SCREENING FOR HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CHRONIC VIRAL HEPATITIS IN UZBEKISTAN

K.S. Brigida[1], A.S. Khikmatullaeva[1], N.M. Mirrakhimova[1], M.E. Khodjaeva[2], N.S. Ibadullaeva[1], M.A. Abdukadirova[1]

Research Institute of Virology of the of the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases, Tashkent, Uzbekistan[1]
Tashkent State Medical University, Tashkent, Uzbekistan[2]
https://doi.org/10.5281/zenodo.18627905

Abstract. *Chronic viral hepatitis B, C, and D are important risk factors for hepatocellular carcinoma (HCC), and screening patients with chronic viral hepatitis for tumor markers is essential for early diagnosis. This study aimed to improve early detection of HCC among patients with chronic viral hepatitis. A total of 121 patients with HCC, 300 patients with liver cirrhosis resulting from viral hepatitis, and 90 patients with chronic viral hepatitis without liver cirrhosis were enrolled. Analysis of HCC etiology showed that hepatitis B virus infection was present in 45% of patients and hepatitis C virus infection in 25%. According to the TNM classification, most patients were diagnosed at advanced stages (stages III and IV), while stage I HCC was not detected. Screening of 300 patients with viral hepatitis–related liver cirrhosis using PIVKA-II and AFP revealed an HCC detection rate of 11%. Additionally, in 2.2% of patients with chronic viral hepatitis without liver cirrhosis, early-stage HCC was detected through PIVKA-II screening. These findings indicate that all patients with chronic viral hepatitis and viral cirrhosis should undergo regular ultrasound examination and tumor marker testing, and that screening of all patients with chronic viral hepatitis can enable early detection of HCC, timely treatment, and improved survival outcomes.*

Keywords: *HCC, liver cirrhosis, viral hepatitis, AFP, PIVKA-II*

Introduction

Hepatocellular carcinoma (HCC) represents the most common histotype of liver cancer accounting for 80-85% of all cases of liver cancer (1). HCC is the fourth most common cause of cancer-related death in the worldwide (2). Although HCC does not top the list of the most common cancers, the high mortality rate is associated with poor operability, high likelihood of recurrence after surgical resection, and poor response to conventional treatment, making HCC a global health problem (3). Studies of natural history in untreated patients have shown that the annual incidence of HCC is 0.3-0.6% in patients without liver cirrhosis and 2.2-3.7% in patients with compensated liver cirrhosis, with the highest rates in Asia (4). Actually, despite the variety of approaches to HCC therapy, the 5-year survival rate does not reach 15% (5). This is due to the fact that most cases of HCC are detected at advanced stages, when resection or transplantation is not possible and only palliative treatment is available (6). The increase of HCC morbidity since the late 1990s is due to the growth of the population and the number of elderly people among them (7). Patient

categories and screening methods are discussed in numerous publications, the main ones being the joint guidelines and recommendations of European Association for the Study of the Liver (EASL), American Association for the Study of Liver Diseases (AASLD) and the European Organization for Research and Treatment of Cancer (EORTC) (9-11). Patients with liver cirrhosis who have a risk of HCC ≥1.5%/year and hepatitis B virus (HBV) carriers without liver cirrhosis who have a risk of HCC ≥0.2%/year should be monitored. HBV carriers are at risk for HCC even without liver cirrhosis. Although the risk of HCC is reduced in patients with hepatitis C virus (HCV) infection who have a sustained virologic response to direct-acting antivirals, patients with liver cirrhosis are still at risk of HCC and should continue monitoring using imaging examinations (12).

One of the commonly used methods for HCC screening is liver ultrasound, despite the fact that the sensitivity of the method varies, according to different authors, from 33% to 96% (13). Patients with indications for HCC screening are recommended to have ultrasound examination with an interval of 6 months. The above recommendations are due to the fact that tumor node size increases 2-fold in the time interval from 80 to 117 days (14). More frequent ultrasound (every 3 months) does not give advantages in detecting small-sized HCC nodules, increasing the intervals between examinations up to 9-12 months, on the contrary, reduces the sensitivity of the method (15).

Most researchers have focused their activities on determining the diagnostic and prognostic role of biomarkers in the progression of HCC. At the same time, the data of studies aimed at predicting HCC before the progression of clinical manifestations of the tumor are practically absent. Thus, the World Gastroenterological Organization (WGO) practice guidelines on HCC recommend alpha-fetoprotein (AFP) as the main marker (16). Retrospective analysis has shown that the optimal threshold values that provide the best likelihood ratio of a positive result are 200 ng/mL (17). Another tumor marker of HCC is des-gamma-carboxyprothrombin, which is an altered form of prothrombin expressed due to impaired post-translational carboxylation caused by vitamin K deficiency (18). This biomarker is also referred to as protein induced by vitamin K deficiency (PIVKA-II), which represents abnormal, deprived prothrombin activity (19). Prothrombin, which contains 10 γ-carboxyglutamic acid residues in the amino-terminal domain, is synthesized in the liver.

