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IS PIVKA-II A PROMISING BIOMARKER FOR HEPATOCELLULAR CARCINOMA?

Broz Pavel, MUDr., Ph.D., Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen, Charles University; Institute of Clinical Biochemistry and Haematology, University Hospital in Pilsen, alley Svobody 1655/76, Pilsen, 32300, Czech Republic, tel.: (+420) 377-104-243, e-mail: brozp@fnplzen.cz.

Svobodova Sarka, MUDr., Ph.D., Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen, Charles University, 13 Dr. E. Benese, Pilsen, 30100, Czech Republic, tel.: (+420) 224-966-695; Third Internal Medicine Department and First Medical Faculty, Charles University, 1 U Nemocnice, Prague 2, 12800, Czech Republic, tel.: (+420) 224-966-694, e-mail: sarka.svobodova@lf1.cuni.cz.

Karlíkova Marie, RNDr., Ph.D., Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen, Charles University, 13 Dr. E. Benese, Pilsen, 30100, Czech Republic, tel.: (+420) 377-401-121, e-mail: karlikovam@fnplzen.cz.

Topolcan Ondrej, professor, MUDr., CSc, Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen, Charles University, 13 Dr. E. Benese, Pilsen, 30100, Czech Republic, tel.: (+420) 377-401-366, e-mail: topolcan@fnplzen.cz.

Treska Vladislav, professor, MUDr., DrSc, Department of Surgery, University Hospital and Faculty of Medicine in Pilsen, Charles University, alley Svobody 80, Pilsen, 32300, Czech Republic, tel.: (+420) 377-104-270, e-mail: treska@fnplzen.cz.

Kucera Radek, doctor PharmDr., Ph.D., Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen, Charles University, 13 Dr. E. Benese, Pilsen, 30100, Czech Republic, tel.: (+420) 377-402-948.

Aim. To evaluate the clinical contribution of protein induced by vitamin K absence (PIVKA-II) for the diagnosis of hepatocellular carcinoma (HCC). The second aim was to compare PIVKA-II with routinely used alpha-fetoprotein (AFP) for the same indication.

Materials and methods. 310 participants were enrolled in our study: 60 with HCC, 40 with liver metastases of colorectal cancer origin, 40 with liver cirrhosis, 20 with pancreatic cancer (PC) and 150 healthy individuals. Serum levels of PIVKA-II were measured using a chemiluminescent assay of the Architect 1000i System (“Abbott”, USA) and AFP levels using a chemiluminescent assay by DxI 800 (“Beckman Coulter”, USA). Serum concentrations of PIVKA-II and AFP were compared between the group with HCC and the other mentioned groups.

Results. PIVKA-II achieved better clinical sensitivity in comparison with AFP. PIVKA-II achieved its best sensitivity (96,9 %) in distinguishing between the HCC and control group with the proposed cut-off value of 60 mAU/ml.

Conclusion. PIVKA-II can be used alongside routinely established AFP as a valuable marker in the diagnosis of HCC.

Key words: *PIVKA-II, AFP, hepatocellular carcinoma, biomarkers.*

Introduction. Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide [22]. The prognosis of patients with HCC is relatively poor and its mortality is almost equal to its morbidity [3, 15]. The prevalence of HCC is higher in Asian countries, e.g. in China. However, the incidence of HCC has also been increasing in developed countries over recent decades. This higher prevalence of HCC is connected with viral hepatitis B and C, liver cirrhosis associated with alcohol consumption and the influence of aflatoxin B [2, 16]. Serum alpha-fetoprotein (AFP) is well known as a tumour marker and routinely used in the detection and staging of HCC disease. However, its possibilities are limited and so new biomarkers are still being tested for this indication. Protein induced by vitamin K absence (PIVKA-II) has been studied in connection with HCC [23]. PIVKA-II concentrations have been described concerning to vitamin K deficiency but also in patients with HCC [7].

