Clinics of Oncology

Research Article

ISSN: 2640-1037 | Volume 5

Predictive Value of Biomarkers Fibrinogen Like Protein-2 and A-Fetoprotein for Hepatocellular Carcinoma Among Patients with Liver Cirrhosis

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Received: 18 July 2021
Accepted: 02 Aug 2021
Published: 06 Aug 2021

Citation:

Elefsiniotis I, Predictive Value of Biomarkers Fibrinogen Like Protein-2 and A-Fetoprotein for Hepatocellular Carcinoma Among Patients with Liver Cirrhosis. Clin Onco. 2021; 5(3): 1-7

Keywords:

FGL-2; AFP; liver cirrhosis; HCC; Child-Pugh score

Abbreviations:

HCC: hepatocellular carcinoma; CP: Child-Pugh; FGL-2: fibrinogen like protein-2; AFP: a-fetoprotein; ELISA: enzyme-linked immunosorbent assay; MELD: model for end-stage liver disease; INR: international normalized ratio; SD: standard deviation; IQR: interquartile range; CI: confidence intervals; OR: odds ratio

1. Abstract

1.1. Aim: Data concerning the utility of biomarkers for accurate early HCC detection in cirrhotic patients are lacking.

1.2. Methods: We evaluated 112 consecutive Caucasian cirrhotic patients with (n=28) or without (n=84) concomitant HCC at baseline for serum AFP and plasma fibrinogen like protein-2 (FGL-2) levels. Patients without confirmed HCC at baseline were further followed up every six months with ultrasound and serum AFP levels, according to HCC surveillance program. Imaging as well as histological confirmation of HCC was established in patients with new lesions. During 5-year surveillance, 14 (16.6%) patients developed HCC.

1.3. Results: Mean plasma FGL-2 levels were 4.04±3.80 pg/ml in the whole population but were significantly higher in patients with CP-B/C cirrhosis compared to those with CP-A ($p<0.0001$) as well as in patients with HCC compared to those without ($p=0.001$). Patients who finally developed HCC had significantly lower platelet count ($p=0.008$), higher FGL-2 levels ($p=0.01$) and were frequently categorized as CP- B/C stage ($p=0.006$) compared to those who did not. In the multivariate analysis, male gender (OR=20.801, $p=0.024$), platelets (OR=0.980, $p=0.025$) and CP score (CP-B/C vs A, OR=27.184, $p=0.004$), but not FGL-2 or AFP levels, were significantly correlated with HCC appearance.

1.4. Conclusion: Plasma FGL-2 levels are significantly elevated in cirrhotic patients with decompensated liver disease compared to those with compensated cirrhosis and presence of HCC as well as HCC staging, seem to further influence them. Liver disease severity as well as low platelet count, but not baseline FGL-2 or AFP levels, could predict HCC emergence among Caucasian cirrhotic patients.

2. Introduction

Liver cancer constitutes a major health problem as it is the seventh most frequently occurring cancer in the world and the second most common cause of cancer- related mortality. Hepatocellular Carcinoma (HCC) is the dominant type of liver cancer, accounting for approximately 75% of all liver cancer cases [1]. It is estimated that 15-20% of patients with chronic liver disease will develop liver cancer during their lifetime. Risk of HCC development varies according to geographic area, etiology, degree of underlying liver disease, and family history [2]. Cirrhosis of any underlying

etiology (chronic viral hepatitis, alcoholic liver disease, metabolic disturbances, autoimmune liver diseases etc.) represents an important risk factor for HCC as all etiologic forms of cirrhosis may be complicated by tumor formation [3].

Long-term follow-up studies have found that approximately 1–8% of patients with liver cirrhosis develop HCC per year, especially those with features of severe liver disease and/or significant portal hypertension [3]. Patients at high risk of developing HCC, such as cirrhotics, should be entered into surveillance programs in order to find HCC at early stages in which potentially curative treatments are feasible. Liver ultrasound every six months, with or without concomitant use of serum a-fetoprotein (AFP) levels, is strongly recommended by EASL/AASLD/APASL guidelines for all cirrhotic patients participating in HCC surveillance programs [3, 4, 5]. Data concerning the utility of several proposed tumor biomarkers for accurate early HCC detection are still lacking.

