Tumor Biol.
DOI 10.1007/s13277-014-1858-4

RESEARCH ARTICLE

Development of a novel score for early detection of hepatocellular carcinoma among high-risk hepatitis C virus patients

Hatem A. El-mezayen · Hossam Darwish

Received: 18 February 2014 / Accepted: 17 March 2014

Abstract Hepatocellular carcinoma (HCC) is often diagnosed at advanced stage where effective therapies are lacking. Identification of new scoring system is needed to discriminate HCC patients from those with chronic liver disease. Based on the link between vascular endothelial growth factor (VEGF) and HCC progression, we aimed to develop a novel score based on combination of VEGF and routine laboratory tests for early prediction of HCC. VEGF was assayed for HCC group (123), liver cirrhosis group (210), and control group (50) by enzyme-linked immunosorbent assay (ELISA). Data from all groups were retrospectively analyzed including α-fetoprotein (AFP), international normalized ratio (INR), albumin and platelet count, transaminases, and age. Areas under receiving operating curve (ROC) were used to develop the score. A novel index named hepatocellular carcinoma-vascular endothelial growth factor score (HCC-VEGF score)=1.26 (numerical constant+0.05×AFP (U l^{-1})+0.038×VEGF (ng ml^{-1})+0.004×INR−1.02×albumin (g l^{-1})−0.002×platelet count×10^9 $l-^{1}$ was developed. HCC-VEGF score produce area under ROC curve of 0.98 for discriminating HCC patients from liver cirrhosis with sensitivity of 91 % and specificity of 82 % at cutoff 4.4 (i.e., less than 4.4 considered cirrhosis and greater than 4.4 considered HCC). Hepatocellular carcinoma-VEGF score could replace AFP in HCC screening and follow up of cirrhotic patients.

Keywords Hepatocellular carcinoma · Cirrhosis · HCV · Diagnosis · Tumor markers

H. A. El-mezayen (✉)
Chemistry Department, Helwan University, Cairo, Egypt
e-mail: hatem_mezayen@yahoo.com

H. Darwish
Medical Oncology Department, Damietta Cancer Institute, Damietta, Egypt

Published online: 01 April 2014

Introduction

Hepatocellular carcinoma (HCC) is often diagnosed at advanced stage where effective therapies are lacking [1]. More than 90 % of HCC cases develop in chronically inflamed liver as a result of viral hepatitis including virus C and B [2].

The current diagnostic tools for HCC among high risk patients includes clinical, laboratory, imaging, and biopsies [3]. The most common HCC biomarker used to screen patients with liver cirrhosis is serum α-fetoprotein (AFP), which is measured at 6-month intervals [4]. Serum AFP test has a low sensitivity, and about one third of patients with early stage HCC and small tumors have low level of AFP as that in normal individuals, which makes the AFP test insufficient for the early detection of HCC in at-risk populations [5]. In addition, AFP test has a high false-positive rate of ~20 % among patients with chronic hepatitis and 20–50 % among those with liver cirrhosis [6]. Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP) have been proposed as complement or substitutes for AFP in the diagnosis of HCC [7]. The sensitivity of AFP-L3 and DCP were better than that of AFP only in large tumors and hence is limited benefit in clinical practice [8].

In this regard, there is an urgent need to identify more sensitive and reliable serum biomarkers for early detection of HCC among high risk patients. One of the notable features of HCC is hypervascularity [9], and it has been reported that vascular endothelial growth factor (VEGF) expression is correlated with tumor vascularity [10]. Recently, it was reported that serum levels of VEGF might be useful predictor of the presence of HCC in patients with chronic liver cirrhosis [8].

To this end, we performed a prospective clinical study in which noninvasive, simple, and more accurate diagnostic score namely HCC-VEGF were developed. That score was based on combination of VEGF, AFP, and routine laboratory test related to liver impairment including, albumin, platelet

count, and international normalized ratio (INR) for HCC detection in compression with AFP alone.

Material and methods

Patients

Two groups of patients were studied. The first group consisted of 123 patients with clinical or biopsy-confirmed cirrhosis complicated by HCC on top hepatitis C virus infection. All patients of that group were prospectively diagnosed with HCC at Damietta Cancer Institute, Damietta, Egypt. The diagnosis of HCC was carried out according to American Association for the Study of Liver Diseases (AASLD) practice guidelines. The diagnosis was confirmed by at least one of the following: histopathology or new hepatic focal lesion by ultrasound and confirmed by computed tomography accompanying with AFP of >200 U L^{-1} [11]. The second group included 210 patients with compensated HCV cirrhosis and no evidence of HCC that were admitted to internal medicine department, Damietta General Hospital, Damietta, Egypt. Diagnosis of cirrhosis was based on clinical, biochemical and ultrasound, and/or histological criteria. All these patients had biopsies revised by a single pathologist and had Metavair [12] fibrosis stage 4 (F4) and variable degree of inflammation. The cirrhosis group had at least 2 year of follow-up from the time of serum been obtained for this study. The follow-up included ultrasound and AFP every 6 months for at least with no evidence of malignancy. In addition, 53 normal healthy control individual with sex- and age-matched, they were negative for HCV antibody and have normal abdominal ultrasonography with no evidence of liver disease and/or of neoplasm, for overall compression purpose. Exclusion criteria include infection with hepatitis B virus, history of drug hepatotoxicity, autoimmune liver disease, bilharzial infection, and metabolic liver diseases. None of HCC patients had received transarterial embolization chemotherapy or radiofrequency or surgical interference. The study protocol was approved by the Institutional Review Committee.

