Biomarkers in Diagnosis and Prediction of Hepatocellular Carcinoma Recurrence (Review)

DOI: 10.17691/stm2019.11.2.23
Received March 21, 2019

S.I. Malov, MD, PhD, Assistant, Department of Infectious Diseases[1]; Senior Researcher, Central Scientific Research Laboratory[2];
I.V. Malov, MD, DSc, Professor, Head of the Department of Infectious Diseases[1];
V.V. Dvornichenko, MD, DSc, Professor, Head of the Department of Oncology and Radiotherapy[1]; Head of the Oncology Department[2];
P.N. Marche, PhD, Professor, Vice Director of Research Center[3];
T. Decaens, PhD, Professor, Research Director, Laboratory Head of Department of Hepatology and Gastroenterology[4];
Z. Macek-Jilkova, PhD, Researcher of Department of Hepatology and Gastroenterology[4];
N.D. Yushchuk, MD, DSc, Professor, Academician of the Russian Academy of Sciences, Head of the Department of Infectious Diseases and Epidemiology[5]

[1]Irkutsk State Medical University, 1 Krasnogo Vosstaniya St., Irkutsk, 664003, Russia;
[2]Irkutsk State Medical Academy of Postgraduate Education, a Branch of the Russian Medical Academy of Continuing Professional Education, 100 Yubileyny Microdistrict, Irkutsk, 664049, Russia;
[3]Institute for Advanced Biosciences, Research Center Inserm U1209, CNRS 5309, Univ. Grenoble-Alpes, La Tronche, 38700, France;
[4]CHU-Grenoble, Clinique Universitaire d'Hépato-Gastroentérologie, La Tronche, 38700, France;
[5]A.I. Yevdokimov Moscow State University of Medicine and Dentistry, 20 Delegatskaya St., Moscow, 127473, Russia

Hepatocellular carcinoma (HCC) is the second leading cause of death in oncological patients. The prognosis of the disease outcome depends directly on its timely detection. Currently, in the majority of countries, the diagnostic algorithm at the preclinical stage of tumor development includes determination of alpha-fetoprotein in combination with instrumental imaging techniques. This approach allows the detection of about 65–80% of liver tumors at an early stage (A according to the BCLC classification), whereas at a very early stage (0 according to the BCLC classification) only 32–50% of cases, the result which cannot be considered satisfactory. In this regard, the search for effective biomarkers of hepatocellular carcinoma is an important challenge that faces the world healthcare.

Advances in proteomics and genomics have led to the discovery of numerous promising markers which are now being clinically tested. Molecules of protein nature proposed as hepatocellular carcinoma tumor markers in different periods of time are described in this review. Comparative data on their effectiveness and specificity are also presented. The possibility of isolated or combined use of these biomarkers for risk assessment and early diagnosis of primary liver cancer is considered.

Key words: hepatocellular carcinoma; screening; biomarkers; proteomics.

Introduction

Annually, about 700,000 diseases of hepatocellular carcinoma (HCC) are registered worldwide [1, 2]. Prevalence of liver cancer stands fifth among oncological diseases [3, 4] and in recent years, HCC moved from the third to the second place among the causes of death of oncological patients [5–8]. HCC-related morbidity rate (per 100,000 population) varies in different countries from 1.7 in Northern Europe and Canada to 30.0 in China where more than half of all cases of liver cancer is registered [9–11]. In Russia, 6–8 thousand new HCC cases are registered every year (4.0–5.0 cases per 100,000 population) which corresponds to an average rate of morbidity [12]. Worldwide, HCC occurs 3 times more often in men than in women [9, 13, 14]. Besides, there are substantial differences in HCC incidence in the representatives of various races: Mongoloids suffer from the disease 2 times more often than Negroids, while the Hispanics 2 times more often than white Americans [13].

Hepatocellular carcinoma develops more commonly in patients with liver cirrhosis (LC) caused by hepatitis viruses B and C, chronic alcohol abuse, and non-alcoholic fatty liver disease [12, 15, 16].

Since there are no clinical symptoms at an early stage of the disease, HCC is diagnosed late in 60% of patients and often concurrently with multiorgan metastasizing [1, 3]. If a tumor is detected at an early stage, the prognosis

Corresponding author: Sergey I. Malov, e-mail: LYNX2000@mail.ru

is relatively good and 5-year survival exceeds 70% [5, 17–21].

All the above-said dictates the necessity of conducting active investigations and finding serum HCC biomarkers which are cost-effective and available for a wide screening [15, 22, 23].

Special attention is paid to the search for HCC biomarkers which would allow the tumors to be diagnosed with a high degree of probability at early stages when instrumental methods are yet ineffective [24, 25]. It is useless to apply oncomarkers at the advanced stage as ultrasound examination, computed tomography, and magnetic resonance imaging show high diagnostic sensitivity (Se) [26, 27].

Practical application of biomarkers is connected with the degree of their clinical trial. Five phases of biomarker validation are distinguished and their stepwise implementation provides the possibility to make an objective conclusion about their effectiveness (Table 1) [5, 28, 29].

At present, a great variety of biomarkers are declared at the preclinical stage. But though their possible usage is theoretically grounded and some limited clinical observations have been obtained, wide multicenter studies proving their actual value are absent [3].

The search for HCC biomarkers is being carried out in three principal directions: examination of protein markers (proteomics), nucleic acids and their polymorphisms (genomics), and determination of metabolites in blood and urine (metabolomics) [5, 19, 29–34]. In this work, information about protein oncomarkers, which are now of special interest, is overviewed. This is caused by the fact that methods of various protein indication are rather well automated, have high Se and reproducibility. Application of genomic oncomarkers in clinical practice is less common, the results of their identification vary greatly depending on the way of nucleic acid isolation, biological substrate, indication method. Serum and urine metabolites in cancerogenesis require further investigation, e.g. determination of a cut-off level for some specific metabolite typical for oncological disease. In other words, questions of specificity (Sp) in metabolomics acquire special importance when a diagnostic value of an oncomarker is concerned. Currently, canavanine succinate, glycochenodeoxycholic acid, and other organic acids, including fatty acids, are being studied as metabolomic indicators of cancerogenesis in liver tissues [35].

At early stages of liver cancer (0–A stages according to BCLC and stage I according to TNM classification), the diagnostic effectiveness of using different biological markers varies widely (Table 2).

Table 1

Phases of clinical testing of hepatocellular carcinoma biomarkers

Phase	Stage of investigation	Purpose
I	Preclinical testing	Search for candidate markers and theoretical grounding of their application
II	Clinical analysis and validation	Testing for the possibility of using in clinical practice
III	Retrospective analysis	Assessment of the effectiveness of using a biomarker according to the data of the retrospective studies
IV	Prospective analysis	Assessment of the effectiveness of using a biomarker at various stages of hepatocellular carcinoma and with consideration of false result frequency according to the data of the prospective studies
V	Assessment of the effectiveness of diagnostic application in actual clinical practice	Analysis of the results of implementing a biomarker into clinical practice and their impact on the reduction of the hepatocellular carcinoma prevalence and morbidity in different countries and various ethnic groups

Table 2

Diagnostic value of identifying some biomarkers in the blood serum at early and very early stages of liver cancer development

Biomarker	The author suggested it as a HCC biomarker	Threshold value (cut-off)	Sensitivity (%)	Specificity (%)	References
Alpha-fetoprotein (AFP)	Iu.S. Tatarinov, 1964 G.I. Abelev, 1968	10.9–400.0 ng/ml	45.0 (24.0–66.0)	88.0 (76.0–100.0)	[17, 19, 36–42]
Des-gamma carboxyprothrombin (DCP, PIVKA-II)	H.A. Liebman et al., 1984	7.5–10.0 ng/ml 40.0–200.0 mAU/ml	46.0 (15.0–77.0)	89.5 (81.0–98.0)	[14, 17, 43–48]
Glycosylated L3 isoform of alpha-fetoprotein (AFP-L3)	K. Taketa et al., 1990	5.0–15.0%	36.5 (28.0–45.0)	93.5 (90.0–97.0)	[17, 49–52]
Alpha-L-fucoidase (AFU)	M. Giardina et al., 1992	870.0 nmol/L	81.9 (81.7–82.0)	70.5 (70.0–71.0)	[39, 53–56]

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Biomarker	The author suggested it as a HCC biomarker	Threshold value (cut-off)	Sensitivity (%)	Specificity (%)	References
Glypican-3 (GPC3)	H.S. Hsu et al., 1997 M. Capurro et al., 2003	20.0–300.0 ng/ml 26.8–58.8 mAU/ml	44.1 (22.0–66.2)	86.6 (75.0–98.2)	[22, 57–64]
Golgi protein 73 (GP73)	J.A. Marrero et al., 2005	7.0–15.0 ng/ml	65.5 (62.0–69.0)	87.0 (86.0–88.0)	[65–67]
Squamous carcinoma antigen (SCCA)	G. Giannelli et al., 2005	0.12–3.8 ng/ml	40.1 (24.0–56.1)	66.5 (50.0–83.0)	[68–72]
Squamous carcinoma antigen (SCCA) and immune complex SCCA-IgM	L. Beneduce et al., 2005	No data	79.5 (70.0–89.0)	50.0 (50.0)	[71–73]
Osteopontin (OPN)	J. Kim et al., 2006	9.3–642.5 ng/ml	79.0 (61.0–97.0)	77.5 (55.0–100.0)	[38, 46, 74–77]
Annexin A2 (Ann A2)	N.Y. Ji et al., 2009	17.3 ng/μl	84.8 (83.2–86.4)	70.5 (67.5–73.5)	[19, 78–80]
Higly sensitive AFP-L3 (hs-AFP-L3)	H. Toyoda et al., 2011	5.0%	53.5 (50.0–57.0)	74.3 (63.5–85.1)	[81, 82]
Dickkopf-related protein 1 (DKK-1)	E.K. Tung et al., 2012	1.01–2.15 ng/ml	61.9 (50.0–73.8)	87.4 (80.8–94.0)	[13, 46, 83–87]
Receptor tyrosine kinase sAxl (AXL)	Y. Sun et al., 2013	No data	78.9 (76.9–80.8)	79.5 (66.7–92.3)	[19, 79, 88, 89]
Heparin-binding growth factor midkine (MDK)	W.-W. Zhu et al., 2013	0.387–0.654 ng/ml	88.5 (87.0–90.0)	No data	[90–92]
Minichromosome maintenance protein 6 (MCM6)	T. Zheng et al., 2014	No data	71.4 (71.4)	86.2 (86.2)	[19, 93]
Thioredoxin (TRX)	J. Li et al., 2015	No data	74.7 (74.5–74.9)	83.6 (79.6–87.5)	[19, 94]
Soluble urokinase plasminogen activator receptor (suPAR)	A. Chounta et al., 2015	9.56 ng/ml	76.0 (76.0)	90.4 (90.4)	[95]