This pilot study **aims** to evaluate the clinical significance of PIVKA-II used in HCC diagnosis in comparison with AFP in the Czech population.

Material and methods. 310 participants were enrolled in the study. Patients with coagulation diseases, an uptake of vitamin K or vitamin K blocking agents, cancer duplicity, acute inflammatory disease, or renal or liver failure were excluded. The study cohort consisted of patients with HCC at different stages of the disease, patients with liver metastases from colorectal cancer, patients with pancreatic cancer, patients with benign liver disease including patients with liver cirrhosis caused by previous alcohol intake and a control group of healthy individuals who underwent a medical examination at the Department of Preventive cardiology. In the healthy control group, there were no signs of the presence of tumour of inflammatory disease. The characteristics of our study cohort are summarized in Table 1.

Table 1

Characteristics of participants enrolled in the study

Group	Number of individuals	Age (years)
Hepatocellular carcinoma	60	69,5 (33–80)
Stage I	5 (8 %)	
Stage II	10 (17 %)	
Stage III	17 (28 %)	
Stage IV	28 (47 %)	
Metastatic colorectal carcinoma	40	64,0 (36–78)
Benign liver disease – liver cirrhosis	40	63,0 (37–79)
Pancreatic cancer	20	69,0 (48–80)
Control group of healthy individual	150	61,5 (29–81)
Total	310	–

Note: Data are presented as median (min–max)

All samples were collected at the time of the diagnosis and before therapy. Blood samples were collected from the antecubital vein using the VACUETTE system (“Greiner Bio-one”, Kremsmünster, Austria). After collection, blood samples were centrifuged at 1300 g for 10 minutes. Samples were stored at -80° C before analysis.

Serum levels of PIVKA-II were analyzed using a chemiluminescent assay of the Architect 1000i System (“Abbott”, Libertyville, IL, USA) and serum concentrations of AFP were measured using a chemiluminescent assay of the Dxl 800 (“Beckman Coulter”, Brea, CA, USA). S.A.S. software (Statistical Analysis Software release 9.2; “SAS Institute Inc.”, Carry, NC, USA) was used for statistical analysis. Data are presented as median (min-max). Cut-off values were calculated at a 95 % level of specificity. The Wilcoxon two-sample test was used for comparison of the assessed parameters. The level of statistical significance was set at $\alpha = 0,05$.

Results. Data including PIVKA-II and AFP concentrations are summarized in Table 2. Proposed cut off values with sensitivity at 95 % specificity for HCC diagnosis are stated in Table 3 in a comparison involving all assessed groups.

Table 2

Summary of the results for individual groups

Group	n	PIVKA-II (mAU/ml)			AFP (IU/ml)		
		Median	Min	Max	Median	Min	Max
Hepatocellular carcinoma	60	7412	27,2	300000	6,96	1,00	118140
Metastatic colorectal carcinoma	40	36,1	32,4	275	2,55	1,00	6,80
Benign liver disease	40	123	20,1	541	7,82	1,00	23,00
Pancreatic cancer	20	37,5	20,6	724	3,00	1,00	6,00
Control group	150	32,5	10,2	88,5	3,00	1,00	9,00

Note: PIVKA-II (protein induced by vitamin K absence), AFP – alpha-fetoprotein

Table 3

Optimal cut-off for biomarkers at 95 % specificity

Differential diagnosis	PIVKA-II		AFP	
	Cut-off (mAU/ml)	Sensitivity (%)	Cut-off (IU/ml)	Sensitivity (%)
HCC *vs*. control group	60	92,1	15	39,0
HCC *vs*. metastatic colorectal carcinoma	135	95,0	6	47,0
HCC *vs*. benign liver disease	265	88,6	22	36,5
HCC *vs*. pancreatic cancer	225	95,3	15	37,5

Note: Wilcoxon test. HCC – hepatocellular carcinoma; PIVKA-II – protein induced by vitamin K absence, AFP – alpha-fetoprotein

Discussion. In the time of introduction, AFP was considered as a milestone in the HCC diagnostics. During the time, advanced imaging techniques like sensitive ultrasonography started to be "a hard competition" in regard to the first line methods of the HCC examination. Nowadays, new biomarker was discovered and there is a possibility to increase the sensitivity of traditionally used biomarker using the combination with the newly introduced molecule. PIVKA II could serve as an example how the development moved forward.