Samples and biomarkers

Blood samples were collected from all patients by vein-puncture, before any treatment. Each sample divided into three portions; the first part of the blood was treated immediately with EDTA-K_3 for platelets counting which done by an automated hematology analyzer (D-Cell 60, Diagon, Hungary); the second treated immediately with sodium citrate for prothrombin-INR identification; the third portion was collected without any anticoagulant. Sera were collected for liver function tests including, albumin (Alb), aspartate aminotransferase (AST), alanine aminotranferase (ALT), and total bilirubin (Bil). All liver function tests were done by an automated biochemistry analyzer (A15, Biosystem, Spain). Alpha fetoprotein (AFP) was evaluated with fully automated chemiluminescence system (Mini Vidas, Biomerieux, France). All serum samples had been screened for HCV using anti-HCV antibody ELISA kit test (Ortho, USA). HCV-RNA was estimated using quantitative polymerase chain reaction (PCR) assay (Cobas/Taqman; Roche Disgnostic, CA, USA). The AST/ALT ratio was calculated for each patient. AST platelet ratio index (APRI) was calculated as following: {[AST U l^{-1}/40 (upper limit of normal)]/(platelet count 10^9 l^{-1})} × 100 [13]. Serum concentrations of VEGF were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Diaclone, France). All samples were obtained with informed consent.

Statistical analysis

Statistical analysis was performed with the Medcalc version 11.3.3.0 statistical software package. Continuous parameters were expressed as arithmetic mean±standard deviation (X± SD) while categorical parameters were expressed as number (percentage), and they were considered statistically significant if the two-sided p value was <0.05. The diagnostic value of each blood marker was assessed by the area under the receiving operating curve (ROC). We determined the turning point of the curve to the best cutoff value for the diagnosis, and it was also a maximal value at the sum of the sensitivity and specificity. The diagnostic accuracy was calculated by sensitivity, specificity, positive predicative value (PPV), and negative predicative value (NPV) and was expressed as percentage. Blood markers with a high area under the curve (AUC) or a high significance on univariate analysis were added to create different multivariable models. The value (0 or 1) in ROC curve was expressed for liver cirrhosis (LC) and hepatocellular carcinoma (HCC) groups. The variables with $p<0.05$ were analyzed by multiple logistic regressions to assess independent variables for predicting HCC.

Results

Patient's characteristics

The main demographic, laboratory, and histological features of LC and HCC patients are depicted in Table 1. Patients with HCC were associated with reduce in mean albumin and platelet count and highest mean of INR, AST/ALT ratio, APRI, bilirubin, APF, and VEGF.

Table 1 added that patients with HCC were classified into 69.9 % with stage I+II and 30.1 % with stage III+IV.

Table 1 Clinicopathological data of healthy individuals and patients with liver cirrhosis and hepatocellular carcinoma

Variable	Healthy (n=53)	LC (n=210)	HCC (n=123)	p*
Age (years)	45.6±7.4	54.3±4.3	59.4±12.5	<0.0001
AST (U l^{-1})	26.6±6.3	74.2±10.2	124.2±12.5	<0.0001
ALT (U l^{-1})	31.4±4.5	65.1±12.4	72.3±13.4	0.033
AST/ALT	0.83±0.54	1.4±0.89	2.1±59.5	<0.0001
Albumin (g dl^{-1})	4.5±2.7	3.7±1.5	2.9±0.87	<0.0001
Total bilirubin (mg dl^{-1})	0.74±0.06	1.7±1.4	2.01±1.08	0.035
Platelet count ($\times 10^9$ l^{-1})	210±49	187±72	93±41	<0.0001
APRI	0.21±0.08	1.9±0.6	2.1±0.8	0.01
Prothrombin-INR	0.89±0.23	1.29±0.54	1.83±0.36	<0.0001
α-fetoprotein (U l^{-1})	4.3±1.04	44.6±15.3	1,894±349	<0.0001
VEGF (pg ml^{-1})	14.9±1.89	47.5±34.7	110.7±59.3	<0.0001
Tumor stage, n, (%)				
I+II			86 (69.9)	
III+IV			37 (30.1)	
Tumor encapsulation, n (%)				
None			59 (48)	
Complete			64 (52)	
Tumor grade, n (%)				
I			57 (46.3)	
II+III			66 (53.7)	
Tumor size, n (%)				
<5 cm			28 (22.7)	
>5 cm			95 (77.3)	
Vascular invasion, n (%)				
Absent			74 (60.2)	
Present			49 (39.8)	
Number of lesion, n (%)				
Single			43 (34.9)	
Multiple			80 (64.1)	