Alpha-fetoprotein (AFP). In spite of the fact that AFP has already been used in clinical practice for over 50 years, this biomarker remains most widely used to predict the development and monitor the efficacy of HCC treatment [5, 31, 39, 40]. AFP represents a glycoprotein with a 70 kDa molecular mass synthetized by endodermal cells of the fetal yolk sac and later by the embryonic hepatocytes [86].

Increase of the AFP level in the blood is observed in degenerative processes in the liver tissues and in various oncological diseases. The analysis of the literature assessing AFP as a HCC biomarker in patients with LC showed the range of Se and Sp from 41 to 65% and from 80 to 94%, respectively [96, 97]. Monitoring of the AFP level may be used to predict HCC recurrence after the operative treatment [98]. Data of AFP and US findings taken separately show Se to be below 50% at an early HCC stage but their combined application improves Se to 65% [23, 38].

In the regions with a high HCC prevalence, application of serological screening alone is permissible and justified [99]. There has been described a successful experienced of HCC screening in patients with chronic hepatitis B in Alaska when US examination was impossible [100]. As a result, HCC was detected in part of the patients at the operable stage and early medical aid was provided [100].

Alpha-fetoprotein is a specific marker not only for HCC, its level rises in LC and tumors of the lungs, biliary tract, stomach, and pancreas [13, 39, 98]. Due to a low Se, the recent versions of European and American clinical recommendations excluded AFP identification from the diagnostic algorithm [19, 26, 40].

Glycosylated L3 isoform of alpha-fetoprotein (AFP-L3). AFP exists in three glycosylated isoforms [5, 7], and each possesses different capability to bind with lectin (lens culinaris agglutinin, LCA): AFP-L1 (non-LCA-bound fraction), AFP-L2 (weakly bound fraction), and AFP-L3 (LCA-bound fraction). AFP-L1 increases in chronic hepatitis and LC, whereas the elevation of AFP-L3 level is noted in tumor processes in the liver [17, 86]. Predominance of AFP-L3 in the total AFP level by more than 10–15% allows HCC to be suspected at an early stage of its development. A large multicenter prospective study [101] showed that Sp of AFP-L3 is approximately 92%, whereas Se is only about 37% irrespectively of the HCC stage.

A wide use of this biomarker is limited in clinical practice because the separation of the total AFP into fractions is possible only if its level exceeds 30 ng/ml [99]. Therefore, tumors producing no AFP cannot be detected by this method [23]. To overcome this disadvantage, a highly sensitive assay (hs-AFP-L3) has been developed in Japan which is applicable at low values of total AFP in the blood [82].

Des-gamma carboxyprothrombin (DCP, PIVKA-II). DCP is an abnormal form of prothrombin which is expressed due to the defect of post-translational carboxylation with underlying vitamin K deficit [102]. The other name of this oncomarker is a protein induced by

vitamin K absence (PIVKA II). Functionally, DCP is a pathological inactive prothrombin [103]. Application of this biomarker with a diagnostic purpose showed good results in Eastern Asia, North America, China [104–106]. In Europe, the results were more controversial as its level was established to depend on the race and etiology of HCC [17, 48, 105, 106].

Des-gamma carboxyprothrombin may be used to assess not only the risk of HCC development but to predict its recurrence after surgical treatment. In the prospective study [107], application of DCP with AFP made it possible to suspect HCC 2 years before the diagnose verification. Further investigations are necessary in order to evaluate the effectiveness of this marker combination in the diagnosis of HCC [44]. It should be also kept in mind that DCP was mainly studied in Asian countries while the experience of using it in Europe remains insufficient.

Alpha-L-fucosidase (AFU). AFU is a lysosomal enzyme which breaks down glycoconjugates containing a carbohydrate — fructose [54]. Its activity has been shown to be higher in patients with HCC than with chronic hepatitis and healthy individuals [56, 108]. Application of AFU for screening purposes is not perspective due to a low Sp [109]. A high activity of this enzyme is found not only in HCC patients but in people with diabetes, pancreatitis, hypothyroidism as well [53, 109]. Besides, an average level of the enzyme activity depends on the person's race and ethnic predisposition [53]. The AFU level has been reported to elevate in 85% of patients who 6 months later were diagnosed HCC on the basis of US findings [13, 110]. In its combination with AFP, Se increased to 95% and Sp to 99% during examination of HCC patients including those with advanced stage IV [55, 56].

Glypican-3 (GPC3). GPC3 belongs to a family of glypicans-proteoglycans. GPC3 is bound to the cellular membrane with a glycosyl-phosphatidyl-inositol anchor [3, 63]. GPC3 interacts with some growth factors [22, 63, 86] participating in cell proliferation and inhibition of cancer cell growth which characterizes it as an oncosuppressor [62, 63, 86]. An elevated GPC level is revealed in 50–55% of HCC patients [61] and only in 5% of those with LC [22, 63, 111]. At an early HCC stage (0 and A according to BCLC or I according to TNM), Se and Sp of serum GPC3 identification were 55.1% (47.9–66.2%) and 97.0% (95.2–98.2%), respectively [60].

An independent significance of the GPC3 for HCC diagnosis is restricted due to low Se [61, 86]. GPC3 is increased in 1/3 of patients with normal AFP indices in the blood [64]. Absence of correlation between these biomarkers gives grounds for their combined use [59, 77], improving Se to 76% for the tumors below 3 cm in size [64, 112]. GPC3 is determined immunohistochemically in liver bioptates which are used in clinics for differential diagnosis of HCC and other liver damages [13, 113].

Golgi protein 73 (GP73). GP73 is a specific membrane protein of Golgi complex which is usually expressed in the epithelial cells of different organs [114]. In the liver, it is synthesized mainly in the epithelium of the biliary tracts but when an inflammatory process develops its level in hepatocytes rises sharply [38, 86, 114, 115]. HCC is also accompanied by GP73 increase in the blood [13, 54, 86, 116]. GP73 is more sensitive than AFP and the elevation of its level concurrently with HCC formation begins earlier [67, 70]. GP73 level does not depend on the etiological causes of tumor development, the stage of cancerogenesis, or functional state of the liver, however, it, like many other biomarkers, does not show high Sp. GP73 rises in other liver tumors as well including cholangiocarcinoma [67, 114]. Another drawback of practical application of this biomarker is a considerable inaccuracy in identification of this protein at low concentrations in the blood [54]. If combined with other markers, e.g. with AFP and AFP-L3, the diagnostic value of GP73 increases [87, 116, 117].

Squamous carcinoma antigen (SCCA) and immune complex SCCA-IgM. SCCA antigen is a member of the family of high-molecular serine protease inhibitors which is present in squamous epithelium [5, 72, 86]. SCCA is synthesized in a large amount in epithelial tumors including cervix cancer cells. Giannelli et al. [69] have found a higher SCCA level in HCC patients relative to those suffering from LC. The identification of SCCA showed high Se but low Sp [71, 72]. The possibility of detecting SCCA in histological sections of tumor bioptates allowed it to be recommended as an immunohistochemical marker [118]. Guido et al. [119] have found that SCCA expression in neoplastic nodes is much higher than in regenerative ones.

The application of the immune complex SCCA-IgM rather than SCCA itself became an alternative variant. This complex is present normally in the desquamating epithelium and is not determined in the blood [54]. SCCA detection in the structure of the immune complex turned out to be more effective than identification of a free antigen [71, 118]. The frequency of SCCA-IgM identification in patients with chronic hepatitis, LC, and HCC was 18, 26, and 70%, respectively [54, 120] but Sp remained sufficiently low (50%) [71]. Evidently, these HCC markers will not have an independent value but their application in future as supplementary indicators in combination with some highly specific component is not excluded [72, 118].

Osteopontin (OPN). OPN, also known as transformed protein phosphatase (SPP1), represents an integrin-binding glycophosphoprotein which is produced in increased amount in many types of malignant neoplasms including cancer of the lungs, breast, intestine, pancreas, kidneys, gallbladder, prostate, ovaries [121–123]. In the physiological state, OPN is synthetized in the epithelium of biliary ducts, Kupffer cells but is not expressed in hepatocytes [35]. In 1999, an increased OPN production was found in the focus of hepatocyte necrosis caused by carbon

tetrachloride. Later, the elevated OPN was reported in many diseases of the liver such as viral hepatitis, acute liver insufficiency, non-alcoholic and alcoholic fatty liver disease [13, 76, 123]. Its key role has been suggested in the induction of inflammation and fibrosis of the liver. Thus, spontaneous liver fibrosis develops during a year in transgenic mice with OPN hyperproduction [123, 124]. Kim et al. [74] were among the first to assess the value of OPN in HCC. The OPN level rises long before (from 6 months to several years) the episode of the instrumental detection of HCC and has better Se than AFP [121, 125].

