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Revolution in the Surveillance of Hepatocellular Carcinoma (HCC): A Mini Review

Lai Thing Wei [1,6], Nur Karyatee Kassim[1,2,6], Julia Omar [2,6], Lee Yeong Yeh [3,6], Mohd Shafie Abdullah [4,6], Muhammad Hafiz Hanafi[5,6]

[1]Basic Sciences and Oral Biology Unit, School of Dental Sciences, Health Campus, UniversitiSains Malaysia, Malaysia
[2]Department of Chemical Pathology, School of Medical Sciences, Health Campus, UniversitiSains Malaysia, Malaysia
[3]Department of Internal Medicine, School of Medical Sciences, Health Campus, UniversitiSains Malaysia, Malaysia
[4]Department of Radiology, School of Medical Sciences, Health Campus, UniversitiSains Malaysia, Malaysia
[5]Department of Neurosains, School of Medical Sciences, Health Campus, UniversitiSains Malaysia, Malaysia
[6]Hospital UniversitiSains Malaysia, Health Campus, UniversitiSains Malaysia, Malaysia

Abstract— **Background:** Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, yet it is typically diagnosed late via imaging. PIVKA-II has recently gained renewed interest as a potential HCC surveillance tool, however literature before 2016 only involves small retrospective studies from western countries and Japan where the aetiology for HCC is HCV infection. **Aim:** This article aims to review studies on PIVKA-II to diagnose or survey for HCC in Asian countries where HBV is the main aetiology. A search was performed using Scopus, PubMed, and Web of Sciences database published from the year 2016 to 2021 focusing on studies on PIVKA-II for early HCC surveillance. **Result:** Seven articles including one review done were selected for this review. **Conclusion:** PIVKA-II is comparable to AFP in terms of sensitivity and specificity for early HCC diagnosis, but the combination of both has the best performance. PIVKA-II can be a promising surveillance tool for HCC, but more studies need to be done on the prognostic potential of PIVKA-II as well.

Keywords: Hepatocellular carcinoma, HCC, PIVKA-II, surveillance, AFP, biomarker, diagnosis, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver malignancy worldwide. According to World Health Organization (WHO), HCC is the sixth leading cause of newly diagnosed cancer cases (905 677 new cases) and the fourth leading cause of cancer death worldwide (830180 cases) (1). Unfortunately, HCC is also known as one of the deadliest cancers ever diagnosed with the number of deaths nearly equaling the number of newly diagnosed cases (2). This is because HCC is always detected late when curative treatment is no longer an option (30). Thus, HCC surveillance programme for at-risk target populations is very important for early HCC diagnosis and as a result, improving the long-term survival rate of HCC patients cost-effectively. On the other hand, Ultrasonography (USG) is the most widely accepted surveillance tool for HCC according to the most recent HCC surveillance guideline published in 2017 by the American Association for Study of Liver Disease (AASLD), the European Association for the Study of the

Liver (EASL) and the Asian Pacific Association for the Study of Liver Disease (APASL) (3,4,5). USG is highly accurate as evidenced by its sensitivity and specificity of 94%. However, for early staged tumours its sensitivity drops tremendously to 63% and specificity remains the same (6). Several limitations were identified such as the quality of the ultrasound machine, the operator's experience, the coarseness of the liver parenchyma due to underlying liver cirrhosis, the severe fatty liver background and the location of the lesion had significant impact on its sensitivity (7). Despite these limitations, USG is still widely used as the surveillance tool for HCC since there are no better tools superior to ultrasound (6). To date there are no studies showing that another tool is superior to ultrasound.

2. Serum Tumour Marker: Alpha-fetoprotein (AFP)

AFP is a glycoprotein made up of 591 amino acids and a carbohydrate moiety, that is produced by the yolk sac and embryonic liver during foetal development. The serum level rapidly decreases after birth and is completely absence by adulthood. It is the most ordered serum tumour marker for HCC, despite lack of evidence supporting its efficacy in HCC surveillance and diagnosis. The serum levels are elevated as a result of hepatocyte regeneration particularly in HCC and other liver pathologies. Due to its low sensitivity and specificity, AASLD 2010 guidelines discouraged the use of AFP as a surveillance tool. Its performance is significantly lower in early-stage HCC with up to 35% of small HCC (<2cm) showing normal serum AFP level (32). Some studies have found that serum AFP has a low sensitivity (49-71%) and specificity (49-86%) at a cut-off value of 20ng/ml for HCC lesions less than 5cm in size (10). It is also non-specific and it has been reported to be elevated in hepatitis c virus (HCV) infection, cholangiocarcinoma, and liver cirrhosis. This is the main reason why it was not recommended as an HCC surveillance marker tool in AASLD 2010 guidelines (8). Neither USG nor AFP are strong enough to be used alone as an HCC surveillance tool. There was no significant difference found between USG alone or in combination with AFP in terms of earlier HCC diagnosis (3). However, AASLD has reviewed all the evidence and has recommended that they should be used in combination. With the suboptimal performance of USG or AFP, this has led to the emergence of other potential HCC biomarkers, however, most of them have not been rigorously studied to date.