AST aspartate aminotransferase, *ALT* alanine aminotranferase, *APRI* AST platelet ratio index, *INR* international normalized ratio, *VEGF* vascular endothelial growth factor, *LC* liver cirrhosis, *HCC* hepatocellular carcinoma

*p>0.05, considered nonsignificant and p<0.05 considered significant, the reference group for p values were HCC and LC

Complete liver encapsulation was observed in 52 % of total HCC patients. According to tumor grade, HCC patients can be classified into 46.3 % with grade I and 53.7 % with grade II+III. Patients with small tumor size (<5 cm) represents only 22.7 % while those with large tumor size (>5 cm) represent 77.3 %. Evidence of vascular invasion was observed in 60.2 %. Patients with single focal lesion represent 34.9 % while those with multiple focal lesions represent 64.1 %.

Diagnostic performance using area under the ROC curves

Using ROC curves, we assessed and compare the diagnostic accuracy of all parameters including age, AST, ALT, AST/ALT, APRI, platelets count, albumin, INR, AFP, and VEGF. The most effective markers with high AUC were in order of VEGF(0.864)>AFP(0.784)>platelet(0.72)>INR(0.71)>albumin(0.704). Age, AST, ALT, AST/ALT, and APRI were excluded due to the reduce in their AUC values (0.651, 0.594, 0.632, 0.529, and 0.598, respectively) (Fig. 1).

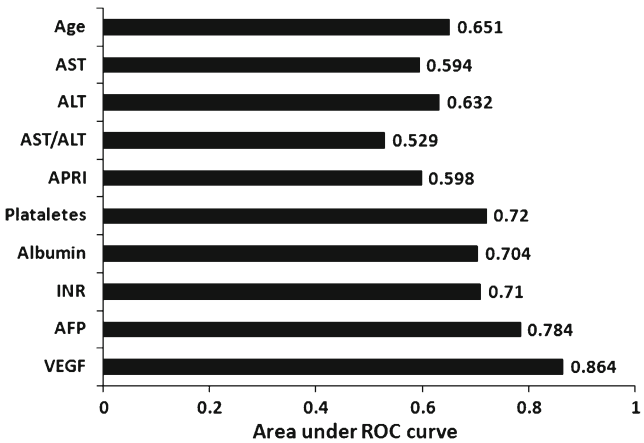

Fig. 1 Area under curve of markers to discriminate patients with HCC from patients with liver cirrhosis

Performance characteristics of multivariate analysis and predictive model

Using multivariate discriminate analysis (MDA), a function based on two parameters (AFP and VEGF), three markers (AFP, VEGF, and INR) and four markers (AFP, VEGF, INR, and albumin), and finally five markers (AFP, VEGF, INR, albumin, and platelet count). The best linear combination of blood markers was selected by MDA using the minimum Wilk's lambda test. The MDA selected a novel noninvasive index for prediction evidence of malignancy among HCV patients based on the above mentioned five markers. We proposed a novel noninvasive index for prediction of HCC-dependent cirrhosis named HCC-VEGF score. HCC-VEGF score=1.26 (numerical constant+0.05×AFP(U l^{-1})+0.038×VEGF (ng ml^{-1})+0.004×INR−1.02×albumin(g l^{-1})−0.002×platelet count×10^9 l^{-1}. The score has ranged from 2.1 to 7.8 and shows a highly significant different ($p<0.001$) between patients with HCC and LC. The mean±SD of score in healthy individual, LC and HCC were 0.5±0.04, 2.9±0.56, and 5.5±1.67, respectively (Fig. 2). The score was calculated for each patients involved in that study. The AUC of our score for prediction of HCC from LC was 0.98 compared to AFP of 0.784 (Fig. 3). The beast sensitivity (91 %) and specificity (82 %) for HCC-VEGF score were at cutoff value of 4.4 for the beast differentiation of patients with HCC from those with LC (i.e., less than 4.4 indicated patients with liver cirrhosis and greater than 4.4 indicated patient with HCC) with efficiency of 89 %. Moreover, the sensitivity of AFP for detection of HCC after combination of VEGF, INR, albumin, and platelet count was increase from 48 to 91 %. Positive predictive value and negative predictive value for HCC-VEFG score were 79 and 83 %, respectively, which higher than those of AFP (61 and 655, respectively) (Table 2). Absolute specificity (100 %) was obtained to discriminate between HCC and healthy individuals.