Tumor microenvironment is the major site for tumor cell proliferation and differentiation that significantly affects carcinogenesis, vascular invasion/metastasis and response to therapy [6]. The HCC tumor microenvironment is divided into cellular (liver sinusoidal endothelial cells, hepatic stellate cells etc) and non-cellular components. Moreover, infiltrating immune cells are present in HCC (neutrophils, lymphocytes, tumor-associated macrophages, dendritic cells, myeloid-derived suppressor cells), creating a unique microenvironment that determines tumor growth as well as prognosis and response to immunotherapy [7]. Fibrinogen-like protein 2 (FGL-2) is a member of the fibrinogen-like protein family that holds a central role in both innate and adaptive immunity. Soluble fibrinogen-like protein 2 (sFGL-2) is the soluble form of fibrinogen-like protein 2, that is mainly secreted by regulatory T cell (Treg) populations and creates a potently immunosuppressive microenvironment, so sFGL-2 might play a role in inhibiting endogenous antitumor immune responses [8]. A recent study suggests that FGL-2 may promote the growth of hepatocellular carcinoma by promoting the accumulation of myeloid-derived suppressor cells in the tumor microenvironment [9].

Data concerning soluble FGL-2 levels in patients with chronic hepatitis B [10], chronic hepatitis C [8], liver cirrhosis and HCC [11] suggest that there is a significant correlation between them and the progression and severity of underlying liver disease as well as with the clinical outcome. A study in cirrhotic patients with HCC concluded that sFGL-2 protein is a novel effector molecule of activated hepatic stellate cells, which suppresses CD8 T cell proliferation and interferon-gamma production and it subsequently might contribute to immune suppression during liver fibrosis and carcinogenesis [11]. To our knowledge the role of soluble FGL-2 levels in the prediction of hepatocellular carcinoma among cirrhotic patients under HCC surveillance program has not been investigated yet.

The aim of our study was to evaluate the predictive value of the biomarkers AFP and FGL-2 in the appearance of HCC among Caucasian cirrhotic patients of various etiologies who were followed-up according to an HCC surveillance program.

3. Materials & Methods

3.1. Patients: We prospectively evaluated serum FGL-2 levels of consecutive compensated or decompensated Caucasian cirrhotic patients with or without concomitant HCC presenting in the outpatient Hepatology Unit of General and Oncology Hospital of Kifisia ‘‘Agioi Anargyroi”. Patient inclusion commenced in September 2014 and ended in January 2015. All patients included had history of chronic liver disease of various etiologies and diagnosis of compensated (confirmed by either liver biopsy or transient elastography) or decompensated liver cirrhosis. All cirrhotic patients with chronic HCV infection participated in the study had received antiviral treatment in the past and exhibited sustained virological response (undetectable serum HCV-RNA twelve weeks following treatment cessation and at least once annually). Moreover, all HBV-related cirrhotic patients participated were appropriately virologically suppressed (undetectable serum HBV-DNA levels using a sensitive PCR assay with a cut-off of 13 IU/ml at least once annually) with long term nucleos(t)ide analogues therapy (entecavir or tenofovir). Cirrhotic patients of any etiology who declare current alcohol use were also excluded.

Decompensated liver cirrhosis was defined by a history of at least one major cirrhosis-related complication, such as portal hypertension related bleeding, hepatic encephalopathy, ascites with Serum Ascites Albumin Gradient (SAAG) >1.1 g/dL or jaundice. Absence of bacterial infection was based on clinical examination, absence of findings on chest X-ray suggestive of lower respiratory tract infections and negativity of blood, urine and ascitic fluid cultures. The neutrophil count in ascitic fluid (<250/mm^3) was also evaluated to exclude spontaneous bacterial peritonitis in cirrhotic patients with ascites. For the baseline characteristics of the study population, demographic data were collected through medical history, while hematological and biochemical tests were performed using standard laboratory methods. For the assessment of severity of chronic liver disease, well defined scores for cirrhotic patients, such as Child-Pugh (CP) and MELD, were used. Contrast enhanced CT or MRI were available, within the last 6 months prior to the baseline visit, in all the included patients in order to suspect or to exclude cirrhotic patients with concurrent HCC. Histological confirmation of HCC was done in all patients with suspected lesions at baseline evaluation. Patients with HCC were further staged according to the Barcelona Clinic Liver Cancer (BCLC) staging system.