Prothrombin is a vitamin K-dependent clotting factor. Vitamin K deficiency can inhibit the activity of vitamin K-dependent carboxylase, resulting in an acquired defect in the posttranslational carboxylation of glutamine on the prothrombin precursor. As a result, abnormal prothrombin (PIVKA-II) is released into the plasma without γ-carboxylation of all glutamine residues. The correlation between PIVKA-II levels and HCC was first reported by Liebman et al. in 1984 (20). Further studies showed that PIVKA-II can be a useful diagnostic and prognostic marker of HCC (21). The use of the described tumor marker for the diagnosis of HCC is widespread in East Asia, North America and China (22-24). Numerous studies have shown that PIVKA-II is applicable for HCC surveillance and has been included in the recommendations of the Japanese Society of Hepatology (25, 26). Volk et al. reported that PIVKA-II was significantly better than total AFP or AFP-L3 in differentiating HCC from liver cirrhosis, with a sensitivity of 86% and specificity of 93% (27). The aim of the study was to improve early diagnosis of HCC in patients with chronic viral hepatitis.

Methods.

Patient enrollment criteria were: age older than 18 years, patient consent, diagnosis of HBV, HBV+HD confirmed by ELISA or PCR, and HCV confirmed by PCR. Exclusion criteria

were patient age under 18 years, refusal of the study and pregnancy. To identify the etiology of HCC, 121 patients with a confirmed diagnosis of HCC hospitalized at the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSPMCOR) were enrolled in the study. For HCC screening 300 patients with liver cirrhosis of viral etiology (LC HBV - 100 patients, LC HCV - 100 patients, LC HBV+HDV - 100 patients) and 90 patients with chronic viral hepatitis without liver cirrhosis hospitalized at the Research Institute of Virology (RIV) were examined. This study was approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan No. 6/31-881 dated September 25, 2018 in accordance with the Declaration of Helsinki.

Patients with increased levels of tumor markers were examined by ultrasound and 4-phase MSCT. In case of confirmation of a volumetric tumor, patients were referred for additional consultation with an oncologist. AFP level was determined by ELISA method using AFP-IFA-BEST kit "Vector Best" (Russian Federation) using Biotech spectrophotometer. To determine the level of PIVKA-II by immunochemiluminescent analysis, we used the PIVKA-II 100 test Maglumi kit (China) on the analyzer Maglumi 800 (Snibe, China). Statistical analysis was carried out with determination of mean values of indicators (M), calculation of standard errors of mean values (m) and using Student's t-test, Chi-square test and Fisher's exact test. Differences were considered significant at $p<0.05$.

Results

To establish the etiology of HCC, we retrospectively analyzed the data of 121 patients with HCC whose diagnosis was confirmed by histological methods. The results of the study showed a prevalence of viral etiology of HCC. Patients with HCC in HBV outcome were 55 (45%), with HCC in HCV outcome 30 (25%) and with HCC of non-viral etiology 36 (30%). In 70% of cases HCC developed in the outcome of viral hepatitis. According to TNM classification, all patients were predominantly classified as stages 3 and 4 and stage 1 was not detected. A pilot viral hepatitis elimination program was started in Uzbekistan in 2019-2020. Within the framework of this project, 53841 people were tested for HBsAg and anti-HCV using rapid tests. The prevalence of HBsAg and anti-HCV was 2.8% (1633/53841) and 2.96% (1727/53841), respectively. Furthermore, HCC was detected by ultrasound in 5 HBsAg positive patients and 3 anti-HCV positive patients. All patients were aware of their status, had advanced stage of liver cirrhosis and 3-4 stages of HCC by TNM.

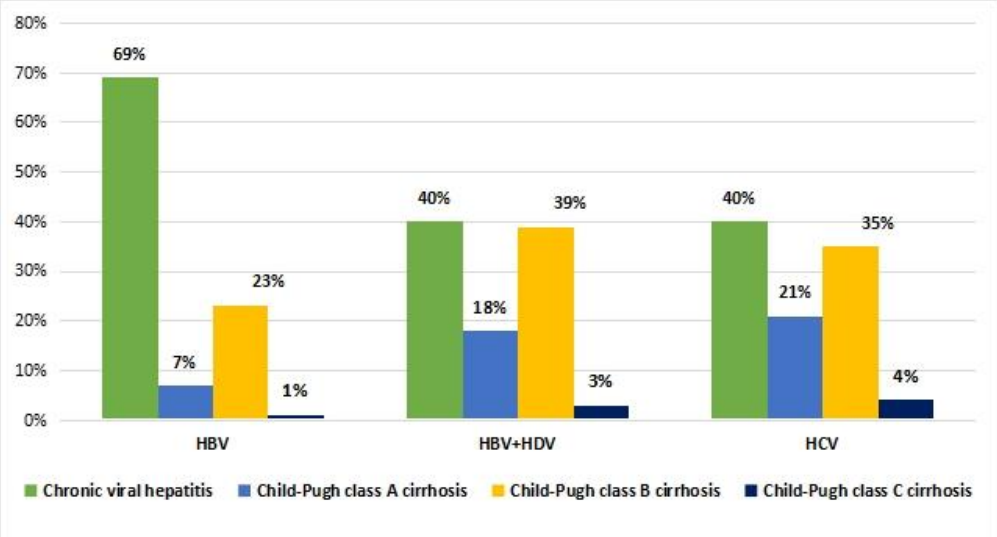


Figure 1: Distribution of patients according to the Child-Pugh classification.

