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Diagnostic role of collagen-III and matrix metalloproteinase-1 for early detection of hepatocellular carcinoma

AM Attallah[a], MS Albannan[a], MS El-Deen[a], K Farid[b], FM Khedr[a], KA Attallah[a] and SO Abdallah[c]

[a]Research & Development Department, Biotechnology Research Center, New Damietta, Egypt; [b]Faculty of Medicine, Mansoura University, Mansoura, Egypt; [c]Faculty of Science, Cairo University, Giza, Egypt

ABSTRACT

Background: As the poor prognosis of hepatocellular carcinoma (HCC) is mostly due to late detection at an advanced stage there is a strong need for establishing more effective strategies for early identification. We hypothesized that collagen-III and matrix metalloproteinase-1 (MMP-1) and their ratio (CMR) are effective markers for identifying early-HCC when used alongside serum AFP, alkaline phosphatase and bilirubin.

Methods: We recruited 148 patients with HCC, 133 with cirrhosis and 121 with fibrosis. Liver fibrosis was staged according to METAVIR, HCC was diagnosed by on histological findings or typical imaging characteristics by ultrasound and computed tomography. Collagen-III and MMP-1 were identified based on Western blotting and quantified in sera using ELISA, liver function tests (LFTs) by routine methods.

Results: Patients with HCC showed a significantly ($P < 0.05$) higher collagen-III and collagen-III /MMP-1 ratio (CMR) than fibrotic and cirrhotic patients. Patients with HCC showed significantly ($P < 0.05$) lower concentration of MMP-1 than those without. As expected, numerous LFTs were also abnormal. A score of AFP, alkaline phosphatase and bilirubin together with CMR (the HCC-ABC test) was then constructed, This yielded ROC area under curves of 0.85 (95% CI 0.79–0.98) for identifying small tumour size (<3 cm), 0.87 (0.79–0.98) for identifying CLIP (0–1) [Cancer of the Liver Italian Program] disease severity, and 0.87 (0.74–0.93) for identifying BCLC disease severity (all $p < 0.001$), which is each case exceeded the predictive value of AFP.

Conclusion: HCC-ABC diagnostic Test is a promising index for HCC early detection with a high degree of accuracy that may facilitate therapy.

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Collagen-III; MMP-1; CLIP; BCLC; HCC-ABC diagnostic; Cirrhosis

Introduction

Globally, hepatocellular carcinoma is considered to be the sixth commonest cancer accounting for approximately 746,000 deaths annually [1]. It is noteworthy that all aetiological forms of liver cirrhosis may be complicated by HCC but the risk varies according to the aetiology of liver cirrhosis, ranging between 1.5% and 4.5% in HCV-related cirrhosis and 2.2–4.3% in HBV-related cirrhosis annually [2,3]. Indeed, curative treatments, such as hepatic resection in addition to liver transplantation, offer good prognosis, but are still limited to early HCC [4]. For these reasons, there is a growing interest for establishing a developed diagnostic strategy for early detection of HCC [5]. Alpha-fetoprotein (AFP) has been traditionally used for routine diagnosis of HCC, but its use as a surveillance test for detecting HCC is unsatisfactory and questionable as only a small proportion (10–20%) of early HCC is associated with elevated AFP [5–9]. In addition, serum levels of AFP may be elevated in patients with chronic liver disease in the absence of HCC [10]. International guidelines suggest ultrasound surveillance for HCC early diagnosis in liver cirrhotic patients (F4). However, 40% of nodules <2 cm are undetectable [11].

Few HCC biomarkers demonstrate a sufficiently precise diagnostic performance for early HCC in clinical practice. These limitations have stimulated the development of surrogate markers which enable clinicians to diagnose asymptomatic patients and thus can be widely used for early detection of HCC. Collagen is considered to be the main component of the connective tissue which is regulated by a family of zinc-dependent neutral proteases called the matrix metalloproteinases (MMPs) [12,13]. Interestingly, Collagen-III is considered to be one of the five collagen subtypes that have been detected in the liver which regulates cell proliferation, migration, polarity and differentiation [14]. Hence, we hypothesized that a new mathematical combination incorporating CMR together with the more significantly elevated liver function tests would have a better prediction efficacy for detecting early HCC.