The role of PIVKA-II in the diagnosis of HCC has been widely studied [5, 9, 11, 12, 13, 17, 20, 21, 24]. Although some previously published studies showed a worse sensitivity and specificity for PIVKA-II in comparison with AFP [9], the results of studies performed later with a revised enzyme immunoassay method showed better sensitivity and/or specificity of PIVKA-II in comparison with AFP [11, 13, 17, 21, 23]. Our results are in accordance with studies evaluating PIVKA-II as a promising biomarker in the diagnosis of HCC. However, several details should be mentioned. The etiology of HCC can differ from country to country and within any examined population. Liver cancer in Asia is mostly caused by viral hepatitis B or C in contrast to steatohepatitis of various origins in the European population [1, 10, 21]. In our opinion, every country, region or responsible reference lab should perform its own study with cut offs based on the particular population and analytical method. Additionally, levels of PIVKA-II and AFP do not always correlate e.g. in patients with HCC caused by chronic hepatitis C infection [14]. The utility of PIVKA-II in a comparison with AFP can be dependent on the size of the tumor [9]. Finally, concentrations of AFP are higher in patients with HBV-related HCC and this etiology is usually connected with more aggressive forms of HCC [14]. Based on these facts, the authors of the mentioned studies usually recommend assessing both complementary markers for the indication of HCC diagnosis. Even though our data suggest there can be a reasonable clinical use of PIVKA-II because of its higher value of sensitivity and specificity in the diagnosis of HCC in comparison with the routinely used AFP, we agree with the mentioned assertion that an assessment of PIVKA-II and AFP concentrations should be combined [4].

Neither PIVKA-II nor AFP are able to fulfil the necessary criteria for screening. However, concentrations of PIVKA-II in the group of metastatic colorectal carcinoma show values that are fairly similar to those in the control group. A maximum concentration of AFP was found in the same group of patients with metastatic colorectal carcinoma 6 IU/mL. We can conclude that both markers can also be useful in the differential diagnosis of HCC. Early diagnosis of HCC is crucial because of advanced surgery techniques and the effective therapy of HCC [6, 8, 19]. Finally, subsequent imaging methods can provide additional information about the extent of disease prior to the surgery.

As we mentioned above, PIVKA-II can also have reasonable values in the differential diagnosis of HCC. To our knowledge, there has only been one published study assessing levels of PIVKA-II in patients with pancreatic cancer [18]. The authors state that according to their results PIVKA-II can serve as an additional biomarker in the diagnosis of PC. We tried to analyze the possibilities of PIVKA-II by comparing levels of PIVKA-II between groups with HCC and those with pancreatic cancer. The results of our study show that PIVKA-II can also offer the possibility of distinguishing between HCC and pancreatic cancer. In contrast, AFP showed poor sensitivity in this setting. There are plans to continue this study in order to evaluate the early stages (I and II) of HCC. Hence we are also planning to investigate the potential of PIVKA-II in the early detection of HCC in the Czech population. Additionally, the correlation of PIVKA-II concentrations with histological findings and results of imaging methods (e.g. ultrasound, positron emission tomography/computed tomography) will be also taken into the consideration.

Conclusion: According to the results of our pilot study, PIVKA-II shows better sensitivity in comparison with AFP, a marker traditionally used in HCC diagnosis. We propose to add PIVKA-II to AFP determination in the routine practice. Use of the both biomarkers together can offer valuable additional information.

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