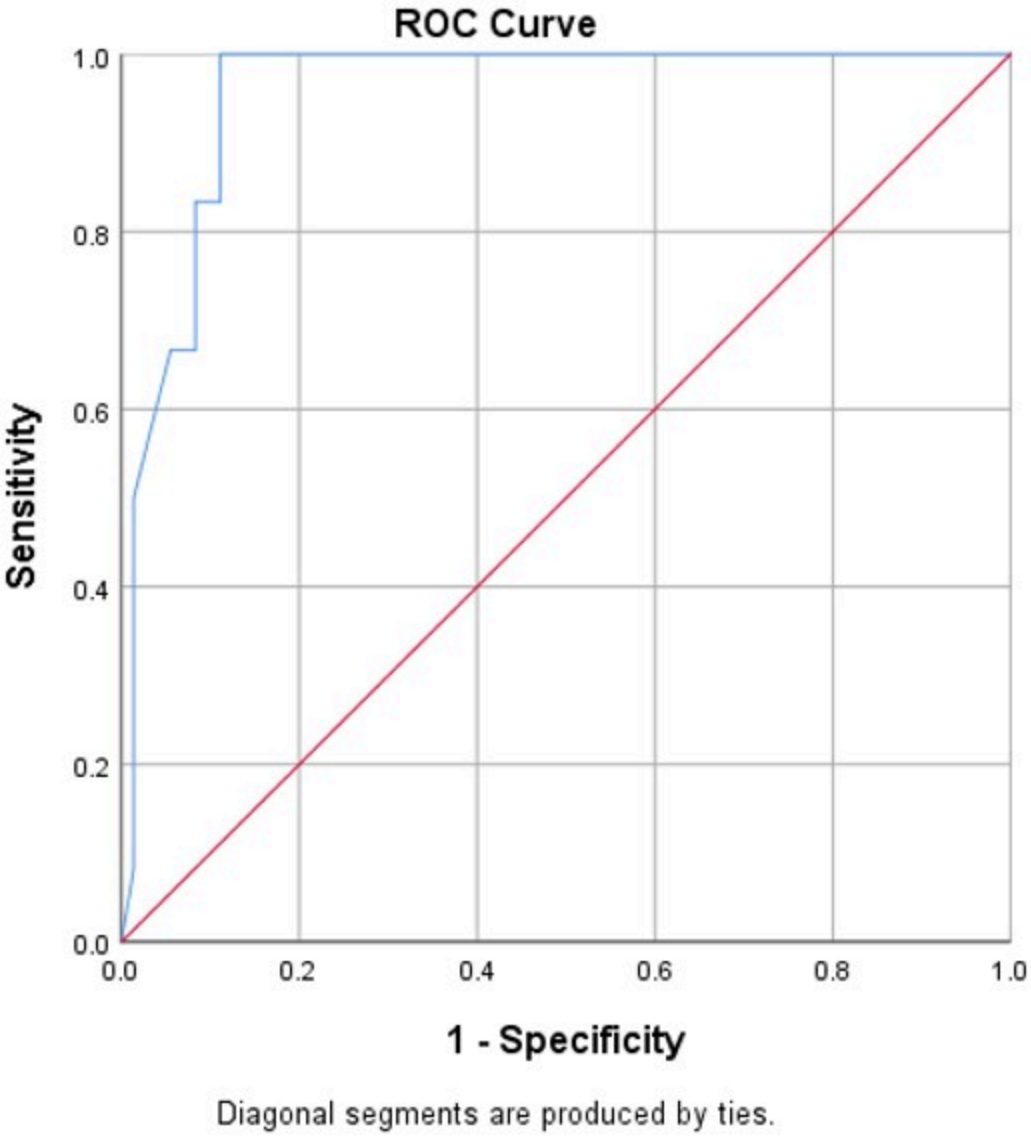


Figure 5

ROC curve showing AUC for using MBL for diagnosing extra hepatic spread of HCC (AUC (95% CI): 0.955 (0.914-0.997), p <0.001.