Consequently, the issue of early diagnosis of HCC was of high priority and, in this regard, screening of patients with liver cirrhosis of HBV, HCV, HBV+HDV etiology for early diagnosis of HCC hospitalized at the Research Institute of Virology was carried out. 300 patients with liver cirrhosis of viral etiology were examined for PIVKA-II and AFP tumor markers. The mean age of patients with LC of HCV etiology was 55.83±3.56 years, LC of HBV etiology - 55.43±4.22 years and LC of HBV+HDV etiology - 53.38±3.88 years and there was no significant difference in the age of patients among the examined patients. However, the analysis of the distribution of patients by gender showed prevalence of male with LC of HBV etiology in comparison with females (61 vs. 39 $p<0.05$), while there was no significant statistical difference in LC of HCV (male - 50, female - 50) and LC of HBV+HDV etiology (male - 45, female - 55). The distribution of Child-Pugh classes of liver cirrhosis is shown in Figure 1.

The results of screening for HCC in patients with liver cirrhosis of viral etiology using different methods showed the highest frequency of HCC detection using PIVKA-II (Fig.2).

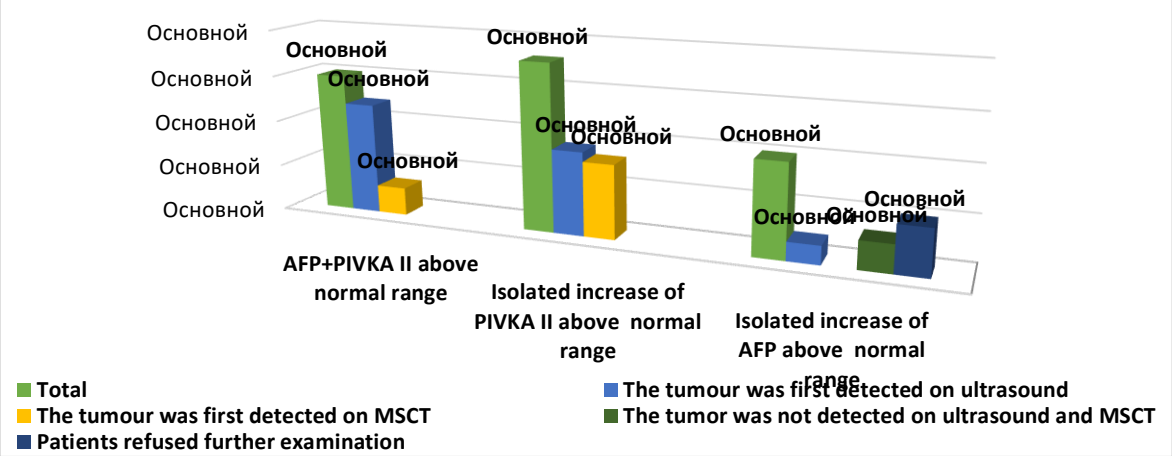


Figure 2: Different methods of screening for HCC in patients with liver cirrhosis of viral etiology

Among 100 LC of HBV etiology patients examined for tumor markers, 4 (4%) had PIVKA-II+AFP levels higher than the normal range. Of these, 3 patients had a volumetric tumor confirmed by ultrasound, and 1 patient had a tumor not visualized by ultrasound but confirmed by MSCT. Of the 4 (4%) patients with isolated increase of PIVKA-II, 2 patients had a volumetric tumor on ultrasound examination, and 2 patients had a volumetric tumor detected by MSCT. In this group, there was not detected an isolated increase of AFP. Of 100 patients with LC of HCV etiology, 6 (6%) had PIVKA-II+AFP higher than the normal range. Of these, 5 patients had a volumetric tumor visualized on ultrasound and 1 patient on MSCT. Isolated increase of PIVKA-II occurred in 8 (8%) patients, of which 4 patients had a volumetric tumor visualized by ultrasound and 4 patients by MSCT. Of the 5 (5%) patients who had an isolated increase of AFP, only 1 patient had a volumetric tumor detected by ultrasound, 2 patients did not have a tumor on MSCT, and 2 patients refused further examination.

Among 100 patients with LC of HBV+HDV etiology, the level of PIVKA-II+AFP was higher than the normal range in 5 (5%) patients. Of these, 4 patients had a volumetric tumor visualized by ultrasound and 1 patient by MSCT. Of the 5 (5%) patients with increased PIVKA-II levels, 3 patients had a volumetric tumor visualized by ultrasound and 2 patients had a volumetric tumor visualized by MSCT. Of the 5 (5%) patients with an isolated increase of AFP, 1 patient had a volumetric tumor identified on ultrasound examination, 1 patient did not have a volumetric tumor identified on MSCT, and 3 patients refused further evaluation.

It is important to note that the frequency of HCC in liver cirrhosis of viral etiology was 11%. At the same time, all patients had tumors of stage 1 or 2 according to TNM. In comparative analysis, the mean tumor node size detected by ultrasound/MSCT in the RIV patient group was significantly smaller than the mean size of HCC in RSPMCOR inpatients who participated in this study, being 31.59±11.68 mm vs. 115.13±36.17 mm. ($p<0.05$), respectively. Next, we compared the frequency of TPR (true positive result), FPR (false positive result), TNR (true negative result) and FNR (false negative result), as well as Se (sensitivity), Sp (specificity), AUC (area under the curve), PPV (positive predictive value) and PNV (negative predictive value) of the studied tumor markers and their combinations in the compared groups (Table 1).