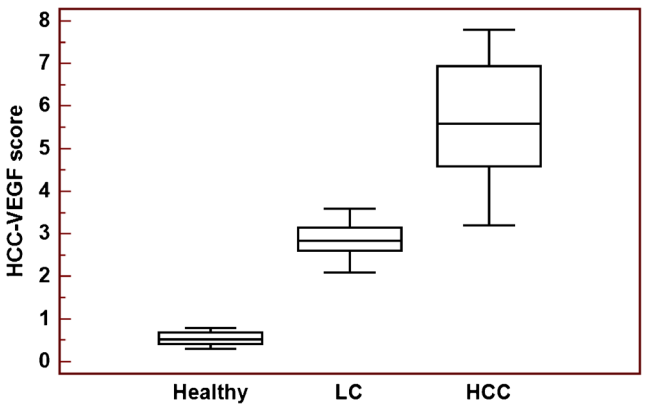

Fig. 2 Box plots of HCC-VEGF score to discriminate HCC patients from those with liver cirrhosis as well as healthy control. The *box* represents the interquartile range. The *whiskers* indicate the highest and lowest values, and the *line* across the box indicates the median value, $p<0.0001$

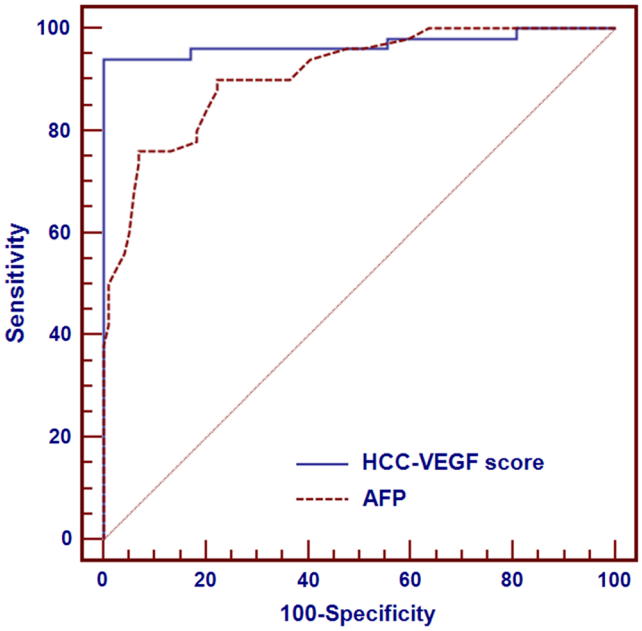

Fig. 3 ROC curve of HCC-VEGF score and AFP for discriminating patients with hepatocellular carcinoma from those with liver cirrhosis (areas under ROC were 0.98 and 0.784, respectively)

Diagnostic performance of HCC-VEGF score versus AFP

The diagnostic power of HCC-VEGF score and AFP against tumor burden characteristics including TNM stage, tumor encapsulation, tumor grade, tumor size, vascular invasion, and number focal lesion were illustrated in Tables 3 and 4. Overall, the diagnostic value of HCC-VEGF score was the best one with an AUC of 0.98 in discriminating patients with HCC from those with liver cirrhosis compared to that of AFP (AUC=0.784). The AUCs of HCC-VEGF score for discriminate patients with low TNM stage, complete encapsulation, low grade, smaller tumor size, absence of vascular invasion, and single focal lesion from cirrhotic patients (0.716, 0.712, 0.697, 0.821, 0.693, and 0.803, respectively) which are higher than AFP (0.613, 0.54, 0.546, 0.697, 0.521, 0.621, respectively) (Fig. 4).

Table 2 Diagnostic value of HCC-VEGF score and AFP in discriminating patients with hepatocellular carcinoma from liver cirrhosis

	HCC-VEGF score[a]	AFP
Sensitivity (%)	91	48
Specificity (%)	82	76
PPV (%)	79	61
NPP (%)	83	65
Accuracy (%)	89	73

AFP α-fetoprotein, at cutoff 200 U l^{-1}, *PPV* positive predictive value, *PPV* positive predictive value

[a] HCC-VEGF score=1.26 (numerical constant+0.05×AFP(U l^{-1})+0.038×VEGF(ng ml^{-1})+0.004×INR−1.02×albumin (g l^{-1})−0.002×platelet count×10^9 l^{-1}, at cutoff 4.4

Table 3 Diagnostic performance of HCC-VEGF score to discriminate HCC patients from patients with liver cirrhosis