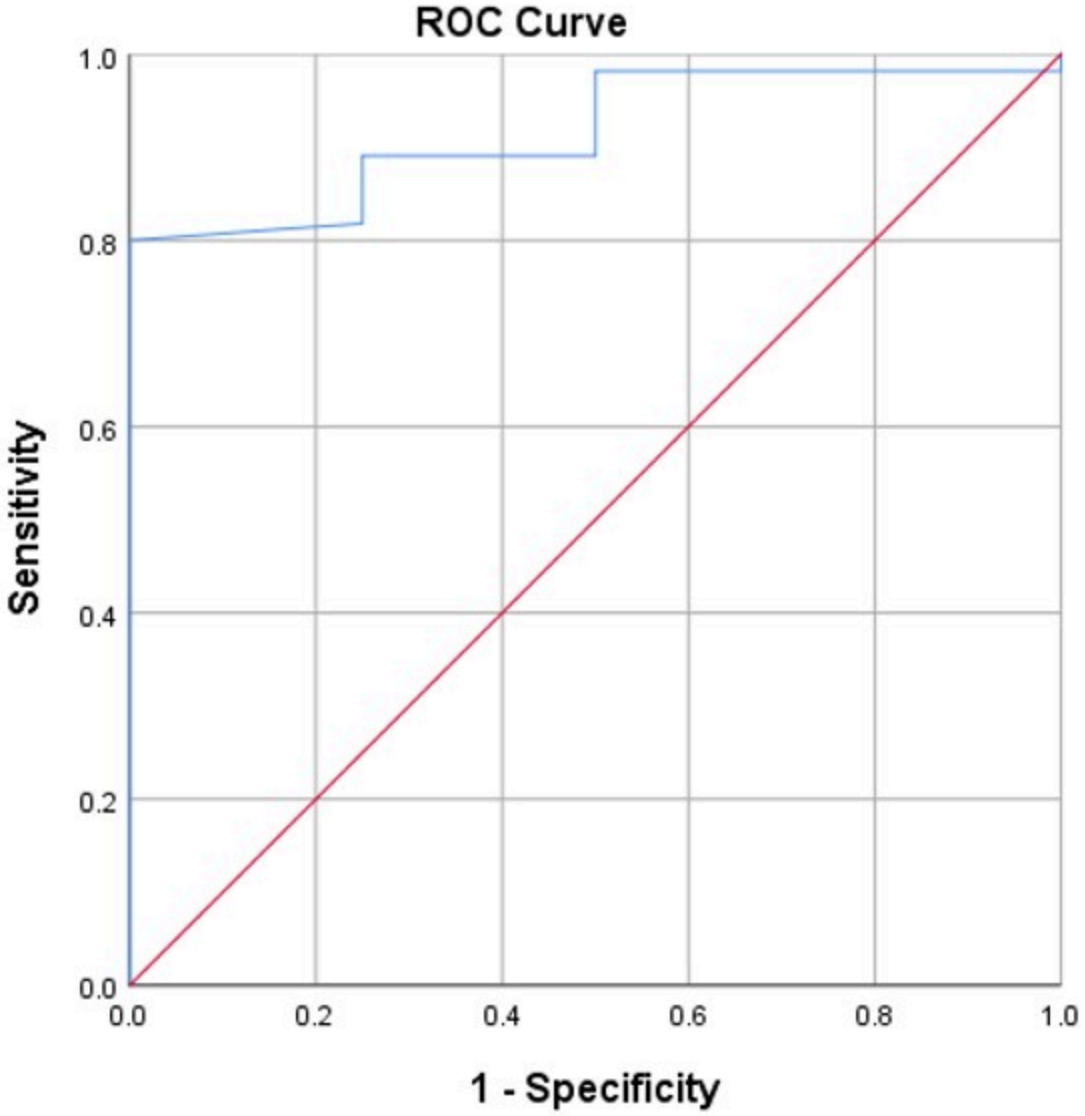


Figure 6

Roc curve analysis for using MBL For identifying ALBI > I