Cirrhotic patients without confirmed HCC at baseline were further followed up every six months with liver ultrasound and serum a-fetoprotein levels, according to the HCC surveillance program that is used in our Department. Patients with new lesions suggestive

of HCC during the surveillance program were further evaluated with contrast enhanced CT or MRI and histological confirmation of HCC was established with imaging guided biopsy of the lesion.

Written informed consent was collected from all patients participating in the current study. The study protocol was in accordance with the Declaration of Helsinki and was evaluated and approved by the Ethics Committee of the School of Health Sciences of the National and Kapodistrian University of Athens, Greece.

3.2. Collection of Samples and Measurements: Collection of samples was performed when the patients presented in the outpatient Hepatology Unit, at the same time as demographical data were recorded and routine laboratory tests were ordered. Whole blood (approximately 10ml) was collected from peripheral veins of patients and serum as well as plasma was immediately separated and stored frozen at -80o C until further analysis. Soluble FGL-2 levels were measured from plasma samples using a commercially available ELISA kit (Cloud-Clone Corp, Houston, TX 77084, USA) following the specific instructions of the manufacturer.

3.3. Statistical Analysis: Continuous variables are presented as mean (standard deviation) or median (range), while categorical variables are presented as numbers (percentages).

We used the Kolmogorov-Smirnov test ($p>0.05$ for all variables) and normal Q-Q plots to test the normality assumption. Univariate analysis between demographic and clinical data and HCC appearance included chi-square test, chi-square trend test and independent samples t-test. Also, the relation between biomarkers and HCC appearance was assessed with Mann-Whitney test since biomarkers did not follow normal distribution. We applied multivariate logistic regression analysis with HCC appearance as the dependent variable to eliminate cofounding. In that case, we estimated odds ratios, 95% confidence intervals and p-values. P-values of less than 0.05 were considered significant. Statistical analysis was performed with the Statistical Package for Social Sciences software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.)

4. Results

Between September 2014 and January 2015, 112 consecutive Caucasian cirrhotic patients (81 males) presenting in the outpatient Hepatology Unit of General and Oncology Hospital of Kifisia ‘‘Agioi Anargyroi” were evaluated. Sixty-eight of them (60.7%) were categorized at CP-A score and 44 (39.3%) at CP-B/C score whereas the median CP score of the whole population was 6 and the median MELD score was 9. Twenty-eight out of 112 cirrhotic patients (25%) were diagnosed with imaging and histologically confirmed concurrent HCC at baseline. Six of them were categorized at stage A, 4 at stage B, 12 at stage C and the rest 6 at stage D, according to BCLC staging system, whereas half of HCC patients (14/28, 50%), presented with portal vein thrombosis.

Overall, mean plasma FGL-2 levels were 4.04±3.80 pg/ml in the whole cirrhotic population but were significantly higher in patients with CP-B/C cirrhosis (6.07±4.80 pg/ml) compared to those with CP-A liver disease (2.82±2.27 pg/ml, $p<0.0001$). Additionally, mean plasma FGL-2 levels were significantly elevated in 28 cirrhotic patients with HCC at baseline (6.41±4.20 pg/ml) compared to 84 cirrhotic patients without HCC (3.24±3.29 pg/ml, $p=0.001$). Moreover, CP-B/C cirrhotic patients with HCC (n=18) exhibited significantly higher FGL-2 levels (7.84±4.24 pg/ml) compared to 25 CP-B/C cirrhotic patients without HCC at baseline (4.87±5.20 pg/ml, $p=0.024$). Patients categorized at advanced and terminal (BCLC-C/D) HCC stages (n=18) exhibited the highest plasma FGL-2 levels (8.17±4.31 pg/ml) that were remarkably higher to the corresponding ones of those of the earlier (BCLC-A/B) HCC stages (3.53±2.20 pg/ml, $p=0.008$). It seems that the presence of HCC as well as the stage of the disease according to BCLC system (which includes the Child-Pugh score) significantly influences plasma FGL-2 levels in cirrhotic patients.