As can be seen from Table 1, the highest number of TPR was in PIVKA II and AFP compared to AFP L3. At the same time, the number of TPR in AFP was slightly lower than in PIVKA II. However, the FPR was higher in AFP than in PIVKA II and AFP L3. PIVKA II had the highest Se, and AFP L3 had the lowest. Sp was equally high for AFP L 3 and PIVKA II, unlike AFP. The highest AUC was observed in PIVKA II. The highest PPV was observed in PIVKA II and AFP L3, compared to AFP. And the highest NPV was observed in PIVKA II compared to AFP and AFP L3. In order to improve diagnostics, we compared the combination of the studied tumor markers. The TPR was the same in the combinations of AFP + PIVKA II and AFP + AFP L3 + PIVKA II and was slightly higher than the FPR of the combination of AFP L3 + PIVKA II. The FPR was lower in the combination of AFP L3 + PIVKA II, compared to AFP + PIVKA II and AFP + AFP L3 + PIVKA II. It should be noted that all FPR were associated with AFP.

Table 1: Indicators of TPR, LPR, TNR, FPR, sensitivity, specificity, PPV, NPV and AUC

Indicators	AFP	AFP L3	PIVKA II	AFP+ PIVKA II	PIVKA II+ AFP L3	AFP+ AFP L3+ PIVKA II
TPR (abs)	21	17	23	26	24	26
FPR (abs)	12	0	0	12	0	12
TNR (abs)	46	58	58	46	58	46
FNR (abs)	9	13	7	4	6	4
Se (%)	70	56,67	76,67	86,67	80	86,67
Sp (%)	79,31	100	100	79,31	100	79,31
AUC	0,747	0,783	0,883	0,83	0,9	0,83
PPV (%)	63,34	100	100	68,42	100	68,42
NPV (%)	83,64	81,69	100	92	90,62	92

AFP + PIVKA II and AFP + AFP L3 + PIVKA II had the highest Se, and PIVKA II + AFP L3 had the lowest. Sp, AUC and PPV were higher for the PIVKA II + AFP L3 combination compared to the AFP + PIVKA II and AFP + AFP L3 + PIVKA II combinations. And NPV was slightly higher for the AFP + PIVKA II and AFP + AFP L3 + PIVKA II combinations. Thus, the use of PIVKA-II+AFP combination as diagnostic tumor markers of HCC in patients with chronic viral hepatitis showed higher sensitivity compared to the use of only one tumor marker and can be recommended for HCC screening in patients with HBV, HCV and HDV infections. In addition, the introduction of the combination of tumor markers in patients with liver cirrhosis of viral etiology into routine practice will allow early detection of HCC.

We further investigated 90 patients with chronic viral hepatitis without liver cirrhosis (chronic HCV - 24 patients, chronic HBV - 45 patients and chronic HBV+HDV - 21 patients). The

mean age of patients with chronic HCV infection was 55.83±3.56 years, chronic HBV infection - 55.43±4.22 years and chronic HBV+HDV infection - 53.38±3.88 years ($p>0.05$). Chronic HBV infection was most common in females relative to males (33 vs. 12 $p<0.05$), and there was no statistical significance in chronic HBV infection (males - 10, females - 14), chronic HBV+HDV infection (males - 11, females -10). Among the examined patients, PIVKA-II level was increased in 2 patients with chronic HCV and HBV+HDV infection. Of them, 1 patient had a volumetric tumor detected on ultrasound and later the diagnosis of HCC was confirmed, and 1 patient had a volumetric tumor that was not detected on ultrasound but later confirmed on MSCT. Thus, HCC was detected in 2.2% of patients with chronic viral hepatitis, and the size of the tumor node did not exceed 5 cm, i.e. the tumor was operable.

Discussion

In recent years, screening of HBV and HCV infection is becoming important (28). According to the practice guidelines of the World Gastroenterological Organization, primary prevention is aimed at eliminating factors contributing to the progression of HCC, primarily HBV and HCV infection (29). A study by Perez et al. emphasized the importance of epidemiological surveillance of HBV and HCV infection and timely therapy of these infections (16). Vaccination against hepatitis B is an important element in the prevention of HBV and consequently HCC. It is known that vaccination against HBV is 95% effective in preventing infection (30). In 2008, the Centers for Disease Control and Prevention and in 2014, the United States Preventive Services Taskforce recommended hepatitis B vaccination for persons born in areas where hepatitis B virus prevalence exceeds 2% (31). A population-based study conducted in Taiwan found that the morbidity of HCC was 4-fold higher in the unvaccinated group compared with those who received the vaccine at birth. The study used data from patients with HCC diagnosed between 1983 and 2011, with the age of HCC debut ranged within 6-26 years. In Uzbekistan, hepatitis B vaccination of newborns began in 2001. In 2011, the hepatitis B vaccination rate was 99% (https://www.globalhep.org/country-progress/uzbekistan).