Clinical data	HCC-VEGF score					
	Sensitivity (%)	Specificity (%)	Accuracy (%)	AUC	PPV (%)	NPV (%)
TNM stage (no., %)						
I+II (86, 69.9)	74	98	73	0.716	67	78
III+IV (37, 30.1)	68	99	72	0.861	72	65
Tumor encapsulation (no., %)						
Complete (64, 52)	70	79	69	0.712	76	57
None (59, 48)	82	87	76	0.783	82	65
Tumor grade (no., %)						
I (57, 46.3)	84	74	75	0.697	65	74
II+III (66, 46.3)	91	87	71	0.732	78	68
Tumor size (no., %)						
<5 cm (28, 22.7	89	99	88	0.821	79	76
>5 cm (95, 77.3)	99	100	97	0.861	64	55
Vascular invasion (no., %)						
Present (49, 39.8)	84	100	82	0.713	72	67
Absent (74, 60.2)	82	100	75	0.693	86	78
Number of lesion (no., %)						
Single (43, 34.9)	69	76	86	0.803	64	65
Multiple (80, 64.1)	64	71	95	0.861	76	78

As shown in Table 4, AFP unable to discriminate HCC patients with nontumor encapsulation from those with liver cirrhosis where the AUC was 0.523 which considered a diagonal marker versus HCC-VEGF score which classified the same characteristic with an AUC of 0.783. In addition, patients without vascular invasion cannot discriminate from those with liver cirrhosis via AFP where the AUC was 0.521; on contrast, HCC-VEGF

Table 4 Diagnostic performance of AFP to discriminate HCC patients from patients with liver cirrhosis

Clinical data	AFP					
	Sensitivity (%)	Specificity (%)	Accuracy (%)	AUC	PPV (%)	NPV (%)
TNM stage (no., %)						
I+II (86, 69.9)	68	89	69	0.613	56	73
III+IV (37, 30.1)	73	81	75	0.644	65	57
Tumor encapsulation (no., %)						
Complete (64, 52)	92	99	71	0.54	76	61
None (59, 48)	89	100	86	0.523	55	75
Tumor grade (no., %)						
I (57, 46.3)	87	71	64	0.546	67	58
II+III (66, 46.3)	81	86	78	0.613	76	76
Tumor size (no., %)						
<5 cm (28, 22.7)	88	81	72	0.697	75	89
>5 cm (95, 77.3)	86	93	84	0.632	67	78
Vascular invasion (no., %)						
Present (49, 39.8)	86	99	94	0.612	66	76
Absent (74, 60.2)	89	89	86	0.521	57	65
Number of lesion (no., %)						
Single (43, 34.9)	79	85	61	0.621	78	68
Multiple (80, 64.1)	74	86	82	0.731	67	78

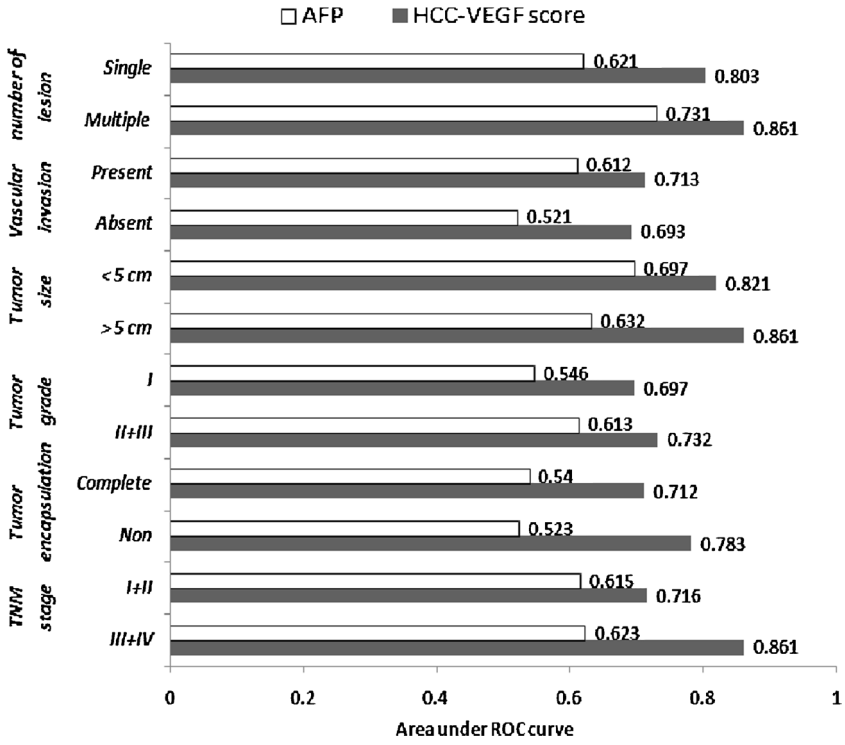

Fig. 4 Area under ROC curve (AUC) of HCC-VEGF score compared with AFP for early diagnosis of HCC with tumor burden features including TNM stage, tumor encapsulation, tumor grade, tumor size, vascular invasion, and number of lesion

score can classify those groups with more accuracy where AUC was 0.693.

Discussion

Chronic HCV infection ultimately progresses to liver cirrhosis, which is thought to play an important role in the development of HCC [14]. Regular surveillance of high-risk individuals is recommended but is presently hindered by the poor performance of the commonly used serum marker, AFP, even in combination with abdominal ultrasonography. A significant effort has been and continues to be applied to the search for improved HCC biomarkers [15].