There is no correlation between the content of OPN and AFP in HCC patients, therefore, their combination is considered as a variant of an effective predictor of HCC risk development [41, 122, 126].

Annexin A2 (Ann A2). Ann A2 is a calcium-dependent protein binding phospholipids [19, 127]. It is expressed in the cells of different tissue tumors and plays a role in realization of the following processes: angiogenesis, proliferation, apoptosis, adhesion, invasion, cell migration [19, 128–131]. Its level changes in the variety of cancers such as cancer of the intestine, lungs, stomach, breast, esophagus [19, 33, 80]. Sun et al. [79] have observed the elevation of Ann A2 level in 83.2% cases in patients with HCC at 0 and A stages (BCLC classification) though Sp of this marker turned out to be low and did not exceed 67.5%. A combination of Ann A2 and AFP slightly improved Se but did not influence Sp (87.4 and 68.3%, respectively) [79]. In the HCC cohort producing no AFP, the Ann A2 level was elevated in 78.4% of cases. In this regard, Ann A2 is considered as a candidate biomarker for early HCC detection in patients with normal AFP indices in the blood serum [5, 33, 79, 80].

Dickkopf-related protein 1 (DKK-1). This is a secreted glycoprotein, inhibitor of intracellular beta-catenin signaling pathway [132, 133]. It participates in cell proliferation, differentiation, and apoptosis [3]. Increase of its content in the blood was found in oncological processes of various localization: cancer of the prostate, skin, liver [86, 134]. It shows a low Se level (65%) during HCC diagnosing but acceptable Sp (94%). Therefore, at early stages of HCC, identification of DKK1 is more effective than AFP [13, 46, 87, 133]. A combined use of AFP, DKK1, and DCP has been reported [87]. It increases Se and, consequently, the effectiveness of diagnosis of small-sized HCC [77, 87, 134].

Receptor tyrosine kinase sAxl (AXL). AXL is a tyrosine kinase which is involved in proliferation processes. It is expressed in many types of cells and its biological effects depend on the tissue specificity of the cells. An AXL form circulating in the blood has a molecular mass of 80 kDa and can be detected by the standard diagnostic methods [135, 136]. Functionally, AXL makes cancer cells resistant to chemopreparations [137–139]. Its increased production is observed in oncological diseases and correlates with poor prognosis [19, 89, 138–140]. In a large multicenter study, a diagnostic value of AXL for early HCC detection has been determined. AXL Se was found to be much higher than that of AFP [88]. A combination of AXL with AFP enables the improvement of Se method and preservation of Sp level within 90% [88].

Heparin-binding growth factor midkine (MDK). MDK has a low molecular mass. In the physiological state, the highest MDK level is observed in the midgestation period which reflects the name of the protein, *midkine* (the middle of kinetics). The MDK level in the serum of healthy people does not usually exceed 0.5–0.6 ng/ml while in patients with malignant diseases it is substantially higher [141]. There has been described an elevated MDK expression in tumors of different tissues and organs such as neuroblastoma, glioblastoma, cancer of the thyroid gland, lungs, esophagus, stomach, and prostate [141, 142]. In the investigation carried out on patients with HCC and LC, it has been established that MDK levels in the first cohort of patients were on average 5 times higher than in the second. The calculated Se for MDK was 90% and for AFP only 50% [141, 142].

Minichromosome maintenance protein 6 (MCM6). This is a component of a protein complex maintaining minichromosome. It takes part in the process of DNA replication during the S-phase of the cell cycle [143]. In a single work [93], a promising outlook of MCM6 application has been shown for early detection of HCC at a satisfactory Se level. However, the cohort of the examined patients in this study differed by clinical criteria from the cohort indicated in the BCLC classification, therefore the value of MCM6 for diagnosis of early and very early HCC stages remains unclear [93, 144, 145].

Thioredoxin (TRX). TRX represents thiol oxidoreductase which reduces disulfide bonds of the proteins. TRX is involved in the biological processes such as regulation of protein synthesis, apoptosis, and cell proliferation and provides protection against oxidative stress [146]. TRX expression is elevated in the tumor cells and its level correlates with the prognosis which has been shown in cancer of the lungs and colorectum [147, 148]. Li et al. [94] have reported the potential possibility of using TRX for early revealing of HCC. In this study, TRX Se and Sp (74.9 and 87.5%, respectively) were higher than for AFP (68.6 and 75.2%, respectively). A combination of TRX and AFP raises the effectiveness of HCC diagnosis (Se — 81.3%; Sp — 93.4%; AUC — 0.889), therefore, their joint application is justified but requires more ample study [94, 149].

Soluble urokinase plasminogen activator receptor (suPAR). SuPar is a circulating form of the membrane protein — a receptor activator of urokinase-type plasmogen [95]. In 2015, suPAR was used to assess tumor progression and cancer metastasizing [79]. The suPAR level in the serum is elevated in patients suffering from cancer of the ovaries, colon, and HCC [79, 95]. In the prospective study [95], the occurrence of HCC in 267 patients with chronic hepatitis has been investigated

for 7 years. This work showed the possibility of using this marker for HCC prediction at Se 76.0% and Sp 90.4%. These results lead to the conclusion that suPAR possesses a certain potential as an early predictor of the risk of HCC development.

Conclusion

The search for hepatocellular carcinoma biomarkers on the basis of proteomics is intensively being carried out in the countries worldwide since the detection of this disease at its early stage substantially influences the results of operative treatment and patients' quality of life. An ideal biomarker of hepatocellular carcinoma must be applicable for screening and reach the level of Se and Sp exceeding 90%. Besides, the methodology of investigation should be minimally invasive and economically grounded [5].

In order to reach this goal, the majority of current strategies make provision for the use of new molecules in combination with previously discovered [5, 19, 29]. It is important that these markers are not associated with each other and do not give diagnostic cross results [5, 31]. Additionally, certain diagnostic algorithms and mathematical formulas are proposed for use combining quantitative results of identification of 2–4 markers and biochemical tests [33, 52, 56, 87, 102, 141]. The diagnostic value of different algorithms and diagnostic scales continue to be assessed in the prospective clinical studies being performed [150–152]. Despite a positive experience of using this approach in Japan, China, Korea, and other countries with a high morbidity from hepatocellular carcinoma, the main limiting factor of its implementation in clinical practice has been until now the cost of the analysis which grows significantly when a combination of markers and the corresponding diagnostic algorithms pertaining to them are applied [153].

Study funding. The work was supported by the Federal Target Program of Research & Development in Priority Areas with participation of research organizations and universities within the frames of the joint French-Russian PHC Kolmogorov project (agreement No.14.616.21.0098; unique project identifier RFMEFI61618X0098).

Conflicts of interests. The authors have no conflicts of interest to declare.

References

1. Siegel R., Naishadham D., Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; 63(1): 11–30, https://doi.org/10.3322/caac.21166.

2. Forner A., Llovet J.M., Bruix J. Hepatocellular carcinoma. *Lancet* 2012; 379(9822): 1245–1255, https://doi.org/10.1016/s0140-6736(11)61347-0.

3. Scaggiante B., Kazemi M., Pozzato G., Dapas B., Farra R., Grassi M., Zanconati F., Grassi G. Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials. *World J Gastroenterol* 2014; 20(5): 1268–1288, https://doi.org/10.3748/wjg.v20.i5.1268.

4. Bertuccio P., Turati F., Carioli G., Rodriguez T., La Vecchia C., Malvezzi M., Negri E. Global trends and predictions in hepatocellular carcinoma mortality. *J Hepatol* 2017; 67(2): 302–309, https://doi.org/10.1016/j.jhep.2017.03.011.

5. Tsuchiya N., Sawada Y., Endo I., Saito K., Uemura Y., Nakatsura T. Biomarkers for the early diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2015; 21(37): 10573, https://doi.org/10.3748/wjg.v21.i37.10573.

6. Duarte-Salles T., Misra S., Stepien M., Plymoth A., Muller D., Overvad K., Olsen A., Tjønneland A., Baglietto L., Severi G., Boutron-Ruault M.C., Turzanski-Fortner R., Kaaks R., Boeing H., Aleksandrova K., Trichopoulou A., Lagiou P., Bamia C., Pala V., Palli D., Mattiello A., Tumino R., Naccarati A., Bueno-de-Mesquita H.B., Peeters P.H., Weiderpass E., Quirós J.R., Agudo A., Sánchez-Cantalejo E., Ardanaz E., Gavrila D., Dorronsoro M., Werner M., Hemmingsson O., Ohlsson B., Sjöberg K., Wareham N.J., Khaw K.T., Bradbury K.E., Gunter M.J., Cross A.J., Riboli E., Jenab M., Hainaut P., Beretta L. Circulating osteopontin and prediction of hepatocellular carcinoma development in a large European population. *Cancer Prev Res (Phila)* 2016; 9(9): 758–765, https://doi.org/10.1158/1940-6207.capr-15-0434.

7. Bruix J., Gores G.J., Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; 63(5): 844–855, https://doi.org/10.1136/gutjnl-2013-306627.

8. *World Cancer Report 2014.* Edited by Stewart B.W., Wild C. Lyon, France: International Agency for Research on Cancer; Geneva, Switzerland: WHO Press; 2014.

9. Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65(2): 87–108, https://doi.org/10.3322/caac.21262.