Another important fact of HCC decrease is the use of antiviral therapy. A meta-analysis including 13 clinical trials and 6350 patients with HBV-associated HCC after resection or ablation showed fewer recurrences and improved survival with the use of nucleos(t)ide analogs (NA), but the authors emphasize that the risk of HCC cannot be completely eliminated by NA, especially in the presence of liver cirrhosis (32). There is still no precise answer to the question at what point patients with eliminated HCV infection can be excluded from the risk group of HCC progression. The risk of HCC formation decreases after treatment compared to untreated patients (33). However, HCV elimination is not a guarantee of HCC prevention, especially if liver cirrhosis has already progressed (15). The inflammatory process caused by viruses plays an important role in the long-term irreversible damage of hepatocytes and, consequently, in the progression of the cancerous process. The anti-tumor function of immunity is a well-known fact. There is a thin line between antitumor and pro-tumor function of the immune system. Direct-acting antiviral drugs (DAADs) modify the function of natural killer cells and the manifestation of interferon action. The use of DAADs promotes rapid decrease of hepatitis C viral load, on the one hand, and disruption of antitumor mechanisms, on the other hand. There is an opinion that disruption of the immune response system can stimulate the formation of metastases (34). Thus, all patients with chronic infection caused by hepatitis B or C virus are candidates for permanent HCC screening.

The results of the study on the etiology of hepatocellular carcinoma (HCC) in this study show that 70% of cases are due to viral hepatitis (45% - HBV and 25% - HCV), consistent with

the data of the world scientific community. Thus, according to the literature, chronic HBV is the cause of approximately 50%-80% of all cases of HCC, especially in regions where HBV is endemic, such as Asia and sub-Saharan Africa. Chronic HCV infection also contributes significantly to HCC, particularly in Western countries where it is the leading cause of liver cancer. A comprehensive review highlighted that chronic HBV and HCV infections lead to cirrhosis in 80%-90% of HCC patients, supporting the observation that a substantial portion of HCC cases in Uzbekistan is linked to these viral infections. The lifetime risk for HBV carriers to develop HCC ranges from 15% to 40%, depending on various factors including the presence of cirrhosis (35,36).

The predominance of advanced stages in the Uzbekistan study reflects global trends where many patients are diagnosed at late stages due to asymptomatic progression of the disease. Studies have shown that early detection remains a challenge, with many patients presenting with advanced disease (37). The higher prevalence of male patients with HBV-related liver cirrhosis noted in the study is consistent with global findings that report a higher incidence of HCC among males compared to females, attributed to various biological and lifestyle factors (38,39).

Secondary prevention of HCC is based on the implementation of screening programs to directly detect of HCC (40). Despite the lack of randomized data, several cohort studies have found a strong and consistent association between HCC screening and improved 3-year survival (41). Considering the limitations of the widespread use of imaging methods for HCC screening, especially in remote regions, there is a need for alternative strategies, such as biomarkers, to increase the sensitivity of early tumor detection.

Currently, the GALAD scale based on sex, age, AFP-L3, AFP and PIVKA-II are widely used worldwide (42). However, the difficulty in using this scale is that the scale cannot be used in the absence of data on one of the indicators. We declined to use the AFP-L3 for two reasons. Firstly, it is a rather expensive tumor marker. Secondly, AFP-L3 level is directly related from total AFP. However, up to 1/3 of HCCs do not produce AFP and, consequently, AFP-L3 (38).

A study from 2007 found that serial measurements of both PIVKA-II and AFP could be beneficial for monitoring patients after curative resection for HCC. In this cohort, PIVKA-II showed a higher sensitivity (75.1%) compared to AFP (48.6%) during initial diagnosis (43).

A 2023 study evaluated the diagnostic properties of PIVKA-II and AFP in a cohort of high-risk patients for HCC. The results indicated that PIVKA-II had a sensitivity of 80.80%, compared to 75.80% for AFP. When both markers were combined, the sensitivity improved to 60.30% for detecting HCC, with a receiver operating characteristic (ROC) curve showing a significant advantage for the combination over AFP alone (44). These findings support our results and underscore the potential of PIVKA-II as a valuable biomarker for HCC detection.

Another study published in 2024 focused on the diagnostic accuracy of PIVKA-II in identifying recurrent HCC after curative resection. The findings demonstrated that PIVKA-II outperformed AFP significantly, with an area under the curve (AUC) of 0.883 for PIVKA-II compared to 0.672 for AFP (45).

Regarding AFP-L3, our observation of its high specificity but lower sensitivity is consistent with earlier literature. A review by Gomaa et al. (2006) reported that AFP-L3 sensitivity can reach 80–90% for tumors >5 cm but only 35–45% for tumors <2 cm. This aligns with our findings and highlights the potential limitations of AFP-L3 as a standalone marker, especially for early-stage HCC detection (46).

Kudo emphasized the urgent need for incorporating PIVKA-II measurements into routine surveillance protocols for HCC, particularly in cases where traditional markers may fail (47). This

reflects a growing consensus on the importance of utilizing multiple tumor markers to improve detection rates across diverse patient populations.

Our specific findings on marker combinations, such as PIVKA-II + AFP-L3 having higher specificity than other combinations, while AFP + PIVKA-II had higher sensitivity, are partially supported by recent literature. A 2024 meta-analysis by Li et al. found that combining PIVKA-II and AFP improved HCC detection accuracy in HBV-related Caucasian cirrhotic patients. This supports our conclusion that the PIVKA-II + AFP combination shows promise for HCC screening in viral hepatitis patients (48).