All HCC patients involved in that study were positive for anti-HCV where that developed on top of HCV-related liver cirrhosis. This is in agreement with previous studies [16]. Diagnosis of HCC not only dependent on tumor stage but also adversely influenced by impaired liver function and hematological indices related to the pathogenic condition [17].

Many scores were designed to guide the prognosis of HCC, each one including parameters reflecting liver dysfunction [18]. None of these previous prognostic indices are considered ideal, because all of them depend on routine laboratory markers, mainly liver function tests, which may altered due to other factor rather than malignancy.

Where the ideal markers of HCC to be specific for HCC and not be detected in cirrhosis. From that point, VEGF was considered the best one where its levels are normal under stable conditions, but in hypoxia, which the main character of tumors arising, causes elevation of VEGF and where oxygen tension play a major role in upregulation of VEGF messenger RNA (mRNA) [19].

Our study was designed to develop of a novel noninvasive score namely hepatocellular carcinoma-vascular endothelial growth factor (HCC-VEGF) for prediction of HCC among HCV infected patients. To maximize the clinical significant of that score, it include the main biomarker involved in angiogenesis process, VEGF [20], in addition to the most significant routine laboratory parameters in discrimination of HCC patient from cirrhotic patients.

It was reported that vascular endothelial cells in tumor tissue showed strong immunostaining for VEGF, whereas these cells do not appreciate staining in nontumor tissues, and tumor vascular endothelial cells may be the main target of VEGF released from HCC cells [21]. In addition, VEGF considered the main factor in neovascularization and dissemination of cancer cells into tumor capsule in HCC patients [22]. So, it was taken as a basic index in the construction of our score.

Herein, for the first time, we report the clinical validation of five biomarkers (VEGF, INR, albumin, and platelet count) in combination with AFP as a means of improving the percentage of accurate and correct diagnosis of HCC patients.

In the present study, there was an insignificant variation of serum VEGF levels between control group and cirrhotic group that mainly due to the two groups had benign liver tissue without hypoxia and there is no need for expression of angiogenic markers.

In contrast, there was a significant elevation of VEGF serum levels in HCC patients compared to both of the control and cirrhotic group. These results indicated that VEGF serum levels can predict development of HCC among patients with liver cirrhosis where that elevation may suggest being due to HCC nodules which tended to have internal hypoxia and necrosis, with upregulation of the expression of VEGF mRNA [23].

It was reported that HCC patients with vascular invasion developed numerous microscopic intrahepatic metastasis, which undetectable by imaging, have high serum levels of VEGF [24]. This might indicate that angiogenesis by microscopic intrahepatic HCC reflected in the serum level of VEGF where it was significant in early detection of HCC patients than imaging techniques.

On univariant analysis, baseline levels of age, AST, ALT, AST/ALT, APRI, albumin, total bilirubin, platelet count, AFP, and VEGF were all associated with the risk of clinical outcome.

Multivariate regression modeling demonstrated that only VEGF, AFP, INR, albumin, and platelets count retained significant when combined with each other. Also, based on AUC for different variables in our study, the previous markers showed superior diagnostic power and were then identified as independent predictive variables to differ significantly between liver cirrhosis and hepatocellular carcinoma patients. Herein, we utilitize routine laboratory tests in addition to VEGF; all parameters were assayed in blood by simple methods which can be applicable in all hospital.

It was reported that, AFP is the most widely used tumor marker but has poor diagnostic accuracy and ethnic variability [25]. Although AFP improves detection of HCC, a significant number of HCC patients present without elevated AFP, and therefore the additional markers were added to increase the sensitivity and specificity.

AFP is produced due to dedifferentiation of cancer cells [26], and that HCC is often well differentiated at an early stage and undergoes dedifferentiation as it grows [27], which explains the low sensitivity of that marker (48 %) observed in our study. These results are consistent with several other reports indicating an AFP sensitivity ranging from 47 to 68 % [28, 29].

Moreover, HCC considered a hypervascular tumor and neovascularization is a hallmark of HCC [22], so it was better to include VEGF in our score to improve sensitivity of AFP in early detection of HCC.

The diagnostic power of AFP in discriminating patients with HCC among cirrhotic patients in our study had an AUC of 0.784 which is similar to other reported by Attallah and his colleagues (AUC=0.7) [30].

The liver has large reserves of albumin synthetic capacity; thus, decreased serum albumin from liver impairment is important and is already used to assess liver carcinogenesis [30]. The serum human albumin is usually normal in chronic liver diseases, until cirrhosis and development of primary liver cancer. Decrease in albumin levels may be due to cause other than liver impairment as nephritic syndrome [31] and heart failure [32].

Our results agreed with Masuzaki et al. [33] where albumin has an AUC of 0.71 for discriminating patient with HCC on top of HCV infection. Moreover, the diagnostic power of albumin in prediction of HCC among cirrhotic patients in the presented study results was lower than that reported by Attallah (AUC=0.8) [34].