Material and methods

We tested our hypothesis in 121 patients with liver fibrosis (fibrosis stages F1-F3), 133 patients with liver cirrhosis (F4) and 148 patients with HCC. Patients were enrolled from Tropical Medicine Unit, Mansoura

CONTACT AM Attallah amattallah@hotmail.com Biotechnology Research Center, P.O. Box (14), 23 July St., Industrial Zone, New Damietta 34517, Egypt

University Hospitals, Mansoura, Egypt. HCC was diagnosed on the basis of liver histological findings or typical imaging characteristics by ultrasound and computed tomography. All patients tested negative for HBsAg (Dia.Pro, Milan, and positive for anti-HCV antibodies (Biomedica, Sorin, Italy). Patients were then confirmed for the presence of HCV-RNA using quantitative polymerase chain reaction assay (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Pleasanton, USA).

HCC staging was determined using Cancer of the Liver Italian Program (CLIP) [15], as well as Barcelona Clinic Liver Cancer (BCLC) staging systems [16]. The American Association for the Study of Liver diseases and the European Association for the Study of the Liver endorsed BCLC system that is considered the standard staging system for use in both clinical trials and routine practice [17]. Overall, multiple clinical indexes are taken into account to stage patients according to CLIP and BCLC scoring systems such as Child-Pugh score, tumour morphology, AFP level and presence of portal vein thrombosis. CLIP (0–1) and BCLC (0-A) were used to define the early stages of HCC. Additionally, diagnosis of non-malignant chronic liver diseases was based on the standard biochemical, clinical, and ultrasonographic criteria, in addition to the pathological data. The METAVIR scoring system [18] was used to stage liver fibrosis as follows: F0, no fibrosis; F1, portal fibrosis alone; F2, portal fibrosis with rare septae; F3, portal fibrosis with many septae; F4, cirrhosis. Overall, liver fibrosis was defined as METAVIR score of ≤3 (F1-F3) whereas cirrhosis was defined as METAVIR score of 4 (F4). This study was approved by the ethical guidelines of the Helsinki Declaration and an informed consent was obtained from all participants.

Liver function tests [LFTs: albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] were all measured on an automated biochemistry analyser (A15, Biosystem, Spain). Complete blood count was performed using KX-21 Sysmex automated haematology analyser (Sysmex Corporation, Kobe, Japan). Serum levels of alpha-fetoprotein (AFP) were measured by chemiluminescence (Immulite 1000, Diagnostic Products Corporation; Los Angeles, USA).

Serum samples were firstly run by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The resolved samples were electro-transferred onto a nitrocellulose membrane in a protein transfer unit according to Towbin et al. [19] and then immunostained using Collagen-III or MMP-1 mono-specific antibodies (ABC Diagnostics, New Damietta, Egypt). Serum concentrations of Collagen-III and MMP-1 were determined by ELISA [20].

Statistical analyses were carried out by SPSS software (SPSS Inc., Chicago, IL) v.15.0 and GraphPad Prism package v.5.0 (GraphPad Software, San Diego, CA, USA). The correlation was evaluated by Spearman's rank correlation. Significant differences between groups were determined based on Jonckheere–Terpstra test. Continuous variables were expressed as mean ± standard deviation. In this study, the main endpoint was concerned with diagnosing the early stage of HCC according to two common staging systems (CLIP and BCLC). The independent discriminative values of candidate markers were evaluated using the area under the receiver operating characteristic (ROC) curves. Then, a statistical index for HCC early diagnosis was developed using the regression function of the most distinct independent factors. The log transformation of the AFP values was performed to correct the deviation of data. The common indicators of the diagnostic performance of the developed index (sensitivity, specificity) were obtained from a 2 × 2 contingency table.