This study showed that HCC screening using a combination of tumor markers AFP+PIVKA-II is optimal. It is important to note that the detection rate of HCC in liver cirrhosis reaches 11%. In the screening study, HCC was detected at stage I, and according to world literature data, five-year survival in HCC significantly depends on the stage of the disease (39). At early stages, it reaches 65.8–72.1% with surgery or radiofrequency ablation. At the same time, at late stages with metastases to lymph nodes or distant organs, this figure decreases to 10% or lower (49).

These data emphasize the importance of early diagnosis to increase the chances of long-term survival and successful treatment. In our studies, HCC was also detected at early stages in 2.2% of patients with chronic viral hepatitis using PIVKA-II. Of course, these data are preliminary, since they are the result of examining a relatively small cohort of patients.Thus, all patients with chronic viral hepatitis and liver cirrhosis of viral etiology should have an ultrasound and tumor marker testing. A nodular tumor larger than 1 cm on ultrasound should be considered malignant and confirmed with 4-phase MSCT or MRT. If PIVKA-II is increased above the normal range, a 4-phase MSCT/MRT study is recommended regardless of ultrasound results. Patients with AFP levels above 200 ng/mL are recommended to be screened for HCC with MSCT/MRT regardless of ultrasound results. All patients with normal tumor markers and absence of volumetric tumors or node less than 1 cm on ultrasound are recommended screening for tumor markers and ultrasound every 6 months. In the Republic of Uzbekistan according to this principle it is planned to examine all patients with chronic viral hepatitis, which will improve early detection of HCC, provide timely treatment, improve survival and quality of life of patients.

Conclusions

Screening Protocols: Implement routine screening for HCC in patients with liver cirrhosis, particularly those with viral hepatitis (HBV, HCV, HBV+HDV). Use ultrasound and serum tumor markers (PIVKA-II and AFP) as part of the screening process.

Consider screening patients with chronic viral hepatitis without cirrhosis, especially if they exhibit risk factors such as advanced age or a family history of liver disease.

Utilization of Tumor Markers: Employ the combination of PIVKA-II and AFP as diagnostic tumor markers for HCC. This combination has shown higher sensitivity compared to using a single marker. Monitor these markers regularly in patients with liver cirrhosis to facilitate early detection of HCC.

Patient Education: Educate patients about the importance of regular check-ups and monitoring for liver health, especially those diagnosed with chronic hepatitis or cirrhosis.

Ensure that patients understand their risk factors for developing HCC and the significance of early detection.

Referral to Specialists: Refer patients with elevated tumor markers or suspicious ultrasound findings to hepatologists or oncologists for further evaluation and management.

Ensure timely access to imaging studies such as MSCT for accurate diagnosis when indicated.

REFERENCES

1. Nevola R, Rinaldi L, Giordano M, Marrone A, Adinolfi LE. Mechanisms and clinical behavior of hepatocellular carcinoma in HBV and HCV infection and alcoholic and non-alcoholic fatty liver disease. Hepatoma Res. 2018;4:55. DOI:10.20517/2394-5079.2018.38.
2. Global Cancer Observatory (2018). https://gco.iarc.fr/today/home.
3. Petruzziello A. Suppl-1, M3: epidemiology of hepatitis B virus (HBV) and hepatitis C virus (HCV) related hepatocellular carcinoma. The open virology journal. 2018;12:26. DOI: 10.2174/1874357901812010026.
4. Kim H, El-Serag H. The epidemiology of hepatocellular carcinoma in the USA. Current gastroenterology reports. 2019;21:1-8. DOI: 10.1007/s11894-019-0681-x.
5. El Serag H, "Hepatocellular carcinoma,". N Engl J Med. 2011;365(12):1118-27. DOI: 10.1056/NEJMra1001683
6. Saitta C, Raffa G, Alibrandi A, Brancatelli S, Lombardo D, Tripodi G, et al. PIVKA-II is a useful tool for diagnostic characterization of ultrasound-detected liver nodules in cirrhotic patients. Medicine. 2017;96(26). DOI: 10.1097/MD.0000000000007266.
7. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA oncology. 2017;3(4):524-48. DOI: 10.1001/jamaoncol.2016.5688.
8. Hsu L, Bowlus CL, Stewart SL, Nguyen TT, Dang J, Chan B, et al. Electronic messages increase hepatitis B screening in at-risk Asian American patients: a randomized, controlled trial. Digestive diseases and sciences. 2013;58:807-14; DOI: 10.1007/s10620-012-2396-9.
9. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology (Baltimore, Md). 2011;53(3):1020. DOI: 10.1002/hep.24199.
10. Davenport MS, Khalatbari S, Liu PS, Maturen KE, Kaza RK, Wasnik AP, et al. Repeatability of diagnostic features and scoring systems for hepatocellular carcinoma by using MR imaging. Radiology. 2014;272(1):132-42; DOI: 10.1148/radiol.14131963.
11. Bae SH. Changes of guidelines diagnosing hepatocellular carcinoma during the last ten-year period. Clinical and molecular hepatology. 2012;18(3):25. DOI: 10.3350/cmh.2012.18.3.258.
12. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Hepatology. 2018;68(2):723-50. DOI: 10.1002/hep.29913.
13. Arif-Tiwari H, Kalb B, Chundru S, Sharma P, Costello J, Guessner RW, et al. MRI of hepatocellular carcinoma: an update of current practices. Diagnostic and Interventional Radiology. 2014;20(3):209. DOI: 10.5152/dir.2014.13370.
14. An C, Choi YA, Choi D, Paik YH, Ahn SH, Kim M-J, et al. Growth rate of early-stage hepatocellular carcinoma in patients with chronic liver disease. Clinical and molecular hepatology. 2015;21(3):279. DOI: 10.3350/cmh.2015.21.3.279.