Thrombocytopenia in patients with HCC may be due to reduced hepatic production of thrombopoietin, increased slenic sequestration of platelets secondary to portal hypertension or the mylosuppressive action of HCV [35]. Results from the current study revealed a significant and sensitive diagnostic power of platelet count in prediction of HCC with an AUC of 0.72, so it was included in our score.

INR index has been used to standardize prothrombin time value in liver diseases and included in some prognostic models of HCC and liver cirrhosis, such as Child-Turcotte-Pugh (CTP) score and the model for end stage liver disease (MELD) [36].

INR index was involved in our combination due its diagnostic performance in discriminating HCC patients with an AUC of 0.72. The increase of INR index in HCC patients may be due to upregulation of VEGF where it is capable of inducing vascular hyperpermeability. That vascular hyperpermeability results in leakage of plasma proteins, including prothrombin [37]. So, the observed elevation of INR index in our HCC patients can be explained in the scope of angiogenesis process; therefore, it has a clinical utility in improve sensitivity of AFP.

Many studies for evaluating combination of serum markers have produced better results in terms of their predictive accuracy for evidence of malignancy [20, 30]. Our study investigated the concept of combining several markers in order to increase sensitivity of AFP for prediction of HCC. Combining alpha-L-fucosidase and AFP increase the sensitivity of the latter from 39 to 83 % [38]. Moreover, the accuracy of AFP was raised from 70 to 90 % when combined with alpha-1-acid glycoprotein [39].

In addition, combination of AFP and routine laboratory tests include, AST/ALT, alkaline phosphatase, and albumin in addition to age raise the sensitivity of AFP form 41 to 97 % [30].

Our model showed an acceptable discriminate power for prediction of HCC among cirrhotic patients on top HCV infection with AUC of 0.98. It was based on combination of the most significant marker involved in angiogenesis process which required for tumor development (VEGF) in combination with routine laboratory tests (AFP, INR, albumin, and platelet count). The sensitivity of AFP for diagnosis of HCC was raised through our model form 48 to 91 %.

In conclusion, HCC-VEGF score could potentially be used to diagnose HCC, especially early stage and will help to resolve the deficiencies of AFP in the testing of AFP-negative patients. The possibility of distinguished HCC from healthy individuals and patients with cirrhosis offers hope for the early detection of HCC. Our score could be used as blood tests for the noninvasive diagnosis of HCC to reduce the need for liver biopsy. Applying that score on other large multicenter cohort for verify its effectiveness is needed to confirm our findings.

Acknowledgments We thank Dr Mohamed El-Laban at Internal Medicine Department, Damietta General Hospital, Damietta, Egypt for providing us with the samples needed in this study.

Conflicts of interest None.