Results

Comparison of laboratory findings of all included participants was estimated and summarized in Table 1. Patients were predominantly male with a mean age (SD) of 48.5 (0.77) years. As anticipated, patients with HCC were older with higher levels of LFTs than those with F1-F3 and F4. Conversely, patients with HCC showed significantly lower levels of albumin.

Collagen-III and MMP-1 were identified based on SDS-PAGE followed by Western blot with a single immunoreactive band was shown at 70 kDa, and 245 kDa corresponding to Collagen-III and MMP-1, respectively, as previously described [21]. Levels of Collagen-III and its degrading enzyme MMP-1 were quantified in patients' sera and the results are shown in Table 1. As a consequence, our findings demonstrated that patients who had HCC were accompanied by a significant ($P < 0.05$) increase in the concentration of Collagen-III when compared to those with F1-F3 and F4. Interestingly, HCC patients displayed a 2.9-fold and 1.3-fold increase in Collagen-III level over F1-F3 and F4 patients, respectively. On the contrary, patients with HCC were associated with a significantly lower MMP-1 concentration when compared to those without ($P < 0.05$). Our findings showed that patients who developed HCC had MMP-1 concentration of 1.8 and 1.2-times lower than those with F1-F3 and F4, respectively.

Collagen-III/MMP-1 ratio (CMR) values increased significantly in patients who developed HCC *versus* those who with F1-F3 and F4 as shown in Table 1, with HCC patients displaying a 4.9-fold and 1.8-fold increase in CMR level over those who have F1-F3 and F4, respectively. The subsequent step in this work was concerned with enhancing the diagnostic power of CMR to detect both early and advanced stages of liver cancer. Consequently, we combined CMR with other blood markers which reflect alteration in hepatic functions that proved to be valuable in liver disease staging.

Table 1. Patient's characteristics.

Variables	F1-F3 (n = 121)	F4 (n = 133)	HCC (n = 148)	*P value by Jonckheere-Terpstra Test		
				F1-F3 *vs* F4	F1-F3 *vs* HCC	F4 *vs* HCC
Age (years)	41.9 ± 8.9	47.7 ± 9.5	57.5 ± 9.1	<0.001	<0.001	<0.001
ALT (U/L)	54 ± 4	65 ± 8	67 ± 3	0.063	<0.001	0.155
AST (U/L)	55 ± 2	65 ± 8	88 ± 6	0.147	<0.001	0.042
AAR	0.89 ± 0.02	1.11 ± 0.13	1.79 ± 0.14	0.042	<0.001	<0.001
ALP (U/L)	78 ± 4	101 ± 16	161 ± 15	0.118	<0.001	0.031
Albumin (g/L)	43 ± 3	36 ± 2	33 ± 6	<0.001	<0.001	0.051
Bilirubin (μmol/L)	19 ± 5	24 ± 3	50 ± 5	<0.001	<0.001	0.006
Log AFP	0.48 ± 0.03	0.80 ± 0.1	1.73 ± 0.1	0.021	<0.001	<0.001
Collagen III (μg/mL)	10.1 ± 2.6	22.7 ± 4.2	29.5 ± 2.1	<0.001	<0.001	0.007
MMP-1 (μg/mL)	5.6 ± 0.6	3.6 ± 0.8	3.1 ± 0.8	0.003	<0.001	0.042
CMR	5.3 ± 0.4	14.4 ± 3.1	26.7 ± 2.8	<0.001	<0.001	0.002

Data mean ± SD. [a] Reference values: aspartate aminotransferase (AST) <40 U/L; alanine aminotransferase (ALT) <45 U/L; alkaline phosphatase (ALP) 22–92 U/L; albumin 38–54 g/L; Total bilirubin <17.1 μmol/L; α -fetoprotein (AFP) <10 U/L. AAR: AST/ALT ratio; MMP-1: matrix metalloproteinase-1; CMR: collagen III/MMP-1 ratio.

