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Interrelations between elevated alpha-fetoprotein levels and tumor morphology of patients with hepatocellular carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer related deaths worldwide, while at the same time having a constant growth in incidence. A commonly used biomarker in managing liver cancer cases, alpha-fetoprotein (AFP) is losing clinical ground in favor of imaging studies and emerging biomarkers. The study aims to reassess potential prognosis indicators and risk factors for an elevated level of this glycoprotein by analyzing its relationship with macroscopic morphology tumor-related features. *Patients, Materials and Methods*: One hundred and thirty-one newly diagnosed HCC patients had their clinical, tumor and liver disease features investigated in contrast to elevated AFP levels with 200 IU/mL being used as preferred cut-off. *Results*: Tumor size ≥5 cm [odds ratio (OR) 3.36, 95% confidence interval (CI): 1.29–8.74, p=0.013] is an independent tumor-related predictor of markedly elevated AFP values. Noteworthy connections with the type of tumor, multinodular appearance and portal vein thrombosis were also found through univariate analysis. *Conclusions*: AFP could still be a reliable tool in diagnosis and prognosis of HCC patients especially in developing countries due to its relevant association with aspects of advanced tumor and liver disease, gender and a poor functional status.

Keywords: biomarkers, cancer, liver neoplasms.

Introduction

Hepatocellular carcinoma (HCC) accounts for up to 90% of primary liver cancer cases, being one of the deadliest types of malignant tumors, ranking 2nd in cancer-related deaths in men and 6th in women [1]. The aggressiveness of this tumor is justified by the increasing trend in incidence, while the survival rate has remained relatively constant over the past 15 years despite newer treatment options [2, 3].

Alpha-fetoprotein (AFP) has been the standard tumor biomarker for HCC for many decades, being useful in terms of diagnosis, monitoring and tumor recurrence [4]. Nowadays, new challenges emerge that threaten its clinical existence, by being replaced with newer, more sensitive and specific serological biomarkers. Furthermore, *American Association for the Study of Liver Diseases* (AASLD) and *European Association for the Study of the Liver* (EASL) do not rely anymore on this tool in HCC screening and diagnosis due to limited sensitivity and specificity [5, 6]. However, developing and newly industrialized countries, which hold the majority of HCC cases worldwide, are still dependent on the combination between serum AFP and tumor imaging techniques due to cost-effectiveness and logistical reasons, being cheaper and easier to perform [7]. Recently, growing support for this biomarker led to a change in the *AASLD* perspective, returning serum AFP in the screening program as an adjunct to ultrasound (US) examination [8]. While the data about the prognostic usefulness is still unclear, debatable and variable among study groups, more insight about the relationship between AFP and prognostic and predictive factors is required in order for this classical biomarker to maintain its clinical relevance. Potential findings might be especially important for Romania, a developing country with a high incidence of HCC that also clusters many risk factors for developing this disease, such as viral hepatitis (highest proportion of hepatitis B (HEP B) virus cases and 2nd highest for hepatitis C (HEP C) virus in the general population in the European Union) [9] and alcohol-related liver disease (2nd biggest consumer of alcohol in the European Union) [10]. Unfortunately, many hospitalized patients already reached an advanced stage of HCC, which further alters their survival rate.

The aim of this study was to analyze the relationship between serum AFP levels and certain tumor-related findings that could be regarded as prognostic indicators for HCC including the most used European and American staging systems [11], so that the clinician could use such 'bedside' markers to ease risk stratification and management of HCC diagnosed patients.

Patients, Materials and Methods

Patients

The study included data of patients managed in a tertiary center – the Institute of Gastroenterology and Hepatology of Iaşi, Romania. One hundred and eighty-four consecutive patients diagnosed with HCC between January 2016 and December 2017 were initially included in the study. Cases were further excluded due to incomplete

data files (24), older treated/recurrent cases (22) and on account of initial misdiagnosis with benign or other malignant liver tumors (7). Hence, 131 newly diagnosed patients with HCC were enrolled in the study (Figure 1). HCC diagnosis was made by following the *National Comprehensive Cancer Network* (NCCN) guidelines, evidence blocks for hepatobiliary cancers non-histopathology pathway published in 2016. HCC was therefore confirmed in at-risk patients with elevated AFP levels and a hepatic mass identified on imaging studies.