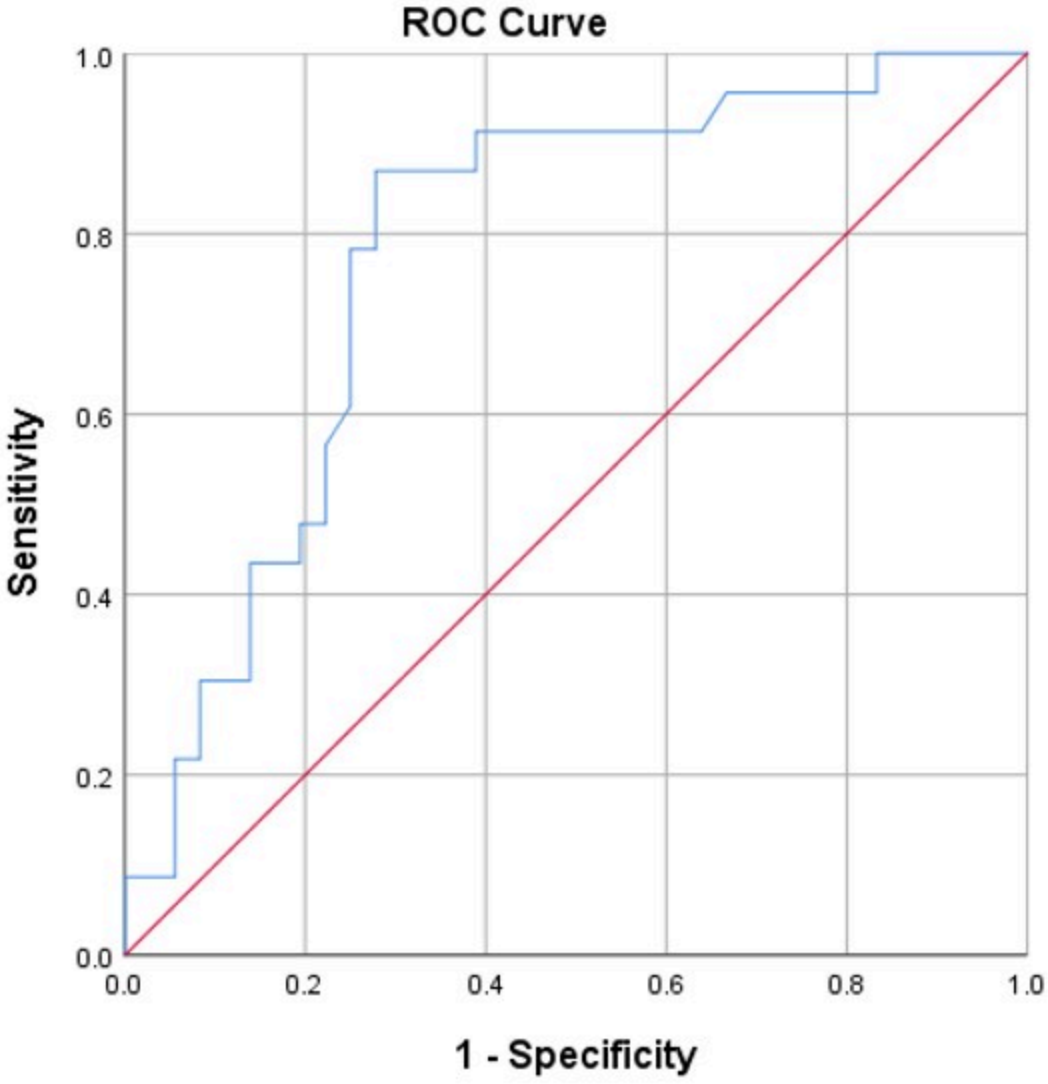


Figure 7

Roc curve an0.alysis for using MBL For identifying ALBI >II