Univariate analysis of all variables tested demonstrated that ALP, AFP, total bilirubin, AST, and AAR were all significantly raised ($P < 0.05$) and thus were identified as predictors of HCC. The area under the ROC curve estimated and compared diagnostic accuracies of individual markers. Based on ROC analysis, CMR was the most efficient for identifying hepatic cancer with an AUC of 0.80, followed by Log AFP (AUC = 0.77) and ALP (AUC = 0.70). Thus, a more sophisticated index for accurate diagnosis of early stage of HCC was developed; HCC-ABC diagnostic Test = 3316 + (0.007 × ALP) + (0.744 × total bilirubin) + (0.037 × CMR) + (2.726 × Log AFP).

The distribution of HCC-ABC diagnostic levels in patients with HCC in relation to those who have F1-F3 and F4 is shown in Table 2. As anticipated, patients who developed HCC were associated with a significant increase ($P < 0.0001$) in the HCC-ABC diagnostic levels when compared to fibrotic of cirrhotic patients. The HCC-ABC diagnostic test correlated significantly with the histological disease progression with a Spearman's rank correlation coefficient of 0.82 ($P < 0.0001$).

HCC patients were then categorized according to two common scoring systems; CLIP system (CLIP 0, 6.1%; CLIP 1, 14.2%; CLIP 2, 30%; CLIP 3, 12.1%; CLIP 4, 18.6%; CLIP 5, 12.9%; CLIP 6, 6.1%) and BCLC system (BCLC A, 22.9%; BCLC B, 24.3%; BCLC C, 26.4%; BCLC D, 26.4%). The distribution of HCC-ABC diagnostic levels in patients with different stages of HCC is shown in Table 2. The diagnostic performances of HCC-ABC diagnostic Test for identifying different categories of HCC compared to AFP were determined and are summarized in Tables 3 and 4. Overall, our developed index yielded an AUC of 0.98 for discriminating HCC patients from those who have liver fibrosis and 0.96 for discriminating HCC patients from cirrhotic patients. Notably, HCC-ABC diagnostic Test has a specificity of 95% for separating liver cirrhotic patients from those with small HCCs (<3 cm). In addition, HCC-ABC diagnostic Test could discriminate patients with early HCC who had CLIP 0–1 and BCLC (0-A) from those who developed cirrhosis with an identical AUC of 0.87 compared to 0.71 and 0.69, respectively, for AFP. The diagnostic value of our developed index was still high providing superior AUCs for distinguishing different stages of HCC according to CLIP and BCLC scoring systems.

A simplifed HCC-ABC diagnostic test metric of sHCC-ABC = ALP × total bilirubin ×CMR × Log AFP had 81% sensitivity and 93% specificity and a superior AUC of 0.92 in discriminating patients who developed HCC. These data are superior to those provided by AFP for identifying early-stages of HCC (CLIP (0–1) and BCLC (0-A)) (Tables 3 and 4).

Table 2. Distribution of HCC-ABC diagnostic test levels in patients with different stages of liver disease.

Categories	Mean ± SD	P value
F1-F3*	5.9 ± 1.2	
F4	7.3 ± 1.3	<0.001
HCC	14.2 ± 4.1	<0.001
CLIP stage		
CLIP 0–1 (early)	11.9 ± 3.0	
CLIP2–3 (intermediate)	13.7 ± 3.5	0.051
CLIP ≥ 4 (advanced)	14.8 ± 4.2	0.046
BCLC stage		
Stage 0-A (early) *	11.4 ± 2.8	
Stage B (intermediate)	13.4 ± 3.8	0.045
Stage C (advanced)	14.3 ± 4.1	0.031
Stage D (end-stage)	19.2 ± 6.1	0.0007

*Reference group.