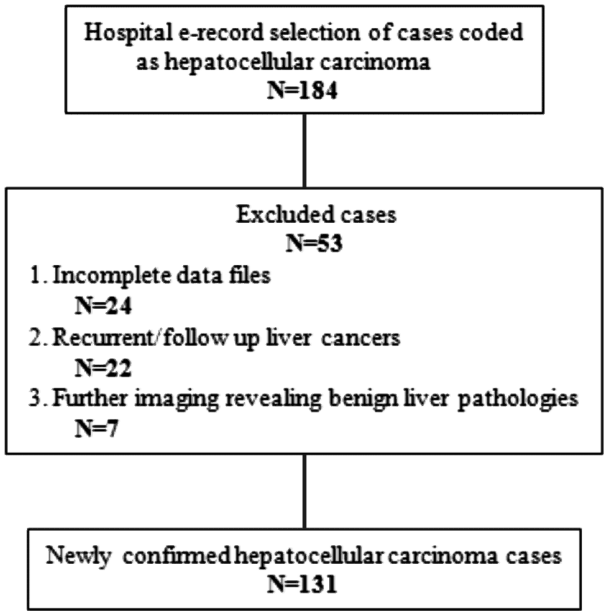

Figure 1 – ***Study flow diagram.***

Methods

Chemiluminescence enzyme immunoassay (CLEIA) was the method used to determine AFP levels, with values ranging from 0.5 to >300 IU/mL. Patients were initially divided based on a 20 and 200 IU/mL cut-off level. The latter value was finally selected to separate patients into two subgroups (normal cases and moderate elevations *vs.* markedly raised AFP levels).

Laboratory parameters taken in to account beside AFP levels were liver function tests, such as alanine transaminase (ALT), aspartate transaminase (AST), albumin, bilirubin, gamma-glutamyl transferase (GGT), alkaline phosphatase (APL) and platelet levels. Viral infection was confirmed based on the positivity of surface antigen of the hepatitis B virus (HBsAg) or anti-hepatitis C virus (anti-HCV) antibodies and viral load. The alcoholic etiology of HCC was determined according to a history of alcohol abuse (>3/4 units per day) and a suggestive biochemical profile [12]. Liver US, computed tomography (CT) or magnetic resonance imaging (MRI) investigations were commenced as part of diagnosis algorithm, as well as to assess tumor features (size, number of nodules, macroscopic aspect, portal vein thrombosis, lymph node involvement, extrahepatic metastasis). All patients were initially assessed with a liver US on presentation. A follow-up CT or MRI scan were further issued either to determine tumor extension or to confirm HCC, where US was not conclusive. Liver metastases were ruled out based on their specific appearance on CT/MRI (doughnut-like ring enhancement). Likewise, hypervascular metastases were differentiated from HCC based upon confirmation of a primary tumor (*e.g.*, neuroendocrine tumors, breast, renal cell carcinoma) alongside a non-cirrhotic liver. Tumor size was reported as the value of the longest measurable axis of the largest nodule (if multinodular mass present). From a macroscopic point of view, as seen on imaging tests, tumor morphology was described as either single nodular (Figure 2), multifocal (Figure 3) or diffusely infiltrative/invasive (Figure 4). Imaging studies also revealed the presence of cirrhosis along with signs of hepatic decompensation (ascites and esophageal varices). Additionally, patients were clinically checked for encephalopathy and their functional status was assessed by using the *Eastern Cooperative Oncology Group* (ECOG) performance score [13]. The Child–Pugh score was used to appraise the severity of the liver disease and liver cancer was further staged based on the Tumor-Node-Metastasis (TNM) (*American Joint Committee on Cancer* 2010) and *Barcelona Clinic Liver Cancer* (BCLC) Classifications.

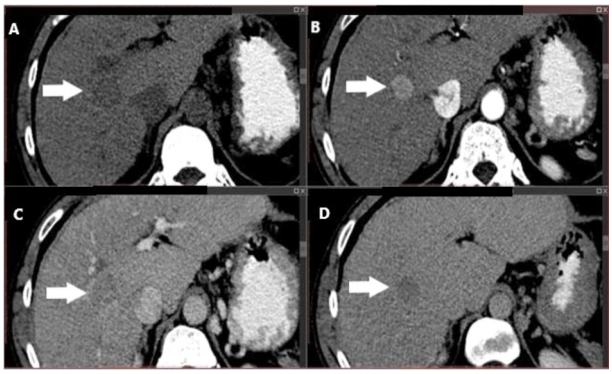

Figure 2 – ***62-year-old man with unifocal HCC: (A) Native, non-enhanced CT; (B) Arterial enhancing nodule; (C) Portal venous phase; (D) Washout of contrast seen in the delayed venous phase. HCC: Hepatocellular carcinoma; CT: Computed tomography.***