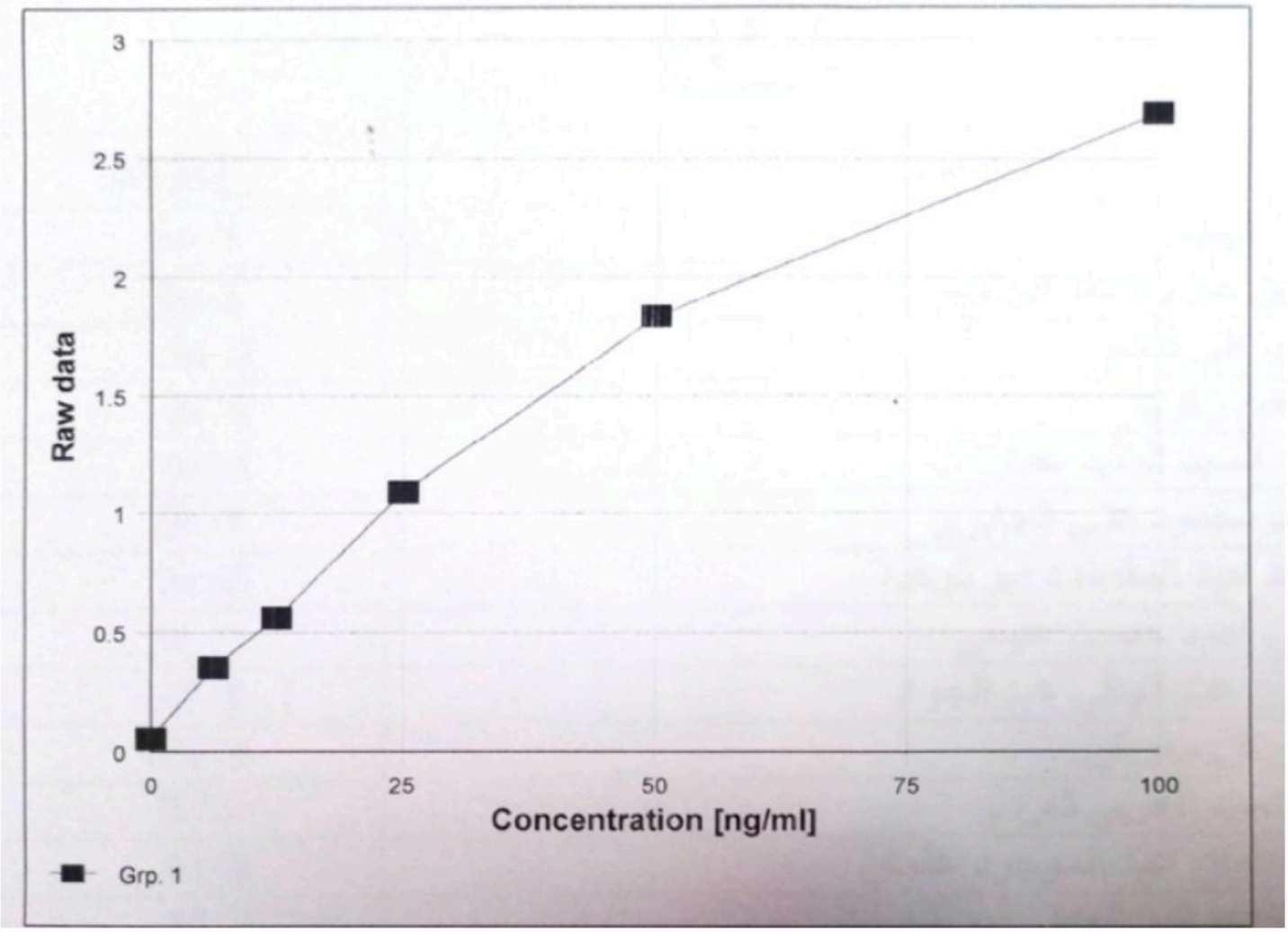


Figure 8

Unnumbered image in the Result section.