Discussion

Early diagnosis of HCC is an important factor in initiating potentially curative interventions and so achieve a favourable outcome. However, HCC early detection strategies are ineffective and HCC biomarkers do not provide adequate diagnostic accuracy for early HCC in clinical practice [22]. Therefore, simultaneous detection of HCC markers derived from different cancer pathways is needed which in turn could improve sensitivity even individually or in various combinations [23,24]. It is noteworthy that the extracellular matrix (ECM) is essential for supporting the architecture of the liver and constantly interacts with the environment, allowing cell

Table 3. Diagnostic performances for HCC-ABC test for identifying different stages of HCC according to tumour size and CLIP staging system.

Categories [a]	AUC (95% CI), *P* value*	Sn (%)	Sp (%)	OR (95% CI), *P* value*
F4 (n = 133) *vs* HCC (n = 148)				
AFP≥ 400 UL^{-1}	0.70 (0.60–0.78), 0.037	40.0	100	1.4 (1.3–1.6), 0.056
HCC-ABC ≥ 8.5	0.96 (0.93–0.99), <0.001	89.9	95.0	26.8 (3.3–56.8), <0.001
Simplified HCC-ABC ≥ 800	0.92 (0.85–0.97), <0.001	81.0	93.0	11.9 (2.2–40.7), 0.002
Tumour size				
<3 cm (n = 78)				
AFP ≥ 400 UL^{-1}	0.72 (0.54–0.84), 0.058	36.0	100	1.48 (1.3–1.7), 0.052
HCC-ABC ≥ 8.5	0.85 (0.79–0.98), 0.013	85.7	95.0	18.0 (3.8–39.9), 0.014
Simplified HCC-ABC ≥ 800	0.83 (0.74–0.97), <0.001	79.7	93.3	13.3 (2.2–28.8), 0.021
≥3 cm (n = 70)				
AFP ≥ 400 UL^{-1}	0.76 (0.62–0.86), 0.008	42.3	100	1.6 (1.2–2.1), 0.030
HCC-ABC ≥ 8.5	0.94 (0.82–1.00), <0.001	94.6	95	35.0 (4.2–67.5), <0.001
Simplified HCC-ABC ≥ 800	0.92 (0.84–0.98), <0.001	87.5	93.3	14.6 (3.5–60.4), <0.001
CLIP stage				
CLIP 0–1 (early) (n = 30)				
AFP ≥ 400 UL^{-1}	0.71 (0.55–0.79), 0.061	34.5	100	1.33 (1.1–1.6), 0.101
HCC-ABC ≥ 8.5	0.87 (0.75–0.99), <0.001	88.0	95.0	19.6 (4.2–33.5), <0.001
Simplified HCC-ABC ≥ 800	0.87 (0.79–0.98), <0.001	80.3	93.3	15.3 (3.8–29.9), <0.001
CLIP2–3 (intermediate) (n = 62)				
AFP ≥ 400 UL^{-1}	0.76 (0.64–0.82), 0.012	41.4	100	1.7 (1.3–2.2), 0.036
HCC-ABC ≥ 8.5	0.98 (0.95–1.00), <0.001	92.0	95.0	24.0 (4.5–73.6), <0.001
Simplified HCC-ABC ≥ 800	0.92 (0.84–1.00), <0.001	81.5	93.3	17.6 (3.5–62.7), <0.001
CLIP ≥ 4 (advanced) (n = 56)				
AFP ≥ 400 UL^{-1}	0.78 (0.67–0.85), 0.005	40.0	100	1.7 (1.3–2.4), 0.012
HCC-ABC ≥ 8.5	0.97 (0.92–1.00), <0.001	93.0	95.0	33.1 (5.5–97.3), <0.001
Simplified HCC-ABC ≥ 800	0.90 (0.82–0.99), <0.001	79.0	93.3	14.0 (3.2–59.5), <0.001

[a]**Abbreviations**: AFP: α – fetoprotein; AUC: area under the curve; Sn: sensitivity; Sp: specificity; CI: confidence interval; OR: odds ratio. AUC was generated by comparing HCC patients to liver cirrhotic patients.