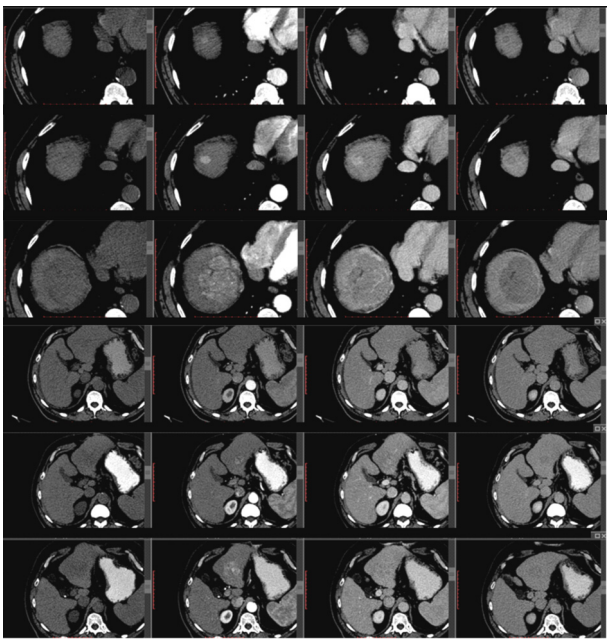

Figure 3 – ***68-year-old man with multifocal HCC involving both right and left lobes of the liver, seen through successive CT phases. HCC: Hepatocellular carcinoma; CT: Computed tomography.***

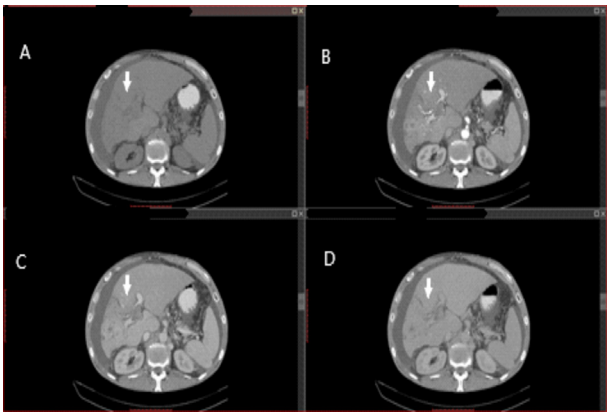

Figure 4 – ***80-year-old man with infiltrative HCC, subsequent portal vein thrombosis and ascites in non-enhancing (A), arterial (B), venous (C) and delayed phase (D) of CT abdomen with contrast. HCC: Hepato-cellular carcinoma; CT: Computed tomography.***

Statistical analysis

Numerical outcomes were reported as mean and standard deviation, while nominal data were expressed as frequencies. χ^2 (*Chi*-square) test was used to compare categorical variables with convenient use of Fisher's exact test or likelihood ratio. Continuous variables, on the other hand, were assessed with the Mann–Whitney *U*-test. Presence and strength of associations between AFP levels and prognosis factors were determined through univariate analysis. Significant variables were consequently introduced in a multivariate model and analyzed *via* binary logistic regression. *P*-values of <0.05 (two-tailed) were considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 20.0 software (IBM Corp. Released 2011; IBM SPSS Statistics for Windows, Version 20.0; Armonk, NY: IBM Corp.).

Results

The baseline characteristics of the 131 patients included in the study are shown in Table 1. The majority of patients were men (72%), with a male to female ratio of 2.5:1. Mean age at diagnosis was 64 years old, with most of the patients (53.4%) coming from urban residential areas. Regarding etiology, liver cirrhosis was prevalent in 76.5% of the patients and chronic hepatitis C was found at the origin of HCC in 55% of the cases. Alcohol-related liver disease was the second most common etiologic factor seen in 47% of patients, closely followed by infection with type B hepatitis virus (43%). In terms of morphological appearance, 59 patients displayed a unifocal tumor archetype (Figure 2) however, in most of cases (65) the tumor was reported as multifocal (Figure 3). Only seven patients displayed an aggressive, infiltrating tumor pattern on imaging studies (Figure 4). AFP levels were markedly elevated (>200 IU/mL) in 43 (33%) patients, however, most of the cases (39%) had normal/lower AFP values (<20 IU/mL).

Comparison of liver and tumor related parameters with markedly elevated AFP levels is reported in Tables 2 and 3.