10. Rowe J., Ghouri Y., Mian I. Review of hepatocellular carcinoma: epidemiology, etiology, and carcinogenesis. *J Carcinog* 2017; 16(1): 1, https://doi.org/10.4103/jcar.jcar_9_16.

11. Ozakyol A. Global epidemiology of hepatocellular carcinoma (HCC epidemiology). *J Gastrointest Cancer* 2017; 48(3): 238–240, https://doi.org/10.1007/s12029-017-9959-0.

12. Breder V.V., Kosyrev V.Y., Kudashkin N.E., Laktionov K.K. Hepatocellular carcinoma as a social and medical problem in the Russian Federation. *Medicinskij sovet* 2016; 10: 10–18, https://doi.org/10.21518/2079-701x-2016-10-10-18.

13. Chávez-López M.G., Zúñiga-García V., Pérez-Carreón J.I., Avalos-Fuentes A., Escobar Y., Camacho J. Eag1 channels as potential early-stage biomarkers of hepatocellular carcinoma. *Biologics* 2016; 10: 139–148, https://doi.org/10.2147/btt.s87402.

14. Ette A.I., Ndububa D.A., Adekanle O., Ekrikpo U. Diagnostic utility of alpha-fetoprotein and des-gamma-carboxyprothrombin in nigerians with hepatocellular carcinoma. *Niger J Clin Pract* 2017; 20(10): 1267–1272, https://doi.org/10.4103/njcp.njcp_398_16.

15. Kiriienko V.T., Zaitsev I.A., Hrushkevych V.V., Potii V.V. Screening and early diagnosis of hepatocellular carcinoma. *Aktual'naa infektologia* 2018; 6(2): 70–76, https://doi.org/10.22141/2312-413x.6.2.2018.131091.

16. Durand F., Antoine C., Soubrane O. Liver

transplantation in France. *Liver Transpl* 2019; 25(5): 763–770, https://doi.org/10.1002/lt.25419.

17. Bertino G., Ardiri A., Malaguarnera M., Malaguarnera G., Bertino N., Calvagno G.S. Hepatocellualar carcinoma serum markers. *Semin Oncol* 2012; 39(4): 410–433, https://doi.org/10.1053/j.seminoncol.2012.05.001.

18. Singal A.G., Pillai A., Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. *PLoS Med* 2014; 11(4): e1001624, https://doi.org/10.1371/journal.pmed.1001624.

19. Reichl P., Mikulits W. Accuracy of novel diagnostic biomarkers for hepatocellular carcinoma: an update for clinicians (review). *Oncol Rep* 2016; 36(2): 613–625, https://doi.org/10.3892/or.2016.4842.

20. Singal A.G., Mittal S., Yerokun O.A., Ahn C., Marrero J.A., Yopp A.C., Parikh N.D., Scaglione S.J. Hepatocellular carcinoma screening associated with early tumor detection and improved survival among patients with cirrhosis in the US. *Am J Med* 2017; 130(9): 1099–1106, https://doi.org/10.1016/j.amjmed.2017.01.021.

21. White D.L., Thrift A.P., Kanwal F., Davila J., El-Serag H.B. Incidence of hepatocellular carcinoma in all 50 United States, from 2000 through 2012. *Gastroenterology* 2017; 152(4): 812–820, https://doi.org/10.1053/j.gastro.2016.11.020.

22. Montalbano M., Rastellini C., McGuire J.T., Prajapati J., Shirafkan A., Vento R., Cicalese L. Role of glypican-3 in the growth, migration and invasion of primary hepatocytes isolated from patients with hepatocellular carcinoma. *Cell Oncol (Dordr)* 2018; 41(2): 169–184, https://doi.org/10.1007/s13402-017-0364-2.

23. Abdel-Rahman O., Cheung W.Y. Population-based assessment of the national comprehensive cancer network recommendations for baseline imaging of hepatocellular carcinoma. *Med Oncol* 2019; 36(3): 26, https://doi.org/10.1007/s12032-019-1248-2.

24. Farvardin S., Patel J., Khambaty M., Yerokun O.A., Mok H., Tiro J.A., Yopp A.C., Parikh N.D., Marrero J.A., Singal A.G. Patient-reported barriers are associated with lower hepatocellular carcinoma surveillance rates in patients with cirrhosis. *Hepatology* 2017; 65(3): 875–884, https://doi.org/10.1002/hep.28770.

25. Simmons O., Fetzer D., Yokoo T., Marrero J.A., Yopp A., Kono Y., Parikh N.D., Browning T., Singal A.G. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. *Aliment Pharmacol Ther* 2017; 45(1): 169–177, https://doi.org/10.1111/apt.13841.

26. Aubé C., Oberti F., Lonjon J., Pageaux G., Seror O., N'Kontchou G., Rode A., Radenne S., Cassinotto C., Vergniol J., Bricault I., Leroy V., Ronot M., Castera L., Michalak S., Esvan M., Vilgrain V.; CHIC Group. EASL and AASLD recommendations for the diagnosis of HCC to the test of daily practice. *Liver Int* 2017; 37(10): 1515–1525, https://doi.org/10.1111/liv.13429.

27. Ronot M., Fouque O., Esvan M., Lebigot J., Aubé C., Vilgrain V. Comparison of the accuracy of AASLD and LI-RADS criteria for the non-invasive diagnosis of HCC smaller than 3 cm. *J Hepatol* 2018; 68(4): 715–723, https://doi.org/10.1016/j.jhep.2017.12.014.

28. Masuzaki R., Karp S.J., Omata M. New serum markers of hepatocellular carcinoma. *Semin Oncol* 2012; 39(4): 434–439, https://doi.org/10.1053/j.seminoncol.2012.05.009.

29. Sengupta S., Parikh N.D. Biomarker development for hepatocellular carcinoma early detection: current and future perspectives. *Hepat Oncol* 2017; 4(4): 111–122, https://doi.org/10.2217/hep-2017-0019.

30. She S., Xiang Y., Yang M., Ding X., Liu X., Ma L., Liu Q., Liu B., Lu Z., Li S., Liu Y., Ran X., Xu X., Hu H., Hu P., Zhang D., Ren H., Yang Y. C-reactive protein is a biomarker of AFP-negative HBV-related hepatocellular carcinoma. *Int J Oncol* 2015; 47(2): 543–554, https://doi.org/10.3892/ijo.2015.3042.

31. Waidely E., Al-Yuobi A.R., Bashammakh A.S., El-Shahawi M.S., Leblanc R.M. Serum protein biomarkers relevant to hepatocellular carcinoma and their detection. *Analyst* 2016; 141(1): 36–44, https://doi.org/10.1039/c5an01884f.

32. Sia D., Llovet J.M. Liver cancer: translating '-omics' results into precision medicine for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2017; 14(10): 571–572, https://doi.org/10.1038/nrgastro.2017.103.

33. Zhao S., Su G., Yang W., Yue P., Bai B., Lin Y., Zhang J., Ba Y., Luo Z., Liu X., Zhao L., Xie Y., Xu Y., Li S., Meng W., Xie X., Li X. Identification and comparison of differentiation-related proteins in hepatocellular carcinoma tissues by proteomics. *Technol Cancer Res Treat* 2017; 16(6): 1092–1101, https://doi.org/10.1177/1533034617732426.

34. Torres-Mena J.E., Salazar-Villegas K.N., Sánchez-Rodríguez R., López-Gabiño B., Del Pozo-Yauner L., Arellanes-Robledo J., Villa-Treviño S., Gutiérrez-Nava M.A., Pérez-Carreón J.I. Aldo-keto reductases as early biomarkers of hepatocellular carcinoma: a comparison between animal models and human HCC. *Dig Dis Sci* 2018; 63(4): 934–944, https://doi.org/10.1007/s10620-018-4943-5.

35. Calvaruso V., Cabibbo G., Cacciola I., Petta S., Madonia S., Bellia A., Tinè F., Distefano M., Licata A., Giannitrapani L., Prestileo T., Mazzola G., Di Rosolini M.A., Larocca L., Bertino G., Digiacomo A., Benanti F., Guarneri L., Averna A., Iacobello C., Magro A., Scalisi I., Cartabellotta F., Savalli F., Barbara M., Davì A., Russello M., Scifo G., Squadrito G., Cammà C., Raimondo G., Craxì A., Di Marco V.; Rete Sicilia Selezione Terapia–HCV (RESIST-HCV). Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. *Gastroenterology* 2018; 155(2): 411–421, https://doi.org/10.1053/j.gastro.2018.04.008.

36. Tatarinov Iu.S. Detection of embryo-specific alpha-globulin in the blood serum of a patient with primary liver cancer. *Voprosy meditsinskoi khimii* 1964; 10: 90–91.

37. Abelev G.I. Production of embrional serum alpha-globulin by hepatomas: review of experimental and clinical data. *Cancer Res* 1968; 28(7): 1344–1350.

38. Rich N., Singal A.G. Hepatocellular carcinoma tumour markers: current role and expectations. *Best Pract Res Clin Gastroenterol* 2014; 28(5): 843–853, https://doi.org/10.1016/j.bpg.2014.07.018.

39. Bai D.S., Zhang C., Chen P., Jin S.J., Jiang G.Q. 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, https://doi.org/10.1038/s41598-017-12834-1.

40. Gao J., Song P. Combination of triple biomarkers AFP, AFP-L3, and PIVAKII for early detection of hepatocellular carcinoma in China: expectation. *Drug Discov Ther* 2017; 11(3): 168–169, https://doi.org/10.5582/ddt.2017.01036.

41. Li J., Chen X., Dai M., Huang S., Chen J., Dai S. Diagnostic accuracy of osteopontin plus alpha-fetoprotein in the hepatocellular carcinoma: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2017; 41(5): 543–553, https://doi.org/10.1016/j.clinre.2017.01.010.