Eighty-four cirrhotic patients (57 males) without HCC at baseline control were followed-up from January 2015 until December 2020 according to the HCC surveillance program (liver ultrasound and serum AFP levels every 6 months). Diabetes was present in 21 (25%) and esophageal varices in 41 (49%) of them. The main cause of the underlying chronic liver disease was chronic hepatitis C virus infection in 31/84 (37%) of cases, chronic hepatitis B virus infection in 10/84 (12%) of cases, non-alcoholic and/or alcoholic liver disease in 28/84 (33%) of cases and autoimmune liver disease in 13/84 (15%) of them. The vast majority of cirrhotic patients (59/84, 70%) were categorized as CP-A and the rest 25 (30%) as CP-B/C (19 CP-B and 6 CP-C).

Fourteen patients (14/84, 16.6%) developed HCC during the 5-years follow-up period (HCC group) and 70 (83.3%) did not (control group). HCC diagnosis was supported by compatible dynamic CT and MRI findings and was also histological confirmed in all cases. Demographic and clinical data of the 14 patients who developed HCC during surveillance as well as HCC stage at diagnosis were presented in table 1. As expected the majority of HCC cases appeared in CP-B/C cirrhotic patients (9/14, 64%). Nine out of 25 CP-B/C cirrhotic patients (36%) developed HCC during the follow-up period in contrast to only 5/59 (8.5%) CP-A patients. It is important to note that all five patients who were categorized as CP-A cirrhotics at baseline and developed HCC during the surveillance period were categorized in very early (BCLC-0, n=2) or early (BCLC-A, n=3) HCC stage and were treated with potentially curative procedures (2 with liver resection and 3 with radiofrequency ablation). Three of them were alive without HCC recurrence or liver disease deterioration at the end of follow-up and the other 2 died from liver-related complications following HCC recurrence. On the other hand the majority of cirrhotic patients categorized at CP-B/C stage (6/9, 66.6%) at baseline were staged at intermediate BCLC-B (n=3) or advanced BCLC-C (n=3) stage

at the time of HCC diagnosis and only 3 of them at early BCLC-A stage. Only two of them are still alive (one following successful orthotopic liver transplantation and one following locoregional treatment, both at BCLC-A stage) whereas 7 have died from liver related events and/or HCC progression during follow-up period.

Demographic, clinical and laboratory data among patients from the HCC group and control group are presented in table 2. As we can clearly see from this table patients age (p=0.60), gender (p=0.20), etiology of chronic liver disease (p=0.90), presence of diabetes (p=0.10) or varices (p=0.07) as well as MELD score were comparable between the two groups. In the univariate analysis only the Child-Pugh score (CP-B/C vs A, p=0.006) and the platelet count (p=0.008) were significantly correlated with HCC appearance. Patients from HCC group had significantly lower platelet count (mean 98.700 vs 147.400, p=0.008) and were frequently categorized at CP B/C stage (64.3% vs 22.9%, p=0.006) at baseline compared to control group. Concerning plasma FGL-2 and serum AFP levels at baseline, only FLG-2 levels (p=0.01) but not AFP levels (p=0.15) significantly differed between the two groups of patients. The median values of FGL-2 and AFP among patients from HCC and control group were (3.6 pg/ml vs 2.1 pg/ml, p=0.01) and (6.0 ng/ml vs 4.0 ng/ml, p=0.15), respectively.