15. Walker DR, Pedrosa MC, Manthena SR, Patel N, Marx SE. Early view of the effectiveness of new direct-acting antiviral (DAA) regimens in patients with hepatitis C virus (HCV). Advances in therapy. 2015;32:1117-27. DOI: 10.1007/s12325-015-0258-5.
16. Ferenci P, Fried M, Labrecque D, Bruix J, Sherman M, Omata M, et al. Hepatocellular carcinoma (HCC): a global perspective. Arab J Gastroenterol. 2010;11(3):174-9. DOI: 10.1097/MCG.0b013e3181d46ef2.
17. Toan BN, Tan C-K, Hasan I, Setiawan L, Yu M-L, Izumi N, et al. Utility of combining PIVKA-II and AFP in the surveillance and monitoring of hepatocellular carcinoma in the Asia-Pacific region. Clinical and molecular hepatology. 2023;29(2):277. DOI: 10.3350/cmh.2022.0212.
18. Unić A, Derek L, Duvnjak M, Patrlj L, Rakić M, Kujundžić M, et al. Diagnostic specificity and sensitivity of PIVKAII, GP3, CSTB, SCCA1 and HGF for the diagnosis of hepatocellular carcinoma in patients with alcoholic liver cirrhosis. Annals of Clinical Biochemistry. 2018;55(3):355-62. DOI: 10.1177/0004563217726808.
19. Hemken PM, Sokoll LJ, Yang X, Dai J, Elliott D, Gawel SH, et al. Validation of a novel model for the early detection of hepatocellular carcinoma. Clinical proteomics. 2019;16(1):1-9. DOI: 10.1186/s12014-018-9222-0.
20. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N. Engl. J. Med. 1984;310:1427–31. DOI: 10.1056/NEJM198405313102204.
21. Poté N, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, et al. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. Journal of hepatology. 2015;62(4):848-54. DOI: 10.1016/j.jhep.2014.11.005.
22. Best J, Bilgi H, Heider D, Schotten C, Manka P, Bedreli S, et al. The GALAD scoring algorithm based on AFP, AFP-L3, and DCP significantly improves detection of BCLC early stage hepatocellular carcinoma. Zeitschrift für Gastroenterologie. 2016;54(12):1296-305. DOI: 10.1055/s-0042-119529.
23. Wu J, Xiang Z, Bai L, He L, Tan L, Hu M, et al. Diagnostic value of serum PIVKA-II levels for BCLC early hepatocellular carcinoma and correlation with HBV DNA. Cancer Biomarkers. 2018;23(2):235-42. DOI: 10.3233/CBM-181402.
24. Yamashiki N, Sugawara Y, Tamura S, Kaneko J, Yoshida H, Aoki T, et al. Diagnostic accuracy of α-fetoprotein and des-γ-carboxy prothrombin in screening for hepatocellular carcinoma in liver transplant candidates. Hepatology Research. 2011;41(12):1199-207. DOI: 10.1111/j.1872-034X.2011.00871.x.
25. Kudo M, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, et al. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. Digestive diseases. 2011;29(3):339-64. DOI: 10.1159/000111702.
26. Kudo M. Japan's successful model of nationwide hepatocellular carcinoma surveillance highlighting the urgent need for global surveillance. Liver cancer. 2012;1(3-4):141. DOI: 10.1159/000342749.
27. Volk ML, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. Cancer Biomarkers. 2007;3(2):79-87. DOI: 10.3233/cbm-2007-3202.