Table 1 – ***Baseline characteristics of patients included in the study***

Age [years]	64.8±9.5
Gender (M/F) [%]	72/28
Social background (Urban/Rural) [%]	53/47
Etiology (HCV/HBV/Alcohol/Other) [%]	55/43/47/12
Cirrhosis, *n* (%)	101 (76.5%)
AFP [IU/mL]:	
▪ <20, *n* (%)	51 (39%)
▪ 20–200, *n* (%)	37 (28%)
▪ >200, *n* (%)	43 (33%)
Albumin [g/dL]	3.2±0.83
Bilirubin [mg/dL]	2.54±3.1
ALT [IU/L]	112±85
AST [IU/L]	79±59
GGT [IU/L]	220±233
ALP [IU/L]	164±147
Platelets [$\times 10^3$/μL]	151±91
Encephalopathy, *n* (%)	18 (13.7%)
Esophageal varices, *n* (%)	58 (44%)
Ascites, *n* (%)	64 (49%)
Child–Pugh score (A/B/C), *n*	62/42/27
Maximum tumor size [mm]	51±30
No. of nodules (<3/>3), *n*	81/50
Type (Unifocal/Multifocal/Diffuse), *n*	59/65/7
Portal vein thrombosis, *n* (%)	40 (30.5%)
Lymph node invasion, *n* (%)	41 (31%)
Extrahepatic metastasis, *n* (%)	12 (9%)
TNM stage (I/II/III/IV), *n*	35/15/32/49
BCLC stage (0/A/B/C/D), *n*	1/29/8/56/27
Performance score (0/1/2/3/4), *n*	55/42/8/15/11

M: Male; F: Female; HCV: Hepatitis C virus; HBV: Hepatitis B virus; AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase; TNM: Tumor-node-metastasis; BCLC: *Barcelona-Clinic Liver Cancer Group*.

Table 2 – ***Tumor morphology prognostic indicators according to serum AFP levels***

Variables	AFP		OR (95% CI)	*P* value
	<200 [IU/mL] n=88	*>200 [IU/mL] n=43*		
Tumor size ≥5 cm, *n* (%)	26 (30%)	29 (67.5%)	4.94 (2.25–10.8)	.000
≥3 nodules, *n* (%)	26 (30%)	24 (56%)	3.01 (1.14–6.41)	.004
≥2 nodules, *n* (%)	41 (46.6%)	25 (58%)	1.59 (0.76–3.32)	.214
Unifocal, *n* (%)	42 (47.7%)	17 (39.5%)	0.71 (0.34–1.5)	.377
Multifocal, *n* (%)	45 (51%)	20 (46.5%)	0.83 (0.4–1.72)	.619
Portal vein thrombosis, *n* (%)	19 (21.6%)	21 (49%)	3.46 (1.58–7.59)	.001
Lymph node invasion, *n* (%)	28 (32%)	13 (30%)	0.92 (0.42–2.04)	.854
Extrahepatic metastasis, *n* (%)	8 (9%)	4 (9%)	1.02 (0.29–3.61)	.969

AFP: Alpha-fetoprotein; OR: Odds ratio; CI: Confidence interval.

Patients with higher AFP levels had larger tumors (18 *vs.* 52% ≥5 cm in size, $p<0.001$), more nodules (23.5% *vs.* 48%, ≥3 nodules, $p=0.004$) and portal vein thromboses (24.2 *vs.* 52.5%, $p=0.001$). Discussing further about tumor morphology, there was no relationship between increased

AFP secretion and unifocal or multifocal HCC (p=0.37 and p=0.61, respectively). Similarly, despite including binodular HCC cases as part of the higher AFP group (46 *vs.* 58%), the given association was not deemed significant (p=0.21). Concerning signs of tumor progression, no relationship was found between markedly elevated AFP levels and patients identified with lymph node invasion or extra-hepatic metastasis (p=0.85 and p=0.96, respectively).

Table 3 – ***Additional prognostic indicators according to serum AFP levels***

Variables	AFP		OR (95% CI)	*P* value
	<200 [IU/mL] n=88	*>200 [IU/mL] n=43*		
Age [years]	65.3±9	63.7±9	0.98 (0.94–1.02)	.373
Gender (M/F), *n*	69/19	25/18	2.61 (1.18–5.76)	.016
HBV infection, *n* (%)	17 (19.3%)	16 (37.2%)	2.47 (1.09–5.58)	.027
HCV infection, *n* (%)	48 (54.5%)	24 (56%)	1.05 (0.5–2.19)	.891
Alcoholic liver disease, *n* (%)	23 (26%)	13 (30%)	1.22 (0.54–2.74)	.622
Cirrhosis, *n* (%)	64 (72.7%)	37 (86%)	2.31 (0.86–6.17)	.088
Albumin [g/dL]	3.38 ±0.8	2.85 ±0.7	0.43 (0.26–0.71)	.001
Bilirubin [mg/dL]	2.24 ±2.9	3.15 ±3.4	1.09 (0.97–1.22)	.006
ALT [IU/L]	97.5 ±69	141.7 ±106	1 (1–1.01)	.005
AST [IU/L]	77.4 ±63	82.8 ±50	1 (0.99–1)	.236
GGT [IU/L]	208.9 ±229	243 ±244	1 (0.99–1)	.378
ALP [IU/L]	146 ±108	200.7 ±200	1 (1–1)	.17
Platelets [×10^3/μL]	146.4 ±95	160.6 ±82	1 (0.99–1)	.114
Encephalopathy, *n* (%)	11 (12.5%)	7 (16.3%)	1.36 (0.48–3.8)	.555
Esophageal varices, *n* (%)	40 (45.5%)	18 (42%)	0.86 (0.41–1.8)	.697
Ascites, *n* (%)	34 (38.6%)	30 (70%)	3.66 (1.68–7.99)	.001
Child–Pugh score C, *n* (%)	12 (13.6%)	15 (35%)	3.39 (1.41–8.13)	.005
PS ≥1, *n* (%)	41 (46.6%)	35 (81.4%)	5.02 (2.09–12)	.000
TNM staging (I/II/III/IV), *n*	30/12/13/33	5/3/19/16	–	.001
BCLC staging (0/A/B/C/D), *n*	1/26/14/33/14	0/3/4/23/13	–	.007