In order to evaluate the impact of liver disease severity in HCC appearance as well as its influence on the levels of biomarkers we continue a multivariate, logistic regression analysis taking into account patient age, gender, platelet count, Child-Pugh score, MELD score as well as baseline FGL-2 and AFP levels as linear or dichotomous variable using the median values (3.6 pg/ml for FGL-2 and 6 ng/ml for AFP). In the multivariate logistic regression analysis, only patients gender (OR=20.801, 95%CI: 1.504-287.7, p=0.024), platelets (OR=0.980, 95%CI:0.964-0.997, p=0.025) and baseline CP score (CP-B/C vs A, OR=27.184, 95%CI:2.815-262.5, p=0.004) were significantly correlated with HCC appearance during the follow-up period, as shown in table 3. In this analysis plasma FGL-2 levels did not relate with HCC appearance either using them as linear (OR=1.16, 95%CI:0.96-1.41, p=0.13) or as dichotomous variable (OR=4.18, 95%CI:0.79-22.14, p=0.093) in the multivariate model.

Table 1: Characteristics of 14 patients who developed HCC during surveillance

	Age	Gender	Etiology	ChildPugh score	Meld score	BCLC at HCC diagnosis
1	56	M	HCV	A5	7	0
2	55	M	ALD	A5	9	A
3	68	M	HBV	A5	10	A
4	68	M	HCV	B7	10	A
5	60	M	HCV	B7	13	C
6	76	M	HCV	A6	8	A
7	62	M	HBV	B7	8	B
8	74	M	ALD	C11	18	B
9	71	F	PBC	B8	8	C
10	78	M	NAFLD	A5	6	0
11	60	M	ALD	B7	13	B
12	60	F	PBC	B8	10	A
13	74	M	ALD	C13	19	C
14	70	M	NAFLD	B8	13	B

MELD score: Model For End-Stage Liver Disease, BCLC: Barcelona Clinic Liver Cancer, HCC : hepatocellular carcinoma, HCV: hepatitis C virus, ALD: alcoholic liver disease, HBV: hepatitis B virus, PBC: primary biliary cholangitis, NAFLD: nonalcoholic fatty liver disease

Table 2: Demographic, clinical and laboratory baseline data among cirrhotic patients under HCC surveillance

	HCC				p-value
	NO (n=70)		YES (n=14)		
	N	%	N	%	
Gender					0.2a
Male	45	78.9	12	21.1	
Female	25	92.6	2	7.4	
Age [b]	63.5	11.2	65.1	6.9	0.6c
Cirrhosis Etiology					0.9
HCV	27	87.1	4	12.9	
HBV	8	80	2	20	
NASH/ALD	22	78.6	6	21.4	
AH/PBC	11	84.6	2	15.4	
CRYPTOGENIC	2	100	0	0	
Diabetes					0.1[a]
No	55	87.3	8	12.7	
Yes	15	71.4	6	28.6	

Decompensation					**0.001**[a]
No	55	91.7	5	8.3	
Yes	15	62.5	9	37.5	
CTP Score					**0.006**[d]
A	54	91.5	5	8.5	
B	12	63.2	7	36.8	
C	4	66.7	2	33.3	
Varices					**0.07**[a]
No	39	90.5	4	9.5	
Yes	31	75.6	10	24.4	
PLTs[b] (K/μl)	147.4	62.6	98.7	51.1	**0.008**[c]
MELD score[b]	9.7	5	10.9	3.9	0.4[c]
FGL-2					**0.01**[e]
Median value (range) (pg/ml)	2.1 (18.4)		3.6 (16.7)		
AFP					**0.15**[e]
Median value (range) (ng/ml)	4.0 (73.8)		6(126.0)		

[a]chi-square test [b]mean, standard deviation [c]independent samples t-test [d]chi-square trend test [e]Mann-Whitney test
HCC: hepatocellular carcinoma, HBV: hepatitis B virus, HCV: hepatitis C virus, NAFLD: non -alcoholic fatty liver disease, ALD: alcoholic liver disease, AH: autoimmune hepatitis, PBC: primary biliary cholangitis, CTP score: ChildTurcotte-Pugh score, MELD score: Model For End-Stage Liver Disease score, PLTs: platelets, FGL-2: fibrinogen like protein-2, AFP: a-fetoprotein