Table 4. Diagnostic performances for HCC-ABC test for identifying different stages of HCC using BCLC stagsing system.

Categories [a]	AUC (95% CI); P value*	Sn (%)	Sp (%)	OR (95% CI); P value*
BCLC stage				
Stage A (early) (n = 34)				
AFP ≥ 400 UL^{-1}	0.69 (0.51–0.78), 0.099	30.4	100	1.4 (1.067–1.84), 0.073
HCC-ABC ≥ 8.5	0.87 (0.74–0.93), <0.001	70.0	95.0	24.0 (4.2–59.3), <0.001
Simplified HCC-ABC ≥ 800	0.82 (0.77–97.0), 0.001	77.8	93.3	19.6 (2.7–39.9, 0.009
Stage B (intermediate) (n = 36)				
AFP ≥ 400 UL^{-1}	0.73 (0.61–0.80), 0.046	33.3	100	1.4 (1.09–1.9), 0.031
HCC-ABC ≥ 8.5	0.95 (0.88–1.00), <0.001	89.0	95.0	26.0 (4.4–75.5), <0.001
Simplified HCC-ABC ≥ 800	0.95 (0.88–1.00), <0.001	87.0	93.3	25.0 (3.9–72.4), <0.001
Stage C (advanced) (n = 39)				
AFP ≥ 400 UL^{-1}	0.77 (0.70–0.81), 0.031	37.5	100	1.6 (1.2–2.2), 0.030
HCC-ABC ≥ 8.5	0.96 (0.91–1.00), <0.001	97.0	95.0	28.8 (4.9–79.1), <0.001
Simplified HCC-ABC ≥ 800	0.91 (0.81–1.00), <0.001	78.0	93.3	15.0 (2.8–80.4), <0.001
Stage D (end-stage) (n = 39)				
AFP ≥ 400 UL^{-1}	0.79 (0.64–0.85), 0.019	45.5	100	1.8 (1.3–2.7), 0.023
HCC-ABC ≥ 8.5	0.99 (0.97–1.0), <0.001	100	95.0	30.0 (5.2–88.5), <0.001
Simplified HCC-ABC ≥ 800	0.99 (0.95–1.00), <0.001	100	93.3	26.0 (3.7–82.4), <0.001

[a]**Abbreviations**: AFP: α-fetoprotein; AUC: area under the curve; Sn: sensitivity; Sp: specificity; CI: confidence interval; OR: odds ratio. AUC was generated by comparing HCC patients to liver cirrhotic patients.

adhesion, growth and migration [25]. Moreover, many ECM components including collagen are responsible for promoting the expression of specific liver functions and cell differentiation [26]. ECM remodelling and collagen turnover are regulated by various MMPs and their inhibitors including the tissue inhibitors of metalloproteinases (TIMPs). Therefore, this work was aimed to assess the clinical significance of Collagen-III and its degrading enzyme MMP-1 simultaneously in HCC early diagnosis.

Our results showed that patients who had HCC were accompanied by a higher concentration of collagen-III than fibrotic and cirrhotic patients. Conversely, patients with HCC showed a significantly lower concentration of MMP-1 than those without. This may be explained by the fact that chronic liver damage leads to pathological accumulation of ECM proteins. In disease, the activity of the ECM remodelling enzymes is deregulated, leading to a fibrotic microenvironment characterized by increased stiffness and abundance of growth factors that contribute to tumorgenesis [25]. In liver fibrosis, changes in ECM composition are driven by hepatic stellate cells (HSCs), which activated and trans-differentiated into proliferative myofibroblast cells after exposure to inflammatory indications [27]. Once activated, HSCs up-regulate gene expression of ECM components, matrix-

degrading enzymes, and their respective inhibitors, which in turn results in an excessive ECM accumulation at the sites containing high densities of activated HSCs [27]. It has been reported that liver fibrosis is characterized with excess collagen synthesis with a reduced collagen turnover, while cirrhotic liver may contain up to six times as much collagen as a healthy liver with types I and III collagen being the most abundant [28].