28. Cabibbo G, Maida M, Genco C, Antonucci M, Cammà C, editors. Causes of and prevention strategies for hepatocellular carcinoma. Seminars in oncology; 2012: Elsevier. DOI: 10.1053/j.seminoncol.2012.05.006.
29. Ferenci P., Fried M., Labrecque D., Bruix J., et al. Hepatocellular carcinoma (HCC): a global perspective. Journal of clinical gastroenterology. 2010;44(4):239-45. DOI: 10.1097/MCG.0b013e3181d46ef2.
30. Orlando R, Foggia M, Maraolo A, Mascolo S, Palmiero G, Tambaro O, et al. Prevention of hepatitis B virus infection: from the past to the future. European Journal of Clinical Microbiology & Infectious Diseases. 2015;34:1059-70. DOI: 10.1007/s10096-015-2341-x.
31. Abara WE, Qaseem A, Schillie S, McMahon BJ, Harris AM, Physicians HVCTFotACo, et al. Hepatitis B vaccination, screening, and linkage to care: best practice advice from the American College of Physicians and the Centers for Disease Control and Prevention. Annals of internal medicine. 2017;167(11):794-804. DOI: 10.7326/M17-1106.
32. Huang G, Lau WY, Wang Z-G, Pan Z-Y, Yuan S-X, Shen F, et al. Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. Ann Surg. 2015;261(1):56-66. DOI: 10.1097/SLA.0000000000000858.
33. Di Marco V, Calvaruso V, Ferraro D, Bavetta MG, Cabibbo G, Conte E, et al. Effects of eradicating hepatitis C virus infection in patients with cirrhosis differ with stage of portal hypertension. Gastroenterology. 2016;151(1):130-9. e2. DOI: 10.1053/j.gastro.2016.03.036.
34. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, et al. Unexpected early tumor recurrence in patients with hepatitis C virus-related hepatocellular carcinoma undergoing interferon-free therapy: a note of caution. J Hepatol. 2016;65(4):719-26. DOI: 10.1016/j.jhep.2016.10.035.
35. El-Serag H. B. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. – 2012. – Т. 142. – №. 6. – С. 1264-1273. DOI: 10.1053/j.gastro.2011.12.061.
36. Mak, L. Y., Cruz-Ramón, V., Chinchilla-López, P., Torres, H. A., LoConte, N. K., Rice, J. P., ... & Hwang, J. P. (2018). Global epidemiology, prevention, and management of hepatocellular carcinoma. American Society of Clinical Oncology Educational Book, 38, 262-279. DOI: 10.1200/EDBK_200939.
37. Vo Quang, E., Shimakawa, Y., & Nahon, P. (2021). Epidemiological projections of viral-induced hepatocellular carcinoma in the perspective of WHO global hepatitis elimination. Liver international, 41(5), 915-927. DOI: 10.1111/liv.14843.
38. Kim, D. Y. Changing etiology and epidemiology of hepatocellular carcinoma: Asia and worldwide. J Liver Cancer 2024; 24 (1): 62-70. DOI: 10.1111/liv.14843 DOI: 10.17998/jlc.2024.03.13.
39. Toh, M. R., Wong, E. Y. T., Wong, S. H., Ng, A. W. T., Loo, L. H., Chow, P. K. H., & Ngeow, J. (2023). Global epidemiology and genetics of hepatocellular carcinoma. Gastroenterology, 164(5), 766-782.
40. Feng H, Li B, Li Z, Wei Q, Ren L. PIVKA-II serves as a potential biomarker that complements AFP for the diagnosis of hepatocellular carcinoma. BMC cancer. 2021;21(1):1-10. DOI: 10.1186/s12885-021-08138-3.
41. Frenette CT, Isaacson AJ, Bargellini I, Saab S, Singal AG. A practical guideline for hepatocellular carcinoma screening in patients at risk. Mayo Clinic Proceedings: Innovations, Quality & Outcomes. 2019;3(3):302-10. DOI: 10.1016/j.mayocpiqo.2019.04.005.

42. Berhane S., Toyoda H., Tada, T., Kumada T., et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clinical Gastroenterology and Hepatology. 2016;14(6):875-86. DOI: 10.1016/j.cgh.2015.12.042.
43. Kim, D. Y., Paik, Y. H., Ahn, S. H., Youn, Y. J., Choi, J. W., Kim, J. K., ... & Han, K. H. (2007). PIVKA-II is a useful tumor marker for recurrent hepatocellular carcinoma after surgical resection. Oncology, 72(Suppl. 1), 52-57.
44. Suttichaimongkol, T., Mitpracha, M., Tangvoraphonkchai, K., Sadee, P., Sawanyawisuth, K., & Sukeepaisarnjaroen, W. (2023). PIVKA-II or AFP has better diagnostic properties for hepatocellular carcinoma diagnosis in high-risk patients. Journal of Circulating Biomarkers, 12, 12. DOI: 10.33393/jcb.2023.2453.
45. Zhu, W., Wang, W., Zheng, W., Chen, X., Wang, X., Xie, J., ... & Wang, G. (2024). Diagnostic performance of PIVKA-II in identifying recurrent hepatocellular carcinoma following curative resection: a retrospective cohort study. Scientific Reports, 14(1), 8416. DOI: 10.1159/000111707.
46. Wu, C. S., Lee, T. Y., Chou, R. H., Yen, C. J., Huang, W. C., Wu, C. Y., & Yu, Y. L. (2014). Development of a highly sensitive glycan microarray for quantifying AFP-L3 for early prediction of hepatitis B virus–related hepatocellular carcinoma. PLoS One, 9(6), e99959. DOI: 10.1371/journal.pone.0099959.
47. Kudo, M. (2024). Urgent Global Need for PIVKA-II and AFP-L3 Measurements for Surveillance and Management of Hepatocellular Carcinoma. Liver Cancer, 13(2), 113.DOI: 10.1159/000537897.
48. Loglio, A., Iavarone, M., Facchetti, F., Di Paolo, D., Perbellini, R., Lunghi, G., ... & Lampertico, P. (2020). The combination of PIVKA-II and AFP improves the detection accuracy for HCC in HBV caucasian cirrhotics on long-term oral therapy. Liver International, 40(8), 1987-1996. DOI: https://doi.org/10.1111/liv.14475/
49. Lindberg G, Hamid SS, Malfertheiner P, Thomsen OO, Fernandez LB, Garisch J, et al. World Gastroenterology Organisation global guideline: constipation. 2011;45(6):483-87. DOI: 10.1097/MCG.0b013e31820fb914.