42. Shen Q., Eun J.W., Lee K., Kim H.S., Yang H.D., Kim S.Y., Lee E.K., Kim T., Kang K., Kim S., Min D.H., Oh S.N., Lee Y.J., Moon H., Ro S.W., Park W.S., Lee J.Y., Nam S.W. Barrier to autointegration factor 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3, and splicing factor 3b subunit 4 as early-stage cancer decision markers and drivers of hepatocellular carcinoma. *Hepatology* 2018; 67(4): 1360–1377, https://doi.org/10.1002/hep.29606.

43. Liebman H.A., Furie B.C., Tong M.J., Blanchard R.A., Lo K.J., Lee S.D., Coleman M.S., Furie B. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984; 310: 1427–1431, https://doi.org/10.1056/nejm198405313102204.

44. Poté N., Cauchy F., Albuquerque M., Voitot H., Belghiti J., Castera L., Puy H., Bedossa P., Paradis V. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol* 2015; 62(4): 848–854, https://doi.org/10.1016/j.jhep.2014.11.005.

45. Haque S., Kumari R., Muzaffar A., Kumar U., Sharan A., Kumari B. Estimation of serum alpha feto-protein (AFP), interlukin-6 and des-γ-carboxyprothrombin (DCP) in case of hepatocellular carcinoma. *Bangladesh Journal of Medical Science* 2016; 15(2): 230–233, https://doi.org/10.3329/bjms.v15i2.19602.

46. Jang E.S., Jeong S.-H., Kim J.-W., Choi Y.S., Leissner P., Brechot C. Diagnostic performance of alpha-fetoprotein, protein induced by vitamin K absence, osteopontin, dickkopf-1 and its combinations for hepatocellular carcinoma. *PLoS One* 2016; 11(3): e0151069, https://doi.org/10.1371/journal.pone.0151069.

47. Chen J., Wu G., Li Y. Evaluation of serum des-gamma-carboxy prothrombin for the diagnosis of hepatitis virus-related hepatocellular carcinoma: a meta-analysis. *Dis Markers* 2018 4; 2018; 8906023, https://doi.org/10.1155/2018/8906023.

48. Svobodova S., Karlikova M., Topolcan O., Pecen L., Pestova M., Kott O., Treska V., Slouka D., Kucera R. PIVKA-II as a potential new biomarker for hepatocellular carcinoma — a pilot study. *In Vivo* 2018; 32(6): 1551–1554, https://doi.org/10.21873/invivo.11413.

49. Taketa K., Sekiya C., Namiki M., Akamatsu K., Ohta Y., Endo Y., Kosaka K. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology* 1990; 99(2): 508–518, https://doi.org/10.1016/0016-5085(90)91034-4.

50. Taketa K., Endo Y., Sekiya C., Tanikawa K., Koji T., Taga H., Satomura S., Matsuura S., Kawai T., Hirai H. A collaborative study for the evaluation of lectin-reactive α-fetoproteins in early detection of hepatocellular carcinoma. *Cancer Res* 1993; 53(22): 5419–5423.

51. Marrero J.A., Feng Z., Wang Y., Nguyen M.H., Befeler A.S., Roberts L.R., Reddy K.R., Harnois D., Llovet J.M., Normolle D., Dalhgren J., Chia D., Lok A.S., Wagner P.D., Srivastava S., Schwartz M. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; 137(1): 110–118, https://doi.org/10.1053/j.gastro.2009.04.005.

52. Park S.J., Jang J.Y., Jeong S.W., Cho Y.K., Lee S.H., Kim S.G., Cha S.W., Kim Y.S., Cho Y.D., Kim H.S., Kim B.S., Park S., Bang H.I. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. *Medicine (Baltimore)* 2017; 96(11): e5811, https://doi.org/10.1097/md.0000000000005811.

53. Giardina M., Matarazzo M., Varriale A., Morante R., Napoli A., Martino R. Serum alpha-L-fucosidase. A useful marker in the diagnosis of hepatocellular carcinoma. *Cancer* 1992; 70(5): 1044–1048, https://doi.org/10.1002/1097-0142(19920901)70:5<1044::aid-cncr2820700506>3.0.co;2-u.

54. Sergeev M.N., Shevaldin A.G., Rakhmanova A.G., Sleptsova S.S., Lyashenko E.A., Sharoyko V.V. Molecular markers of hepatocellular carcinoma. perspectives of early diagnostics. *VICh-infektsiya i immunosupressii* 2014; 6(3): 16–23.

55. Trevisani F., D'Intino P.E., Morselli-Labate A.M., Mazzella G., Accogli E., Caraceni P., Domenicali M., De Notariis S., Roda E., Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; 34(4): 570–575, https://doi.org/10.1016/s0168-8278(00)00053-2.

56. Junna Z., Gongde C., Jinying X., Xiu Z. Serum AFU, 5'-NT and AFP as biomarkers for primary hepatocellular carcinoma diagnosis. *Open Med (Wars)* 2017; 12(1): 354–358, https://doi.org/10.1515/med-2017-0051.

57. Hsu H.C., Cheng W., Lai P.L. Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. *Cancer Res* 1997; 57(22): 5179–5184.

58. Capurro M., Wanless I.R., Sherman M., Deboer G., Shi W., Miyoshi E., Filmus J. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; 125(1): 89–97, https://doi.org/10.1016/s0016-5085(03)00689-9.

59. Qiao S.S., Cui Z.Q., Gong L., Han H., Chen P.C., Guo L.M., Yu X., Wei Y.H., Ha S.A., Kim J.W., Jin Z.T., Li S., Peng J.R., Leng X.S. Simultaneous measurements of serum AFP, GPC-3 and HCCR for diagnosing hepatocellular carcinoma. *Hepatogastroenterology* 2011; 58(110–111): 1718–1724, https://doi.org/10.5754/hge11124.

60. Tangkijvanich P., Chanmee T., Komtong S., Mahachai V., Wisedopas N., Pothacharoen P., Kongtawelert P. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. *J Gastroenterol Hepatol* 2010; 25(1): 129–137, https://doi.org/10.1111/j.1440-1746.2009.05988.x.

61. Jia X., Liu J., Gao Y., Huang Y., Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. *Arch Med Res* 2014; 45(7): 580–588, https://doi.org/10.1016/j.arcmed.2014.11.002.

62. Haruyama Y., Kataoka H. Glypican-3 is a prognostic factor and an immunotherapeutic target in hepatocellular carcinoma. *World J Gastroenterol* 2016; 22(1): 275–283, https://doi.org/10.3748/wjg.v22.i1.275.

63. Montalbano M., Georgiadis J., Masterson A.L., McGuire J.T., Prajapati J., Shirafkan A., Rastellini C., Cicalese L. Biology and function of glypican-3 as a candidate for early cancerous transformation of hepatocytes in

hepatocellular carcinoma (review). *Oncol Rep* 2017; 37(3): 1291–1300, https://doi.org/10.3892/or.2017.5387.

64. Xu D., Su C., Sun L., Gao Y., Li Y. Performance of serum glypican 3 in diagnosis of hepatocellular carcinoma: a meta-analysis. *Ann Hepatol* 2018; 18(1): 58–67, https://doi.org/10.5604/01.3001.0012.7863.

65. Marrero J.A., Romano P.R., Nikolaeva O., Steel L., Mehta A., Fimmel C.J., Comunale M.A., D'Amelio A., Lok A.S., Block T.M. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. *J Hepatol* 2005; 43(6): 1007–1012, https://doi.org/10.1016/j.jhep.2005.05.028.

66. Hu J.S., Wu D.W., Liang S., Miao X.Y. GP73, a resident Golgi glycoprotein, is sensibility and specificity for hepatocellular carcinoma of diagnosis in a hepatitis B-endemic Asian population. *Med Oncol* 2010; 27(2): 339–345, https://doi.org/10.1007/s12032-009-9215-y.

67. Jiao C., Cui L., Piao J., Qi Y., Yu Z. Clinical significance and expression of serum Golgi protein 73 in primary hepatocellular carcinoma. *J Cancer Res The*r 2018; 14(6): 1239–1244, https://doi.org/10.4103/0973-1482.199784.

68. Giannelli G., Marinosci F., Trerotoli P., Volpe A., Quaranta M., Dentico P., Antonaci S. SCCA antigen combined with alpha-fetoprotein as serologic markers of HCC. *Int J Cancer* 2005; 117(3): 506–509, https://doi.org/10.1002/ijc.21189.

69. Giannelli G., Fransvea E., Trerotoli P., Beaugrand M., Marinosci F., Lupo L., Nkontchou G., Dentico P., Antonaci S. Clinical validation of combined serological biomarkers for improved hepatocellular carcinoma diagnosis in 961 patients. *Clin Chim Acta* 2007; 383(1–2): 147–152, https://doi.org/10.1016/j.cca.2007.05.014.

70. Witjes C.D., van Aalten S.M., Steyerberg E.W., Borsboom G.J., de Man R.A., Verhoef C., Ijzermans J.N. Recently introduced biomarkers for screening of hepatocellular carcinoma: a systematic review and meta-analysis. *Hepatol Int* 2013; 7(1): 59–64, https://doi.org/10.1007/s12072-012-9374-3.

71. Pozzan C., Cardin R., Piciocchi M., Cazzagon N., Maddalo G., Vanin V., Giacomin A., Pontisso P., Cillo U., Farinati F. Diagnostic and prognostic role of SCCA-IgM serum levels in hepatocellular carcinoma (HCC). *J Gastroenterol Hepatol* 2014; 29(8): 1637–1644, https://doi.org/10.1111/jgh.12576.