AFP: Alpha-fetoprotein; OR: Odds ratio; CI: Confidence interval; M: Male; F: Female; HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase; PS: Performance score; TNM: Tumor-node-metastasis; BCLC: *Barcelona-Clinic Liver Cancer Group*.

AFP levels >200 IU/mL were associated with higher ALT (97.5 *vs.* 141.7 IU, p=0.05) and bilirubin levels (2.24 *vs.* 3.15, p=0.06) and lower albumin values (2.85 *vs.* 3.38, p=0.01). Female gender also played a role in the elevation of this biomarker (21.6% *vs.* 41.9%, p=0.16) along with HBsAg-positive cases (19.3% *vs.* 37.2%, p=0.27). Among clinical signs of hepatic decompensation, presence of ascites was significantly associated with higher AFP values (19.5% *vs.* 47%). Similarly, Child–Pugh score C was more prevalent in the latter group (27 *vs.* 55.5%, p=0.05). Both TNM and BCLC staging systems were significantly related to AFP levels therefore, a greater proportion of patients within the markedly elevated AFP group had advanced/terminal liver cancer when compared to cases with normal/moderate elevations (52.5% *vs.* 81%, with TNM stages III–IV and 53.6% *vs.* 83.7%, with BCLC stages C–D, p<0.05). BCLC was chosen from all European HCC stages because it is considered superior in determining prognosis [14, 15]. Additionally, patients considered fully active, without restrictions in physical labor (Grade 0 *ECOG*) were less common in the group with higher AFP levels (53.4 *vs.* 18.6%, p<0.001).

The results of the multivariate regression analysis (Table 4), which incorporated the significant factors mentioned above, showed that tumor size >5 cm [odds ratio (OR) 3.36, 95% confidence interval (CI): 1.29–8.74, p=0.013], female gender (OR 4.4, 95% CI: 1.57–12.3, p=0.005) and chronic hepatitis B infection (OR 2.78, 95% CI: 1–7.71, p=0.049) are independent predictors of markedly elevated AFP levels (>200 IU/mL).

Table 4 – ***Independent predictors of serum AFP levels >200 IU/mL***

Variable	OR (95% CI)	*P* value
Female gender	4.4 (1.57–12.3)	.005
HBV infection	2.78 (1–7.71)	.049
Albumin	1.54 (0.65–3.65)	.324
ALT	0.99 (0.98–1)	.132
Bilirubin	1.01 (0.84–1.21)	.814
Ascites	2.27 (0.73–7.05)	.154
Child–Pugh score C	0.82 (0.13–4.95)	.834
Tumor size ≥5 cm	3.36 (1.29–8.74)	.013
≥3 nodules	1.6 (0.6–4.25)	.347
Portal vein thrombosis	1.23 (0.36–4.22)	.733
PS ≥1	1.26 (0.34–4.65)	.728

AFP: Alpha-fetoprotein; OR: Odds ratio; CI: Confidence interval; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; PS: Performance score.

Discussions

Among features of advanced liver cancer, tumor size was found to be one of the most significant predictors of elevated AFP levels. While some studies either denied [16] or found limited relationships between these variables [4], most published data about this topic seem to tilt the balance in favor of this molecule as a marker of tumor progression [17–21]. The cut-off point for tumor size used in this study was 5 cm, division consistent with previous studies assessing prognosis implication [22–24]. Multi-nodular and diffuse aspects of tumor, vascular invasion and portal vein thrombosis were also found to be associated with increased AFP levels reflecting the ability of this biomarker to discriminate more aggressive cases of HCC from early stages. Same implication can be seen with regards to HCC staging systems. Albeit limited in assessing prognosis, the TNM staging system still issues a significant relationship with AFP levels >200 IU/mL. Most of the significance however, comes from intrinsic association with T variable, which essentially reflects tumor morphology since is mostly size dependent.