Table 3: Logistic Regression Analysis with HCC appearance as the dependent variable

	Odds ratio	95% confidence interval	p-value
PLTs	0.980	0.964 – 0.997	**0.025**
MELD	0.764	0.583 – 1.002	0.051
AGE	1.047	0.962 – 1.139	0.286
GENDER (Males vs. females)	20.801	1.504 – 287.7	**0.024**
CTP B-C vs A	27.184	2.815 – 262.5	**0.004**
FGL-2	2.834	0.497 – 16.15	0.241
AFP	5.455	0.878 – 33.87	0.069

CTP score: Child-Turcotte-Pugh score, MELD: Model For End-Stage Liver Disease, PLTs: platelets, FGL-2: fibrinogen like protein-2, AFP: a-fetoprotein

5. Discussion

The vast majority of HCC cases occur in the setting of severe chronic liver disease of any etiology and liver cirrhosis is the strongest risk factor of HCC emergence [12]. The cirrhotic background seems to play a crucial role in the development and progression of HCC as chronic liver disease deranges hepatic immune tolerogenic network facilitating tumor development [13]. Cytotoxic T Lymphocytes (CTLs) and Natural Killer (NK) cells are antitumor immune cells that play an integral role in cancer immune surveillance and eradication of tumor cells whereas accumulation of regulatory T cells (Tregs) that frequently occurs in HCCs, is associated with CTLs and NK cells dysfunction, tumor invasiveness as well as progression, and poor patient outcomes [12, 13]. Fibrinogen-like protein 2 (FGL-2), that is mainly secreted by regulatory T cells, has been demonstrated to promote tumor progression by regulating cellular components of the tumor microenvironment [9, 13]. In our study we evaluate soluble FGL-2 levels in Caucasian cirrhotic patients with or without HCC as well as their predictive value for HCC emergence during the surveillance program.

Our results are in accordance with other studies [8, 10] which conclude that liver disease severity is significantly related to plasma FGL-2 levels. Patients with liver cirrhosis exhibited higher FGL-2 levels compared to non-cirrhotics with chronic hepatitis C [8] or chronic hepatitis B [10] but levels were comparable among patients with HBV-related cirrhosis with or without HCC [10], suggesting that mainly cirrhotic background significantly affects them. Additionally, in a study with a small sample size, among 21 HCC patients, those with cirrhosis (n=10) exhibited significantly higher levels of sFGL-2 compared to 11 non-cirrhotic individuals [11]. In our study patients with advanced liver cirrhosis (Child-Pugh stage B/C) with HCC exhibited significantly higher FGL-2 levels (7.84±4.24 pg/ml) compared to CP-B/C cirrhotic patients without HCC (4.87±5.20 pg/ml, p=0.024) and patients at advanced or terminal (BCLC-C/D) HCC stages exhibited the highest plasma FGL-2 levels (8.17±4.31 pg/ml) that were remarkably higher to the corresponding ones of those of earlier (BCLC-A/B) HCC stages (3.53±2.20 pg/ml, p=0.008). It seems that the interaction of advanced liver disease with the high tumor burden and/or vascular invasion observed in HCC cirrhotic patients of advanced (BCLC-C) or terminal (BCLC-D) stage significantly affects plasma FGL-2 levels. It is important to note that portal vein thrombosis was observed only in 14 cirrhotic patients with HCC at baseline control, who by definition were categorized as advanced or terminal stage (BCLC-C/D), so it was impossible to evaluate the specific impact of this major event and its possible consequences (aggravation of

portal hypertension, bacterial translocation etc.) on FGL-2 levels.