72. Liu C.H., Gil-Gómez A., Ampuero J., Romero-Gómez M. Diagnostic accuracy of SCCA and SCCA-IgM for hepatocellular carcinoma: a meta-analysis. *Liver Int* 2018; 38(10): 1820–1831, https://doi.org/10.1111/liv.13867.

73. Beneduce L., Castaldi F., Marino M., Quarta S., Ruvoletto M., Benvegnù L., Calabrese F., Gatta A., Pontisso P., Fassina G. Squamouse cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. *Cancer* 2005; 103(12): 2558–2565, https://doi.org/10.1002/cncr.21106.

74. Kim J., Ki S.S., Lee S.D., Han C.J., Kim Y.C., Park S.H., Cho S.Y., Hong Y.J., Park H.Y., Lee M., Jung H.H., Lee K.H., Jeong S.H. Elevated plasma osteopontin levels in patients with hepatocellular carcinoma. *Am J Gastroenterol* 2006; 101(9): 2051–2059, https://doi.org/10.1111/j.1572-0241.2006.00679.x.

75. Shang S., Plymoth A., Ge S., Feng Z., Rosen H.R., Sangrajrang S., Hainaut P., Marrero J.A., Beretta L. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2012; 55(2): 483–490, https://doi.org/10.1002/hep.24703.

76. Wan H.G., Xu H., Gu Y.M., Wang H., Xu W., Zu M.H. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; 38(6): 706–714, https://doi.org/10.1016/j.clinre.2014.06.008.

77. Ge T., Shen Q., Wang N., Zhang Y., Ge Z., Chu W., Lv X., Zhao F., Zhao W., Fan J., Qin W. Diagnostic values of alpha-fetoprotein, dickkopf-1, and osteopontin for hepatocellular carcinoma. *Med Oncol* 2015; 32(3): 59, https://doi.org/10.1007/s12032-014-0367-z.

78. Ji N.Y., Park M.Y., Kang Y.H., Lee C.I., Kim D.G., Yeom Y.I., Jang Y.J., Myung P.K., Kim J.W., Lee H.G., Kim J.W., Lee K., Song E.Y. Evaluation of annexin II as a potential serum marker for hepatocellular carcinoma using a developed sandwich ELISA method. *Int J Mol Med* 2009; 24(6): 765–771, https://doi.org/10.3892/ijmm_00000290.

79. Sun Y., Gao G., Cai J., Wang Y., Qu X., He L., Liu F., Zhang Y., Lin K., Ma S., Yang X., Qian X., Zhao X. Annexin A2 is a discriminative serological candidate in early hepatocellular carcinoma. *Carcinogenesis* 2013; 34(3): 595–604, https://doi.org/10.1093/carcin/bgs372.

80. Shaker M.K., Abdel Fattah H.I., Sabbour G.S., Montasser I.F., Abdelhakam S.M., El Hadidy E., Yousry R., El Dorry A.K. Annexin A2 as a biomarker for hepatocellular carcinoma in Egyptian patients. *World J Hepatol* 2017; 9(9): 469–476, https://doi.org/10.4254/wjh.v9.i9.469.

81. Toyoda H., Kumada T., Tada T., Kaneoka Y., Maeda A., Kanke F., Satomura S. Clinical utility of highly sensitive lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/ml. *Cancer Sci* 2011; 102(5): 1025–1031, https://doi.org/10.1111/j.1349-7006.2011.01875.x.

82. Oda K., Ido A., Tamai T., Matsushita M., Kumagai K., Mawatari S., Saishoji A., Kure T., Ohno K., Toyokura E., Imanaka D., Moriuchi A., Uto H., Oketani M., Hashiguchi T., Tsubouchi H. Highly sensitive lens culinaris agglutinin-reactive α-fetoprotein is useful for early detection of hepatocellular carcinoma in patients with chronic liver disease. *Oncol Rep* 2011; 26(5): 1227–1233, https://doi.org/10.3892/or.2011.1425.

83. Tung E.K., Ng I.O. Significance of serum DKK1 as a diagnostic biomarker in hepatocellular carcinoma. *Future Oncol* 2012; 8(12): 1525–1528, https://doi.org/10.2217/fon.12.147.

84. Shen Q., Fan J., Yang X.R., Tan Y., Zhao W., Xu Y., Wang N., Niu Y., Wu Z., Zhou J., Qiu S.J., Shi Y.H., Yu B., Tang N., Chu W., Wang M., Wu J., Zhang Z., Yang S., Gu J., Wang H., Qin W. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. *Lancet Oncol* 2012; 13(8): 817–826, https://doi.org/10.1016/s1470-2045(12)70233-4.

85. Zhang J., Zhao Y., Yang Q. Sensitivity and specificity of dickkopf-1 protein in serum for diagnosing hepatocellular carcinoma: a meta-analysis. *Int J Biol Markers* 2014; 29(4): 403–410, https://doi.org/10.5301/jbm.5000101.

86. Van Hees S., Michielsen P., Vanwolleghem T. Circulating predictive and diagnostic biomarkers for hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol* 2016; 22(37): 8271–8282, https://doi.org/10.3748/wjg.v22.i37.8271.

87. Qin Q.F., Weng J., Xu G.X., Chen C.M., Jia C.K. Combination of serum tumor markers dickkopf-1, DCP and AFP for the diagnosis of primary hepatocellular carcinoma. *Asian Pac J Trop Med* 2017; 10(4): 409–413, https://doi.org/10.1016/j.apjtm.2017.03.016.

88. Reichl P., Fang M., Starlinger P., Staufer K., Nenutil R., Muller P., Greplova K., Valik D., Dooley S., Brostjan C., Gruenberger T., Shen J., Man K., Trauner M., Yu J., Gao C.F., Mikulits W. Multicenter analysis of soluble Axl reveals diagnostic value for very early stage hepatocellular carcinoma. *Int J Cancer* 2015; 137(2): 385–394, https://doi.org/10.1002/ijc.29394.

89. Dengler M., Staufer K., Huber H., Stauber R., Bantel H., Weiss K.H., Starlinger P., Pock H., Klöters-Plachky P., Gotthardt D.N., Rauch P., Lackner C., Stift J., Brostjan C., Gruenberger T., Kumada T., Toyoda H., Tada T., Weiss T.S., Trauner M., Mikulits W. Soluble Axl is an accurate biomarker of cirrhosis and hepatocellular carcinoma development: results from a large scale multicenter analysis. *Oncotarget* 2017; 8(28): 46234–46248, https://doi.org/10.18632/oncotarget.17598.

90. Zhu W.-W., Guo J.-J., Guo L., Jia H.L., Zhu M., Zhang J.B., Loffredo C.A., Forgues M., Huang H., Xing X.J., Ren N., Dong Q.Z., Zhou H.J., Ren Z.G., Zhao N.Q., Wang X.W., Tang Z.Y., Qin L.X., Ye Q.H. Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma. *Clin Cancer Res* 2013; 19(14): 3944–3954, https://doi.org/10.1158/1078-0432.ccr-12-3363.

91. Shaheen K.Y., Abdel-Mageed A.I., Safwat E., AlBreedy A.M. The value of serum midkine level in diagnosis of hepatocellular carcinoma. *Int J Hepatol* 2015; 2015: 146389, https://doi.org/10.1155/2015/146389.

92. Vongsuvanh R., van Der Poorten D., Iseli T., Strasser S.I., McCaughan G.W., George J. Midkine increases diagnostic yield in AFP negative and NASH-related hepatocellular carcinoma. *PLoS One* 2016; 11(5): e0155800, https://doi.org/10.1371/journal.pone.0155800.

93. Zheng T., Chen M., Han S., Zhang L., Bai Y., Fang X., Ding S.Z., Yang Y. Plasma minichromosome maintenance complex component 6 is a novel biomarker for hepatocellular carcinoma patients. *Hepatol Res* 2014; 44(13): 1347–1356, https://doi.org/10.1111/hepr.12303.

94. Li J., Cheng Z.J., Liu Y., Yan Z.L., Wang K., Wu D., Wan X.Y., Xia Y., Lau W.Y., Wu M.C., Shen F. Serum thioredoxin is a diagnostic marker for hepatocellular carcinoma. *Oncotarget* 2015; 6(11): 9551–9563, https://doi.org/10.18632/oncotarget.3314.

95. Chounta A., Ellinas C., Tzanetakou V., Pliarhopoulou F., Mplani V., Oikonomou A., Leventogiannis K., Giamarellos-Bourboulis E.J. Serum soluble urokinase plasminogen activator receptor as a screening test for the early diagnosis of hepatocellular carcinoma. *Liver Int* 2015; 35(2): 601–607, https://doi.org/10.1111/liv.12705.

96. Gupta S., Bent S., Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; 139(1): 46–50, https://doi.org/10.7326/0003-4819-139-1-200307010-00012.

97. Yang J.D., Dai J., Singal A.G., Gopal P., Addissie B.D., Nguyen M.H., Befeler A.S., Reddy K.R., Schwartz M., Harnois D.M., Yamada H., Gores G.J., Feng Z., Marrero J.A., Roberts L.R. Improved performance of serum alpha-fetoprotein for hepatocellular carcinoma diagnosis in HCV cirrhosis with normal alanine transaminase. *Cancer Epidemiol Biomarkers Prev* 2017; 26(7): 1085–1092, https://doi.org/10.1158/1055-9965.epi-16-0747.

98. Sauzay C., Petit A., Bourgeois A.M., Barbare J.C., Chauffert B., Galmiche A., Houessinon A. Alpha-foetoprotein (AFP): a multi-purpose marker in hepatocellular carcinoma. *Clin Chim Acta* 2016; 463: 39–44, https://doi.org/10.1016/j.cca.2016.10.006.