Hepatitis B infection is known to be at the origin of HCC in up to 85% of cases in endemic regions [25]. However, chronic HBV infection was only the 3rd most frequent etiology for liver cancer in our study after alcohol and chronic HCV infection, which resemble a pattern associated with HCC in developed areas [26]. Nonetheless, it has been shown that hepatitis B virus infection can independently predict higher AFP levels in our study and a few others [17, 27, 28]. Underlying chronic hepatocyte inflammation and deoxyribonucleic acid (DNA) mutagenesis might explain this finding since these mechanisms contribute to necrosis and regeneration processes [29]. These findings along with input from recent published data suggest that AFP is still a reliable tool in diagnosis and prognosis of HCC patients with adjacent HBV chronic infection [30].

Female gender is considered a favorable prognosis factor due to a higher survival rate and features associated with early disease [31]. On the other hand, it has also been shown to elevate AFP levels, fact that might suggest an implication of sexual hormones in AFP synthesis to a certain extent. Given the menopausal status of women included in the study group, chances of direct hormonal involvement in AFP production are unlikely, unless patients were subjected to hormone replacement therapy. This parameter along with serum hormone panel was not assessed in our study. However, it has been noted that menopause is associated with a greater degree of necro-inflammatory activity mediated by tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) cytokines, the latter being involved in severe fibrotic changes. The intensity of the inflammatory processes influenced by these molecules was found to be especially higher in HCV infection [32]. Our study confirms that female gender is significantly associated with anti-HCV positivity and might explain the nature of elevated AFP-levels through the previous mentioned pathway (crossover with necro-inflammatory process). The associations between female gender and AFP levels at different cut-offs were not significant for values less than 100 IU/mL (*e.g.*, 20 IU/mL, 50 IU/mL). This might prove that female gender accounts only for moderate to considerably higher AFP elevations although sample size might play a part in this finding as well. Moreover, a recent large-scale study has found that the relationship persists with each degree of AFP elevation. Regardless, more research is required in order to find common ground regarding cut-off values (gender related) and the overall hormone influence on AFP synthesis in women suffering or being at-risk for HCC.

As part of the *British Society of Gastroenterology* (BSG), AFP plays a vital role in the active surveillance for liver cancer in combination with abdominal US, both performed at six months. A raised AFP value in a patient with pre-existing cirrhosis and a liver mass >2 cm confirms the diagnosis of HCC and other investigations are needed only to assess for further treatment. Furthermore, a high AFP would confirm the HCC diagnosis even in patients with a newly identified liver mass that is not known with cirrhosis, as long as a primary testicular tumor is excluded [33].

Recommended by both *EASL* and *AASLD* as a standard guide for HCC management, the BCLC classification is known to possess a higher prognosis value over most staging systems. In the given study, there was a significant relationship between BCLC and higher AFP levels that can be explained by the underlying connection with parameters included in the classification. These include features of advanced liver cancer (tumor size, number of nodules, portal invasion, performance status) and adjacent liver disease (Child–Pugh score). The latter, especially stage C, is also associated with markedly elevated AFP levels through destruction of liver tissue that releases more bilirubin and decreases the amount of albumin in the circulation. The pressure build-up in the portal circulation further leads to development of ascites, the only clinical sign of hepatic decompensation related to AFP secretion found in our study. Performance status was the other clinical element with a relevant association that could be explained by the underlying liver disease and tumor progression, which gradually decreases the functional status of the patient. Similarly, a PS >1 has been described as an independent factor for overall survival in HCC patients with associated liver cirrhosis [34]. These findings suggest that AFP might have the potential to enhance the prognosis ability of HCC patients by being further included in actual or newer staging systems. AFP is currently used in the *Cancer of the Liver Italian Program* (CLIP), the *Chinese University Prognostic Index* (CUPI), *Groupe d'Etude et de Traitement du Carcinoma Hépatocellulaire* (GRETCH) and a few other emerging systems [35] but the actual prognosis effect is either unclear or limited. Consequently, future large scale, comparative studies or systematic reviews might prove useful in this direction.

There are a few limitations in our study. One of them consisted of solely including AFP in the comparative analysis with no possibility of combining multiple novel biomarkers. Alternatives like des-gamma-carboxy prothrombin (DCP), AFP-L3, micro-ribonucleic acids (miRNAs), chemokine (C-X-C motif) receptor (CXCR), C-C chemokine receptor type 2 (CCR2) or E1A-binding protein P400 (EP400) might outperform AFP on their own however, when these novel biomarkers are combined with AFP, diagnosis performance could be significantly enhanced [36]. Results could not be appropriately generalized due to the fact that the given analysis only included a limited number of patients in Romania. Other drawbacks included the absence of tumor differentiation as a prognosis factor due to unavailability of liver biopsies and the omission of other HCC staging systems.