Surveillance programs for hepatocellular carcinoma aim to detect liver tumors at an early stage when they are amenable to potentially curative therapy that is known to improve patient's survival [14]. Based on HCC volume doublingtime? EASL/AASLD recommendations support a 6-month screening interval, mainly with abdominal ultrasound as the primary surveillance test recommended [3, 4]. Although ultrasound has an acceptable sensitivity (84%) for detecting HCC at any stage, its sensitivity for detection of early stage HCC is significantly lower (47%) and did not always translate into survival benefit [15]. The latter could be influenced by the cirrhotic background of patients with HCC, as classical scores for liver cirrhosis severity such as Child-Pugh score are taken into account in BCLC staging of HCC and impact patients' prognosis. In our study, remarkable survival was observed only in CP-A cirrhotic patients at baseline who finally developed HCC were categorized at BCLC-0/A stage, so liver resection or locoregional therapies with favorable outcome could be implemented. On the contrary intermediate or advanced BCLC staging and poor clinical outcome was noticed in the majority of CPB/C cirrhotic patients with HCC emergence during the surveillance period. For these reasons most clinical practice guidelines [3, 4, 5] did not suggest HCC screening in patients with decompensated cirrhosis (especially those of ChildPugh C stage and/or MELD score above 20), unless they are listed for liver transplantation, because they are unlikely to benefit from HCC treatment. All patients with liver cirrhosis do not have the same risk of developing HCC and it remains difficult to assess the specific risk at an individual level [16]. In order to improve the sensitivity for early HCC detection in cirrhotic patients, several serum biomarkers (AFP, lectin-bound AFP, des-gamma carboxy prothrombin etc.) as well as combinations of them with clinical and routine laboratory data in panels (GALAD, BALAD-2) have been evaluated [17].

Serum AFP levels exhibit poor sensitivity for early HCC detection, when used alone. At a cut-off value of 20 ng/mL, the most commonly used cut-off value in clinical practice, AFP has a sensitivity and specificity of approximately 60% and 80% for HCC, respectively [18]. The accuracy of serum AFP levels for the detection of HCC is limited especially in cirrhotic patients with chronic HCV infection [18]. Concomitant use of liver ultrasound and AFP improved the sensitivity of early HCC detection (63%) compared to ultrasound alone (45%) [19]. Inclusion of serial AFP measurements further improves the predictive value of HCC risk in patients with HCV related cirrhosis. A model that incorporates the rate of AFP change along with the most recent value of AFP taking into account also patient age, serum alanine aminotransferase levels and platelet count is associated with improved sensitivity for early HCC detection compared to the standard of care surveillance program [20]. To our knowledge this study is the first which tries to evaluate the predictive value of plasma FGL-2 levels for HCC emergence among cirrhotic patients. It was hypothesized that cirrhotic patients without HCC but with relatively elevated FGL-2 levels could have created a potentially immunosuppressive microenvironment that could facilitate tumor development in the near future. Eighty-four cirrhotic patients, mainly with compensated Child-Pugh A liver cirrhosis (70%), joined a 5-year follow-up for HCC emergence. Baseline serum AFP levels did not differ between the HCC group and the control group whereas there was a significant difference between the two groups concerning plasma FGL-2 levels. In the multivariate analysis, taking into account several parameters that reflect liver failure and/or portal hypertension, such as platelet count, MELD score and Child-Pugh score, the statistical significance of plasma FGL-2 levels was lost, suggesting once again that liver disease severity probably influences them. The higher levels of baseline FGL2 in patients who develop HCC compared to ones who did not, could be explained by the observation that the majority of patients who finally developed HCC (64%) were categorized in Child-Pugh B/C score. As expected only male gender, Child-Pugh B/C score and low platelet count (as an indirect marker of significant portal hypertension) could predict HCC emergence.

Limitations of our study were the relative small sample size or small number of patients, the mixed etiology of advanced liver disease of patients, the relatively small proportion of those who finally develop HCC, especially from the subgroup of cirrhotic patients with compensated liver disease (Child-Pugh A5), as well as the calculation of FGL-2 levels only at the baseline visit. Thus, random error as indicating by wide confidence intervals in multivariate logistic regression model was unavoidable. This necessitates the replication of the results in larger samples of cirrhotic patients with long-term follow-up and recording of HCC events as well as clinical outcomes. On the other hand this small pilot study was performed in a single Hepatogastroenterology Unit of a General and Oncology Hospital, with the same experts in Hepatology, Gastroenterology, Liver imaging and Liver Histopathology and all the measurements were done in the same laboratory, which possibly reduces observer biases and somehow outweighs the limitations of the study.