Deregulation of collagen cross-linking and ECM stiffness plays a causative role in the pathogenesis of cancer by enhancing integrin signalling [29]. Deregulation of ECM homoeostasis directly affects epithelial cells and leads to cellular transformation and metastasis [30]. Tumour growth requires the breakdown of pre-existing boundaries and rearrangement of liver tissue, a process mainly regulated by MMPs and TIMPs. Overexpression of MMPs can compromise the basement membrane barrier and facilitate tissue invasion by cancer cells.

Indeed, biomarkers that distinguish HCC from inflammation and cirrhosis are needed in order to help improve the prognosis of these patients [5]. Thus, the Collagen-III/MMP-1 ratio was created yielding values that increased significantly in patients who developed HCC than those who have F1-F3 and F4. Hence, the overlap in Collagen-III and MMP-1 among patients with F4 and HCC has been reduced and subsequently, the difference in their values has been amplified. Of course, the aforementioned findings could provide important clues for the possibility of using CMR in diagnosing patients with HCC. Indeed, the proper definition of early HCC is of a clinical importance before a breakthrough appears on HCC surveillance and early intervention. The HCC-ABC diagnostic Test composed of Collagen-III and MMP-1 together with three routine laboratory tests (ALP, AFP and total bilirubin) was then developed. Indeed, combining markers in a single predictive function would exaggerate the effects of these variables and subsequently reduce the overlap in values among patient groups. The HCC-ABC diagnostic Test has a higher AUC and very good sensitivity specificity for HCC detection from nonmalignant liver cirrhosis and enables the correct identification of HCC patients who have tumour size <3 cm, CLIP (0–1) and BCLC (0-A), being superior to that of AFP. The HCC-ABC diagnostic Test showed a higher sensitivity than other HCC diagnostic approaches including ultrasound that exhibit a sensitivity ranging from 60% to 80%, CT (72%) and MRI (79%) for HCC detection [31]. Moreover, the HCC-ABC diagnostic Test outperformed or was comparable to other HCC early detection biomarkers. For example, the combined use of osteopontin and AFP yielded a sensitivity of 83% for early HCC [32], whilst serum midkine showed a sensitivity of 80% in distinguishing early-stage compared with AFP (40%) [33]. Serum Dickkopf-1 (DKK1) was employed to detect HCC, especially early-stage disease. Measurement of DKK1 and AFP together showed a lower sensitivity of 87.5% when compared to HCC-ABC diagnostic test [34]. Further multicenter prospective studies are needed to validate the usefulness of the HCC-ABC index in clinical practice.

In conclusion, this work represents an advance in biomedical science because it provides a promising index for HCC early detection with a high degree of accuracy that may facilitate definitive therapy.

Disclosure statement

No potential conflict of interest was reported by the authors.

Summary table

What is known about this subject:

- HCC early detection is an important factor in initiating potentially curative interventions and achieve a favourable outcome.
- Existing HCC strategies and biomarkers do not provide adequate diagnostic accuracy for early-stage HCC in clinical practice.

What this paper adds:

- The HCC-ABC diagnostic test is a promising index for HCC early detection with a high degree of accuracy that may facilitate therapy
- HCC-ABC diagnostic test identified early HCC [CLIP 0-1 and BCLC (0-A)] from F4 with an AUC of 0.87