Conclusions

The given study proves that in patients diagnosed with HCC, a markedly elevated AFP level (>200 IU/mL) can be correlated with pathological features of advanced liver cancer and underlying liver disease, female gender, hepatitis B infection and a worse functional status. These factors can intervene in clinical decisions for susceptible or known HCC patients especially in what a more thorough follow-up would be concerned. Despite

its long-term clinical use and limitations, serum AFP still plays a significant role in assessing diagnosis and prognosis of HCC especially in developing countries as other biomarkers are out of reach or still subjected to research. Future prospective studies that would extensively analyze the joint relationship of AFP and other biomarkers with HCC prognosis factors are warranted.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' contribution

Elena Toader and Andrei Bancu share first authorship.

References

[1] American Cancer Society (ACS). Global cancer facts & figures. 3rd edition, ACS, Atlanta, Georgia, 2015, 18–20, https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/global-cancer-facts-and-figures/global-cancer-facts-and-figures-3rd-edition.pdf.

[2] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin, 2005, 55(2):74–108.

[3] El-Serag HB. Hepatocellular carcinoma. N Engl J Med, 2011, 365(12):1118–1127.

[4] Behne T, Copur MS. Biomarkers for hepatocellular carcinoma. Int J Hepatol, 2012, 2012:859076.

[5] European Association for Study of Liver; European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. Eur J Cancer, 2012, 48(5):599–641.

[6] Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology, 2011, 53(3):1020–1022.

[7] Erstad DJ, Tanabe KK. Hepatocellular carcinoma: early-stage management challenges. J Hepatocell Carcinoma, 2017, 4: 81–92.

[8] Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology, 2018, 67(1):358–380.

[9] European Centre for Disease Prevention and Control (ECDC). Systematic review on hepatitis B and C prevalence in the EU/EEA. ECDC, Stockholm, 2016, 15, https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/systematic-review-hepatitis-B-C-prevalence.pdf.

[10] World Health Organization (WHO). Global status report on alcohol and health 2018. WHO, Geneva, 2018, 41–46, https://apps.who.int/iris/bitstream/handle/10665/274603/9789241565639-eng.pdf.

[11] Tandon P, Garcia-Tsao G. Prognostic indicators in hepatocellular carcinoma: a systematic review of 72 studies. Liver Int, 2009, 29(4):502–510.

[12] Mailliard ME, Sorrell MF. Alcoholic liver disease. In: Longo DL, Fauci AS, Langford CA (eds). Harrison's gastroenterology and hepatology. 2nd edition, McGraw–Hill Publishing, 2013, 427–430.

[13] Toader E, Bălan GG, Bulgaru Iliescu D, Perju-Dumbrava DP. Ethical and legal medicine aspects related to hepatic encephalopathy. Rom J Leg Med, 2017, 25(1):125–127.

[14] Bataga S, Tanţău M, Cristian G, Stanciu C, Constantinescu G, Goldiş A, Străin M, Eugen D, Frăticiu A, Săftoiu A, Mulder CJ. ERCP in Romania in 2006; a National Programme seems mandatory. J Gastrointestin Liver Dis, 2007, 16(4):431–435.

[15] Gomaa AI, Hashim MS, Waked I. Comparing staging systems for predicting prognosis and survival in patients with hepatocellular carcinoma in Egypt. PLoS One, 2014, 9(3):90929.

[16] Stankevičiūtė L, Sarnelytė J, Masalaitė L. [Correlation of serum alpha fetoprotein with tumor size and number of tumors in hepatocellular carcinoma]. Laboratorinė Medicina, 2016, 18(1):29–32.

[17] Liu C, Xiao GQ, Yan LN, Li B, Jiang L, Wen TF, Wang WT, Xu MQ, Yang JY. Value of α-fetoprotein in association with clinicopathological features of hepatocellular carcinoma. World J Gastroenterol, 2013, 19(11):1811–1819.

[18] Giannini EG, Sammito G, Farinati F, Ciccarese F, Pecorelli A, Rapaccini GL, Di Marco M, Caturelli E, Zoli M, Borzio F, Cabibbo G, Felder M, Gasbarrini A, Sacco R, Foschi FG, Missale G, Morisco F, Svegliati Baroni G, Virdone R, Trevisani F; Italian Liver Cancer (ITA.LI.CA) Group. Determinants of alpha-fetoprotein levels in patients with hepatocellular carcinoma: implications for its clinical use. Cancer, 2014, 120(14):2150–2157.