In conclusion, plasma FGL-2 levels are significantly elevated in cirrhotic patients with decompensated liver disease compared to those with compensated cirrhosis and presence of HCC as well as HCC staging, according to the BCLC staging system, seems to significantly further influence them. Liver disease severity, according to Child-Pugh scoring system as well as low platelet count, but not baseline plasma FGL-2 or serum AFP levels, could predict HCC emergence among Caucasian cirrhotic patients.

References

1. McGlyn KA, Petrick JL, El Serag HB. Epidemiology of hepatocellular carcinoma. Hepatology. 2021; 73(S1): 4-13.
2. Villanueva A. Hepatocellular carcinoma. N Engl J Med. 2019; 380: 1450-62.
3. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018; 69: 182-236.
4. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, Staging and Management of hepatocellular carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Hepatology. 2018; 68(2): 723-50
5. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J et al. AsiaPacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int. 2017; 11: 317-70.
6. Eggert T, Greten TF. Tumor regulation of the tissue environment in the liver. Pharmacol Ther. 2017; 173: 47-57.
7. Tesi RJ. MDSC: the most important cell you have never heard of. Trends Pharmacol Sci. 2019; 40: 4-7.
8. Foerster K, Helmy A, Zhu Y, Khattar R, Adeyi OA, Wong KM et al. The novel immunoregulatory molecule FGL2: A potential biomarker for severity of chronic hepatitis C virus infection. J Hepatol. 2010; 53: 608-15.
9. Liu BQ, Bao ZY, Zhu JY, Liu H. Fibrinogen-like protein-2 promotes the accumulation of myeloid-derived suppressor cells in the hepatocellular carcinoma tumor microenvironment. Oncol Lett. 2021; 21(1): 47.
10. Van Tong H, Van Ba N, Xuan Hoan N, Thanh Binh M, Thanh Quyen D, Anh Son H, et al. Soluble fibrinogen-like protein-2 levels in patients with hepatitis B virus related liver diseases. BMC Infectious Diseases. 2018; 18: 553.
11. Sun Y, Xi D, Ding W, Wang F, Zhou H, Ning Q. Soluble FGL2, a novel effector molecule of activated hepatic stellate cells, regulates T-cell function in cirrhotic patients with hepatocellular carcinoma. Hepatol Int. 2014; 8: 567-75.
12. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021; 7(1): 6.
13. Risvi S, Wang J, El-Khoueiry AB. Liver Cancer Immunity. Hepatology. 2021; 73(S1): 86-103.
14. Singal AG, Lampertico P, Nahon P. Epidemiology and surveillance for hepatocellular carcinoma: New trendsJ Hepatol. 2020; 72: 250-61.
15. Moon AM, Weiss NS, Beste LA, Su F, Ho SB, Jin GY, et al. No association between screening for hepatocellular carcinoma and reduced cancer-related mortality in patients with cirrhosis. Gastroenterology. 2018; 155: 1128-39.
16. Goldberg DS, Taddei TH, Serper M, Mehta R, Dieperink E, Aytaman A, et al. Identifying barriers of hepatocellular carcinoma surveillance in a national sample of patients with cirrhosis. Hepatology. 2017; 65(3): 864-74.
17. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol. 2016; 14: 875-86.
18. Gopal P, Yopp AC, Waljee AK, Chiang J, Nehra M, Kandunoori P, et al. Factors that affect accuracy of a-fetoprotein test in detection of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2014; 12(5): 870-7.
19. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, et al. Surveillance imaging and a-fetoprotein for early detection of hepatocellular carcinoma in patient with cirrhosis: a meta-analysis. Gastroenterology. 2018; 154: 1706-18.
20. White DL, Richardson P, Tayoub N, Davila JA, Kanwal F, El-Serag HB. The updated model: an adjusted serum alpha-fetoprotein based algorithm for hepatocellular carcinoma detection with hepatitis C virus related cirrhosis. Gastroenterology. 2015; 149: 1986-7.