99. Yen C.W., Kuo Y.H., Wang J.H., Chang K.C., Kee K.M., Hung S.F., Chen Y., Tsai L.S., Chen S.C., Hung C.H., Lu S.N. Did AFP-L3 save ultrasonography in community screening? *Kaohsiung J Med Sci* 2018; 34(10): 583–587, https://doi.org/10.1016/j.kjms.2018.05.005.

100. McMahon B.J., Bulkow L., Harpster A., Snowball M., Lanier A., Sacco F., Dunaway E., Williams J. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. *Hepatology* 2000; 32(4 Pt 1): 842–846, https://doi.org/10.1053/jhep.2000.17914.

101. Sterling R.K., Jeffers L., Gordon F., Venook A.P., Reddy K.R., Satomura S., Kanke F., Schwartz M.E., Sherman M. Utility of lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2009; 7(1): 104–113, https://doi.org/10.1016/j.cgh.2008.08.041.

102. Unić A., Derek L., Duvnjak M., Patrlj L., Rakić M., Kujundžić M., Renjić V., Štoković N., Dinjar P., Jukic A., Grgurević I. Diagnostic specificity and sensitivity of PIVKAII, GP3, CSTB, SCCA1 and HGF for the diagnosis of hepatocellular carcinoma in patients with alcoholic liver cirrhosis. *Ann Clin Biochem* 2018; 55(3): 355–362, https://doi.org/10.1177/0004563217726808.

103. Hemken P.M., Sokoll L.J., Yang X., Dai J., Elliott D., Gawel S.H., Lucht M., Feng Z., Marrero J.A., Srivastava S., Chan D.W., Davis G.J. Validation of a novel model for the early detection of hepatocellular carcinoma. *Clin Proteomics* 2019; 16: 2, https://doi.org/10.1186/s12014-018-9222-0.

104. Yamashiki N., Sugawara Y., Tamura S., Kaneko J., Yoshida H., Aoki T., Hasegawa K., Akahane M., Ohtomo K., Fukayama M., Koike K., Kokudo N. Diagnostic accuracy of alpha-fetoprotein and des-gamma-carboxy prothrombin in screening for hepatocellular carcinoma in liver transplant candidates. *Hepatol Res* 2011; 41(12): 1199–1207, https://doi.org/10.1111/j.1872-034x.2011.00871.x.

105. Best J., Bilgi H., Heider D., Schotten C., Manka P., Bedreli S., Gorray M., Ertle J., van Grunsven L.A., Dechene A. The GALAD scoring algorithm based on AFP, AFP-L3, and DCP significantly improves detection of BCLC early stage hepatocellular carcinoma. *Z Gastroenterol* 2016; 54(12): 1296–1305, https://doi.org/10.1055/s-0042-119529.

106. Wu J., Xiang Z., Bai L., He L., Tan L., Hu M., Ren Y. Diagnostic value of serum PIVKA-II levels for BCLC early hepatocellular carcinoma and correlation with HBV DNA. *Cancer Biomark* 2018; 23(2): 235–242, https://doi.org/10.3233/cbm-181402.

107. Yu R., Tan Z., Xiang X., Dan Y., Deng G. Effectiveness of PIVKA-II in the detection of hepatocellular carcinoma based on real-world clinical data. *BMC Cancer* 2017; 17(1): 608, https://doi.org/10.1186/s12885-017-3609-6.

108. Shuang Z., Mao Y., Lin G., Wang J., Huang X., Chen J., Duan F., Li S. Alpha-L-fucosidase serves as a prognostic indicator for intrahepatic cholangiocarcinoma and inhibits its invasion capacity. *Biomed Res Int* 2018; 2018: 8182575, https://doi.org/10.1155/2018/8182575.

109. López Vivancos J., Segura R.M., Oliva G., Vives L., Pascual C., Vilaseca J. Value of the serum measurement of alpha-L-fucosidase in the diagnosis of hepatocarcinoma. *Med Clin (Barc)* 1989; 93(7): 241–243.

110. Ishizuka H., Nakayama T., Matsuoka S., Gotoh I., Ogawa M., Suzuki K., Tanaka N., Tsubaki K., Ohkubo H., Arakawa Y., Okano T. Prediction of the development of hepato-cellular-carcinoma in patients with liver cirrhosis by the serial determinations of serum alpha-L-fucosidase activity. *Intern Med* 1999; 38(12): 927–931, https://doi.org/10.2169/internalmedicine.38.927.

111. Gotoh M., Nakatani T., Masuda T., Mizuguchi Y., Sakamoto M., Tsuchiya R., Kato H., Furuta K. Prediction of invasive activities in hepatocellular carcinomas with special reference to alpha-fetoprotein and des-gamma-carboxyprothrombin. *Jpn J Clin Oncol* 2003; 33(10): 522–526, https://doi.org/10.1093/jjco/hyg096.

112. Attallah A.M., El-Far M., Omran M.M., Abdelrazek M.A., Attallah A.A., Saeed A.M., Farid K. GPC-HCC model: a combination of glybican-3 with other routine parameters improves the diagnostic efficacy in hepatocellular carcinoma. *Tumor Biol* 2016; 37(9): 12571–12577, https://doi.org/10.1007/s13277-016-5127-6.

113. Majeed S., Mushtaq S., Azam M., Akhtar N., Hussain M., Loya A. Diagnostic accuracy of glypican-3 in differentiating hepatocellular carcinoma from metastatic liver tumours. *J Pak Med Assoc* 2018; 68(7): 1029–1031.

114. Liang R., Liu Z., Piao X., Zuo M., Zhang J., Liu Z., Li Y., Lin Y. Research progress on GP73 in malignant tumors. *Onco Targets Ther* 2018; 11: 7417–7421, https://doi.org/10.2147/ott.s181239.

115. Ismail M.M., Morsi H.K., Abdulateef N.A., Noaman M.K., Abou El-Ella G.A. Evaluation of prothrombin induced by vitamin K absence, macrophage migration inhibitory factor and Golgi protein-73 versus alpha fetoprotein for hepatocellular carcinoma diagnosis and surveillance. *Scand J Clin Lab Invest* 2017; 77(3): 175–183, https://doi.org/10.1080/00365513.2017.1286684.

116. Farag R.M.A., Al Ayobi D., Alsaleh K.A., Kwon H.J., EL-Ansary A., Dawoud E.A. Studying the impact of Golgi protein 73 serving as a candidate biomarker in early diagnosis for hepatocellular carcinoma among Saudi patients. *Asian Pac J Cancer Prev* 2019; 20(1): 215–220, https://doi.org/10.31557/apjcp.2019.20.1.215.

117. Xu W.J., Guo B.L., Han Y.G., Shi L., Ma W.S. Diagnostic value of alpha-fetoprotein-L3 and Golgi protein 73 in hepatocellular carcinomas with low AFP levels. *Tumor Biol* 2014; 35(12): 12069–12074, https://doi.org/10.1007/s13277-014-2506-8.

118. Guarino M., Di Costanzo G.G., Gallotta A., Tortora R., Paneghetti L., Auriemma F., Tuccillo C., Fassina G., Caporaso N., Morisco F. Circulating SCCA-IgM complex is a useful biomarker to predict the outcome of therapy in hepatocellular carcinoma patients. *Scand J Clin Lab Invest* 2017; 77(6): 448–453, https://doi.org/10.1080/00365513.2017.1336569.

119. Guido M., Roskams T., Pontisso P., Fassan M., Thung S.N., Giacomelli L., Sergio A., Farinati F., Cillo U., Rugge M. Squamous cell carcinoma antigen in human liver carcinogenesis. *J Clin Pathol* 2008; 61(4): 445–447, https://doi.org/10.1136/jcp.2007.051383.

120. Anwar S.L., Lehmann U. MicroRNAs: emerging novel clinical biomarkers for hepatocellular carcinomas. *J Clin Med* 2015; 4(8): 1631–1650, https://doi.org/10.3390/jcm4081631.

121. Cabiati M., Gaggini M., Cesare M.M., Caselli C., De Simone P., Filipponi F., Basta G., Gastaldelli A., Del Ry S. Osteopontin in hepatocellular carcinoma: a possible biomarker for diagnosis and follow-up. *Cytokine* 2017; 99: 59–65, https://doi.org/10.1016/j.cyto.2017.07.004.

122. Hao C., Cui Y., Owen S., Li W., Cheng S., Jiang W.G. Human osteopontin: potential clinical applications in cancer (review). *Int J Mol Med* 2017; 39(6): 1327–1337, https://doi.org/10.3892/ijmm.2017.2964.

123. Zhao H., Chen Q., Alam A., Cui J., Suen K.C., Soo A.P., Eguchi S., Gu J., Ma D. The role of osteopontin in the progression of solid organ tumour. *Cell Death Dis* 2018; 9(3): 356, https://doi.org/10.1038/s41419-018-0391-6.

124. Bruha R., Jachymova M., Petrtyl J., Dvorak K., Lenicek M., Urbanek P., Svestka T., Vitek L. Osteopontin: a non-invasive parameter of portal hypertension and prognostic marker of cirrhosis. *World J Gastroenterol* 2016; 22(12): 3441–3450, https://doi.org/10.3748/wjg.v22.i12.3441.

125. Fouad S.A., Mohamed N.A.G., Fawzy M.W., Moustafa D.A. Plasma osteopontin level in chronic liver disease and hepatocellular carcinoma. *Hepat Mon* 2015; 15(9): e30753, https://doi.org/10.5812/hepatmon.30753.