References

1. Nafee AM, Pasha HF, Abd El Aal SM, Mostafa NA. Clinical significance of serum clusterin as a biomarker for evaluating diagnosis and metastasis potential of viral-related hepatocellular carcinoma. Clin Biochem. 2012;45:1070–4.
2. Welzel TM, Graubard BI, Zeuzem S, El–Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. Hepatology. 2011;54:463–71.
3. Gonzalez SA, Keeffe EB. Diagnosis of hepatocellular carcinoma: role of tumor markers and liver biopsy. Clin Liver Dis. 2011;15:297–306.
4. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2001;35:421–30.
5. Chen L, Ho DW, Lee NP, et al. Enhanced detection of early hepatocellular carcinoma by serum SELDI-TOF proteomic signature combined with alpha-fetoprotein marker. Ann Surg Oncol. 2010;17:2518–25.
6. Hao K, Luk JM, Lee NP, et al. Predicting prognosis in hepatocellular carcinoma after curative surgery with common clinicopathologic parameters. BMC Cancer. 2009;9:389.
7. Kew MC. Hepatocellular carcinoma in developing countries: Prevention, diagnosis and treatment. World J Hepatol. 2012;4:99–104.
8. Mukozu T, Nagai H, Matsui D, Kanekawa T, Sumino Y. Serum VEGF as a tumor marker in patients with HCV-related liver cirrhosis and hepatocellular carcinoma. Anticancer Res. 2013;33:1013–1021.
9. Yoshiji H, Kuriyama S, Hicklin DJ, et al. KDR/Flk-1 is a major regulator of vascular endothelial growth factor-induced tumor development and angiogenesis in murine hepatocellular carcinoma cells. Hepatology. 1999;30:1179–86.
10. Moon WS, Rhyu KH, Kang MJ, et al. Overexpression of VEGF and angiopoietin 2: a key to high vascularity of hepatocellular carcinoma? Mod Pathol. 2003;16:552–7.
11. Soresi M, Magliarisi C, Campagna P, et al. Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. Anticancer Res. 2003;23:1747–53.
12. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997;349:825–32.
13. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518–26.
14. Anzola M. Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat. 2004;11:383–93.
15. Giannelli G, Fransvea E, Trerotoli P, et al. Clinical validation of combined serological biomarkers for improved hepatocellular carcinoma diagnosis in 961 patients. Clin Chim Acta. 2007;383:147–52.
16. Lehman EM, Wilson ML. Epidemiology of hepatitis viruses among hepatocellular carcinoma cases and healthy people in Egypt: a systematic review and meta-analysis. Int J Cancer. 2009;124:690–7.
17. Tandon P, Garcia-Tsao G. Prognostic indicators in hepatocellular carcinoma: a systematic review of 72 studies. Liver Int. 2009;29:502–10.
18. Camma C, Cabibbo G. Prognostic scores for hepatocellular carcinoma: none is the winner. Liver Int. 2009;29:478–80.
19. Harris SR, Schoeffner DJ, Yoshiji H, Thorgeirsson UP. Tumor growth enhancing effects of vascular endothelial growth factor are associated with increased nitric oxide synthase activity and inhibition of apoptosis in human breast carcinoma xenografts. Cancer Lett. 2002;179:95–101.
20. El-Mezayen HA, el Toson SA, Darwish H, El-Badry E. Discriminant function based on parameters of hyaluronic acid metabolism and nitric oxide to differentiate metastatic from non-metastatic colorectal cancer patients. Tumour Biol. 2012;33:995–1004.
21. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature. 1992;359:845–8.
22. Mise M, Arii S, Higashituji H, et al. Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. Hepatology. 1996;23:455–64.
23. Suzuki K, Hayashi N, Miyamoto Y, et al. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res. 1996;56:3004–9.
24. Matsumata T, Kanematsu T, Takenaka K, Yoshida Y, Nishizaki T, Sugimachi K. Patterns of intrahepatic recurrence after curative resection of hepatocellular carcinoma. Hepatology. 1989;9:457–60.
25. El-Zayadi AR, Badran HM, Barakat EM, et al. Hepatocellular carcinoma in Egypt: a single center study over a decade. World J Gastroenterol. 2005;11:5193–8.
26. Yeh SH, Chen PJ, Lai MY, Chen DS. Allelic loss on chromosomes 4q and 16q in hepatocellular carcinoma: association with elevated alpha-fetoprotein production. Gastroenterology. 1996;110:184–92.
27. Kojiro M. Pathological evolution of early hepatocellular carcinoma. Oncology. 2002;62 Suppl 1:43–7.
28. Sherman M, Klein A. AASLD single-topic research conference on hepatocellular carcinoma: Conference proceedings. Hepatology. 2004;40:1465–73.
29. Marrero JA, Lok AS. Newer markers for hepatocellular carcinoma. Gastroenterology. 2004;127:S113–9.
30. Attallah AM, Omran MM, Attallah AA, et al. HCC-ART score, a simple, highly sensitive and specific test for early diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Br J Cancer. 2013;109:1657–65.
31. Fliser D, Zurbruggen I, Mutschler E, et al. Coadministration of albumin and furosemide in patients with the nephrotic syndrome. Kidney Int. 1999;55:629–34.
32. Djousse L, Rothman KJ, Cupples LA, Levy D, Ellison RC. Serum albumin and risk of myocardial infarction and all-cause mortality in the Framingham Offspring Study. Circulation. 2002;106:2919–24.
33. Masuzaki R, Tateishi R, Yoshida H, et al. Risk assessment of hepatocellular carcinoma in chronic hepatitis C patients by transient elastography. J Clin Gastroenterol. 2008;42:839–43.

34. Attallah AM, El-Far M, Abdel Malak CA, et al. Evaluation of cytokeratin-1 in the diagnosis of hepatocellular carcinoma. Clin Chim Acta. 2011;412:2310–5.
35. Dai CY, Ho CK, Huang JF, et al. Hepatitis C virus viremia and low platelet count: a study in a hepatitis B & C endemic area in Taiwan. J Hepatol. 2009;52:160–166.
36. Saad WE, Darwish WM, Davies MG, et al. Transjugular intrahepatic portosystemic shunts in liver transplant recipients: technical analysis and clinical outcome. AJR Am J Roentgenol. 2013;200:210–8.
37. Daskalow K, Rohwer N, Raskopf E, et al. Role of hypoxia-inducible transcription factor 1alpha for progression and chemosensitivity of murine hepatocellular carcinoma. J Mol Med (Berl). 2010;88:817–27.
38. Tangkijvanich P, Tosukhowong P, Bunyongyod P, et al. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in Thailand. Southeast Asian J Trop Med Public Health. 1999;30:110–4.
39. Bachtiar I, Santoso JM, Atmanegara B, et al. Combination of alpha-1-acid glycoprotein and alpha-fetoprotein as an improved diagnostic tool for hepatocellular carcinoma. Clin Chim Acta. 2009;399:97–101.