[19] Bai DS, Zhang C, Chen P, Jin SJ, Jiang GQ. The prognostic correlation of AFP level at diagnosis with pathological grade, progression, and survival of patients with hepatocellular carcinoma. Sci Rep, 2017, 7(1):12870.

[20] Tangkijvanich P, Anukulkarnkusol N, Suwangool P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Clinical characteristics and prognosis of hepatocellular carcinoma: analysis based on serum alpha-fetoprotein levels. J Clin Gastroenterol, 2000, 31(4):302–308.

[21] Abbasi A, Bhutto AR, Butt N, Munir SM. Correlation of serum alpha fetoprotein and tumor size in hepatocellular carcinoma. J Pak Med Assoc, 2012, 62(1):33–36.

[22] Lim C, Mise Y, Sakamoto Y, Yamamoto S, Shindoh J, Ishizawa T, Aoki T, Hasegawa K, Sugawara Y, Makuuchi M, Kokudo N. Above 5 cm, size does not matter anymore in patients with hepatocellular carcinoma. World J Surg, 2014, 38(11):2910–2918.

[23] Dai CY, Lin CY, Tsai PC, Lin PY, Yeh ML, Huang CF, Chang WT, Huang JF, Yu ML, Chen YL. Impact of tumor size on the prognosis of hepatocellular carcinoma in patients who underwent liver resection. J Chin Med Assoc, 2018, 81(2):155–163.

[24] Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations. Int J Cancer, 2004, 112(1):44–50.

[25] Niu B, Hann HW. Chapter 5: Hepatitis B virus-related hepatocellular carcinoma: carcinogenesis, prevention, and treatment. In: Abdeldayem H (ed). Updates in liver cancer. IntechOpen, 2017, 69–93.

[26] Tarocchi M, Polvani S, Marroncini G, Galli A. Molecular mechanism of hepatitis B virus-induced hepatocarcinogenesis. World J Gastroenterol, 2014, 20(33):11630–11640.

[27] Murugavel KG, Mathews S, Jayanthi V, Shankar EM, Hari R, Surendran R, Vengatesan A, Raghuram K, Rajasambandam P, Murali A, Srinivas U, Palaniswamy KR, Pugazhendhi T, Thyagarajan SP. Alpha-fetoprotein as a tumor marker in hepatocellular carcinoma: investigations in south Indian subjects with hepatotropic virus and aflatoxin etiologies. Int J Infect Dis, 2008, 12(6):e71–e76.

[28] Akuta N, Suzuki F, Kobayashi M, Hara T, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Saitoh S, Ikeda K, Kumada H. Correlation between hepatitis B virus surface antigen level and alpha-fetoprotein in patients free of hepatocellular carcinoma or severe hepatitis. J Med Virol, 2014, 86(1):131–138.

[29] Zamor PJ, deLemos AS, Russo MW. Viral hepatitis and hepatocellular carcinoma: etiology and management. J Gastrointest Oncol, 2017, 8(2):229–242.

[30] Yao M, Zhao J, Lu F. Alpha-fetoprotein still is a valuable diagnostic and prognosis predicting biomarker in hepatitis B virus infection-related hepatocellular carcinoma. Oncotarget, 2017, 7(4):3702–3708.

[31] Farinati F, Sergio A, Giacomin A, Di Nolfo MA, Del Poggio P, Benvegnù L, Rapaccini G, Zoli M, Borzio F, Giannini EG, Caturelli E, Trevisani F; Italian Liver Cancer group. Is female sex a significant favorable prognostic factor in hepatocellular carcinoma? Eur J Gastroenterol Hepatol, 2009, 21(10):1212–1218.

[32] Brady CW. Liver disease in menopause. World J Gastroenterol, 2015, 21(25):7613–7620.

[33] Ryder SD; British Society of Gastroenterology. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. Gut, 2003, 52(Suppl 3):*iii*1–*iii*8.
[34] Nishikawa H, Kita R, Kimura T, Ohara Y, Sakamoto A, Saito S, Nishijima N, Nasu A, Komekado H, Osaki Y. Clinical implication of performance status in patients with hepatocellular carcinoma complicating with cirrhosis. J Cancer, 2015, 6(4):394–402.
[35] Kinoshita A, Onoda H, Fushiya N, Koike K, Nishino H, Tajiri H. Staging systems for hepatocellular carcinoma: current status and future perspectives. World J Hepatol, 2015, 7(3):406–424.
[36] Lou J, Zhang L, Lv S, Zhang C, Jiang S. Biomarkers for hepatocellular carcinoma. Biomark Cancer, 2017, 9:1–9.

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