References

[1] Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018;391:1301–1314.
[2] Danila M, Sporea I. Ultrasound screening for hepatocellular carcinoma in patients with advanced liver fibrosis. An overview. Med Ultrason. 2014;16:139–144.
[3] El-Serag HB. Hepatocellular carcinoma. New Engl J Med. 2011;365:1118–1127.
[4] Rich NE, Yopp AC, Singal AG. Medical management of hepatocellular carcinoma. J Oncol Pract. 2017;13:356–364.
[5] Tsuchiya N, Sawada Y, Endo I, et al. Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2015;21:10573.
[6] Aghoram R, Cai P, Dickinson JA. Alpha-foetoprotein and/or liver ultrasonography for screening of hepatocellular carcinoma in patients with chronic hepatitis B. Cochrane Database Syst Rev. 2012;9:CD002799.
[7] Lou J, Zhang L, Lv S, et al. Biomarkers for hepatocellular carcinoma. Biomark Cancer. 2017;9:1–9.
[8] Sherman M. Current status of alpha-fetoprotein testing. Gastroenterol Hepatol. 2011;7:113–114.
[9] Giannini EG, Marenco S, Borgonovo G, et al. Alpha-fetoprotein has no prognostic role in small hepatocellular carcinoma identified during surveillance in compensated cirrhosis. Hepatol. 2012;56:1371–1379.
[10] Gupta S, Bent S, Kohlwes J. Test characteristics of α-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C: a systematic review and critical analysis. Ann Intern Med. 2003;139:46–50.
[11] Soresi M, Terranova A, Licata A, et al. Surveillance program for diagnosis of HCC in liver cirrhosis: role of ultrasound echo patterns. Biomed Res Int. 2017;2017:4932759.

[12] Walker C, Mojares E, Del Río Hernández A. Role of extracellular matrix in development and cancer progression. Int J Mol Sci. 2018;19:3028.
[13] Di Lullo GA, Sweeney SM, Körkkö J, et al. Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. J Biol Chem. 2002;277:4223–4231.
[14] Alcolado R, Arthur M, Iredale J. Pathogenesis of liver fibrosis. Clin Sci. 1997;92:103–112.
[15] Pererrone F, Daniele B, Gaeta GB, et al. Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatol. 2000;31:840–845.
[16] Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19:329–338.
[17] Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nat Rev Gastroenterol Hepatol. 2010;7:448–458.
[18] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet. 1997;349:825–832.
[19] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci. 1979;76:4350–4354.
[20] Attallah AM, El-Far M, Ghaly MF, et al. Circulating levels of Collagen-III and MMP-1 in patients with chronic hepatitis C co-infected with hepatitis B virus. Br J Biomed Sci. 2017;74:95–100.
[21] Attallah AM, El-Far M, Abdel Malak CA, et al. Fibrocheck: a combination of direct and indirect markers for liver fibrosis staging in chronic hepatitis C patients. Ann Hepatol. 2015;14:225–233.
[22] Sengupta S, Parikh ND. Biomarker development for hepatocellular carcinoma early detection: current and future perspectives. Hepatol Oncol. 2017;4:111–122.
[23] Dimitroulis D, Damaskos C, Valsami S, et al. From diagnosis to treatment of hepatocellular carcinoma: an epidemic problem for both developed and developing world. World J Gastroenterol. 2017;23:5282.
[24] Wang J, Jain S, Chen D, et al. Development and evaluation of novel statistical methods in urine biomarker-based hepatocellular carcinoma screening. Sci Rep. 2018;8:3799.
[25] Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. 2012;196:395–406.
[26] Baiocchini A, Montaldo C, Conigliaro A, et al. Extracellular matrix molecular remodeling in human liver fibrosis evolution. PloS One. 2016;11:e0151736–e0151736.
[27] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterol. 2008;134:1655–1669.
[28] Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol. 2008;214:199–210.
[29] Schrader J, Gordon-Walker TT, Aucott RL, et al. Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells. Hepatol. 2011;53:1192–1205.
[30] Hernandez–Gea V, Toffanin S, Friedman SL, et al. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. Gastroenterol. 2013;144:512–527.
[31] Schraml C, Kaufmann S, Rempp H, et al. Imaging of HCC-current state of the art. Diagnostics. 2015;5:513–545.
[32] Shang S, Plymoth A, Ge S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. Hepatology. 2012;55:483–490.
[33] Zhu WW, Guo JJ, Guo L, et al. Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma. Clin Cancer Res. 2013;19:3944–3954.
[34] Shen Q, Fan J, Yang XR, et al. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol. 2012;13:817–826.