126. Yahya S.M.M., Fathy S.A., El-Khayat Z.A., El-Toukhy S.E., Hamed A.R., Hegazy M.G.A., Nabih H.K. Possible role of microRNA-122 in modulating multidrug resistance of hepatocellular carcinoma. *Indian J Clin Biochem* 2018; 33(1): 21–30, https://doi.org/10.1007/s12291-017-0651-8.

127. Lokman N.A., Ween M.P., Oehler M.K., Ricciardelli C. The role of annexin A2 in tumorigenesis and cancer progression. *Cancer Microenviron* 2011; 4(2): 199–208, https://doi.org/10.1007/s12307-011-0064-9.

128. Tressler R.J., Updyke T.V., Yeatman T., Nicolson G.L. Extracellular annexin II is associated with divalent cation-dependent tumor cell-endothelial cell adhesion of metastatic RAW117 large-cell lymphoma cells. *J Cell Biochem* 1993; 53(3): 265–276, https://doi.org/10.1002/jcb.240530311.

129. Díaz V.M., Hurtado M., Thomson T.M., Reventós J., Paciucci R. Specific interaction of tissue-type plasminogen activator (t-PA) with annexin II on the membrane of pancreatic cancer cells activates plasminogen and promotes invasion in vitro. *Gut* 2004; 53(7): 993–1000, https://doi.org/10.1136/gut.2003.026831.

130. Sharma M.R., Koltowski L., Ownbey R.T., Tuszynski G.P., Sharma M.C. Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. *Exp Mol Pathol* 2006; 81(2): 146–156, https://doi.org/10.1016/j.yexmp.2006.03.003.

131. Shiozawa Y., Havens A.M., Jung Y., Ziegler A.M., Pedersen E.A., Wang J., Wang J., Lu G., Roodman G.D., Loberg R.D., Pienta K.J., Taichman R.S. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J Cell Biochem* 2008; 105(2): 370–380, https://doi.org/10.1002/jcb.21835.

132. Lozada M.E., Chaiteerakij R., Roberts L.R. Screening for hepatocellular carcinoma and cholangiocarcinoma: can biomarkers replace imaging? *Curr Hepatol Rep* 2015; 14(2): 128–138, https://doi.org/10.1007/s11901-015-0261-y.

133. Li J., Gong W., Li X., Wan R., Mo F., Zhang Z., Huang P., Hu Z., Lai Z., Lu X., Zhao Y. Recent progress of Wnt pathway inhibitor dickkopf-1 in liver cancer. *J Nanosci Nanotechnol* 2018; 18(8): 5192–5206, https://doi.org/10.1166/jnn.2018.14636.

134. Zhou Y., Li W., Tseng Y., Zhang J., Liu J. Developing slow-off dickkopf-1 aptamers for early-diagnosis of

hepatocellular carcinoma. *Talanta* 2019; 194: 422–429, https://doi.org/10.1016/j.talanta.2018.10.014.

135. Pinato D.J., Mauri F.A., Lloyd T., Vaira V., Casadio C., Boldorini R.L., Sharma R. The expression of Axl receptor tyrosine kinase influences the tumour phenotype and clinical outcome of patients with malignant pleural mesothelioma. *Br J Cancer* 2013; 108(3): 621–628, https://doi.org/10.1038/bjc.2013.9.

136. Axelrod H., Pienta K.J. Axl as a mediator of cellular growth and survival. *Oncotarget* 2014; 5(19): 8818–8852, https://doi.org/10.18632/oncotarget.2422.

137. Byers L.A., Diao L., Wang J., Saintigny P., Girard L., Peyton M., Shen L., Fan Y., Giri U., Tumula P.K., Nilsson M.B., Gudikote J., Tran H., Cardnell R.J., Bearss D.J., Warner S.L., Foulks J.M., Kanner S.B., Gandhi V., Krett N., Rosen S.T., Kim E.S., Herbst R.S., Blumenschein G.R., Lee J.J., Lippman S.M., Ang K.K., Mills G.B., Hong W.K., Weinstein J.N., Wistuba I.I., Coombes K.R., Minna J.D., Heymach J.V. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. *Clin Cancer Res* 2013; 19(1): 279–290, https://doi.org/10.1158/1078-0432.ccr-12-1558.

138. D'Alfonso T.M., Hannah J., Chen Z., Liu Y., Zhou P., Shin S.J. Axl receptor tyrosine kinase expression in breast cancer. *J Clin Pathol* 2014; 67(8): 690–696, https://doi.org/10.1136/jclinpath-2013-202161.

139. Dunne P.D., McArt D.G., Blayney J.K., Kalimutho M., Greer S., Wang T., Srivastava S., Ong C.W., Arthur K., Loughrey M., Redmond K., Longley D.B., Salto-Tellez M., Johnston P.G., Van Schaeybroeck S. AXL is a key regulator of inherent and chemotherapy-induced invasion and predicts a poor clinical outcome in early-stage colon cancer. *Clin Cancer Res* 2014; 20(1): 164–175, https://doi.org/10.1158/1078-0432.ccr-13-1354.

140. Paccez J.D., Vogelsang M., Parker M.I., Zerbini L.F. The receptor tyrosine kinase Axl in cancer: biological functions and therapeutic implications. *Int J Cancer* 2014; 134(5): 1024–1033, https://doi.org/10.1002/ijc.28246.

141. Hodeib H., ELshora O., Selim A., Sabry N.M., El-Ashry H.M. Serum midkine and osteopontin levels as diagnostic biomarkers of hepatocellular carcinoma. *Electron Physician* 2017; 9(1): 3492–3498, https://doi.org/10.19082/3492.

142. Mashaly A.H., Anwar R., Ebrahim M.A., Eissa L.A., El Shishtawy M.M. Diagnostic and prognostic value of talin-1 and midkine as tumor markers in hepatocellular carcinoma in Egyptian patients. *Asian Pac J Cancer Prev* 2018; 19(6): 1503–1508.

143. Lindner K., Gregán J., Montgomery S., Kearsey S.E. Essential role of MCM proteins in premeiotic DNA replication. *Mol Biol Cell* 2002; 13(2): 435–444, https://doi.org/10.1091/mbc.01-11-0537.

144. Liu M., Hu Q., Tu M., Wang X., Yang Z., Yang G., Luo R. MCM6 promotes metastasis of hepatocellular carcinoma via MEK/ERK pathway and serves as a novel serum biomarker for early recurrence. *J Exp Clin Cancer Res* 2018; 37(1): 10, https://doi.org/10.1186/s13046-017-0669-z.

145. Liu Z., Li J., Chen J., Shan Q., Dai H., Xie H., Zhou L., Xu X., Zheng S. MCM family in HCC: MCM6 indicates adverse tumor features and poor outcomes and promotes S/G2 cell cycle progression. *BMC Cancer* 2018; 18(1): 200, https://doi.org/10.1186/s12885-018-4056-8.

146. Laurent T.C., Moore E.C., Reichard P. Enzymatic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from Escherichia Coli B. *J Biol Chem* 1964; 239: 3436–3444.

147. Mollbrink A., Jawad R., Vlamis-Gardikas A., Edenvik P., Isaksson B., Danielsson O., Stål P., Fernandes A.P. Expression of thioredoxins and glutaredoxins in human hepatocellular carcinoma: correlation to cell proliferation, tumor size and metabolic syndrome. *Int J Immunopathol Pharmacol* 2014; 27(2): 169–183, https://doi.org/10.1177/039463201402700204.

148. Gunes A., Bagirsakci E., Iscan E., Cakan-Akdogan G., Aykutlu U., Senturk S., Ozhan G., Erdal E., Nart D., Barbet F.Y., Atabey N. Thioredoxin interacting protein promotes invasion in hepatocellular carcinoma. *Oncotarget* 2018; 9(96): 36849–36866, https://doi.org/10.18632/oncotarget.26402.

149. Cho S.Y., Kim S., Son M.J., Rou W.S., Kim S.H., Eun H.S., Lee B.S. Clinical significance of the thioredoxin system and thioredoxin-domain-containing protein family in hepatocellular carcinoma. *Dig Dis Sci* 2019; 64(1): 123–136, https://doi.org/10.1007/s10620-018-5307-x.

150. Fox R., Berhane S., Teng M., Cox T., Tada T., Toyoda H., Kumada T., Kagebayashi C., Satomura S., Johnson P.J. Biomarker-based prognosis in hepatocellular carcinoma: validation and extension of the BALAD model. *Br J Cancer* 2014; 110(8): 2090–2098, https://doi.org/10.1038/bjc.2014.130.

151. Johnson P.J., Pirrie S.J., Cox T.F., Berhane S., Teng M., Palmer D., Morse J., Hull D., Patman G., Kagebayashi C., Hussain S., Graham J., Reeves H., Satomura S. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1): 144–153, https://doi.org/10.1158/1055-9965.epi-13-0870.

152. Berhane S., Toyoda H., Tada T., Kumada T., Kagebayashi C., Satomura S., Schweitzer N., Vogel A., Manns M.P., Benckert J., Berg T., Ebker M., Best J., Dechêne A., Gerken G., Schlaak J.F., Weinmann A., Wörns M.A., Galle P., Yeo W., Mo F., Chan S.L., Reeves H., Cox T., Johnson P. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. *Clin Gastroenterol Hepatol* 2016; 14(6): 875–886, https://doi.org/10.1016/j.cgh.2015.12.042.

153. Kotha S., Neong S., Patel K. Serum biomarkers for diagnosis and monitoring viral hepatitis and hepatocellular carcinoma. *Expert Rev Mol Diagn* 2018; 18(8): 713–722, https://doi.org/10.1080/14737159.2018.1496020.