3. Serum Biomarker: Protein-induced by Vitamin K Absence or Antagonist II (PIVKA-II)

PIVKA is a prothrombin, with full capacity coagulation activity. It is synthesized in the liver via vitamin K dependent γ-glutamyl carboxylation as part of post-translational modification. Under normal conditions, the vitamin K-dependent enzyme, γ-glutamyl carboxylase, is responsible of for catalysing the complete conversion of 10 Glu residues in Gla domain of prothrombin precursor to 10 Gla residues. (12) PIVKA-II is synthesized under abnormal conditions whereby there is an absence of vitamin-K or presence of antagonist-II, with less than 10 Glu residues which are converted to Gla residues. Hence, PIVKA-II, also known as Des-γ-carboxy-prothrombin (DCP), is an abnormal prothrombin that lacks coagulation activity (11). Liebman et.al discovered PIVKA-II as a serum biomarker for primary HCC in 1984. A reduction in the activity of γ-glutamyl carboxylase on the prothrombin precursor was observed in HCC patients as compared to healthy subjects (13). The acquired defect in posttranslational carboxylation of the prothrombin precursor resulted in PIVKA-II formation by HCC. (14) PIVKA-II is produced by malignant hepatocytes and results from an acquired defect in posttranslational carboxylation of the prothrombin precursor (33)

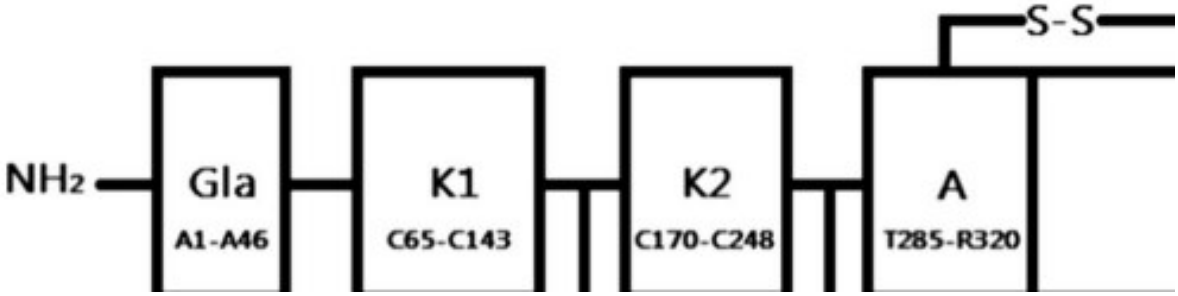

Diagram 1: Structure of PIVKA-II (Adopted from Hao Xing et al, 2016)

The management of HCC has advanced by leaps and bounds over the years, improving the quality of life for HCC patients. Despite this, the prognosis of HCC is still appalling, owing largely to the late presentation and diagnosis of the HCC (27). Hence, there is an urgent need in the medical community to develop an accurate, cost-effective surveillance method that can be implemented and accepted globally.

3.1 Relationship between PIVKA & HCC for Cancer Surveillance

Numerous studies had been conducted since the discovery of the link between PIVKA-II and HCC, focusing on the potential role of PIVKA-II as a serum biomarker for HCC in terms of screening, early diagnosis, and prognosis after treatment. However, PIVKA-II is still not widely accepted as a serum biomarker for HCC surveillance. Currently, only Japan and Taiwan use PIVKA-II in combination with AFP and AFP-L3 (lens culinaris agglutinin (LCA) bound fraction of AFP) as the standard screening tool in their national HCC surveillance programmes (15). Although there is insufficient evidence to prove that PIVKA-II has a higher sensitivity and specificity as compared to other markers. There were several studies that have shown that PIVKA-II has a higher sensitivity with a lower specificity when compared to AFP (ref). PIVKA-IIhad a sensitivity of 14-54% and specificity of 95-99% for tumours <5cm in size at a cut-off level of 40 mau/ml. (16) Thus, the combination of PIVKA-II and AFP has the potential to be used for the detection of HCC, especially in the early stages.

Hao Xing et.al conducted a review of the biological profile and clinical utility of PIVKA-II for diagnosis and treatment. They concluded that PIVKA-II could be the promising clinical serum biomarker for HCC due to its demonstrated sensitivity and specificity in combination with AFP at maximum 94 % and 98.5%. (17) Despite their careful analysis of the literature, Hao Xing et al stated that there is currently insufficient information to recommend the use of PIVKA-II for HCC surveillance. This is because the majority of studies available during the evaluation were conducted in Western countries and Japan, where the main causes of HCC were HCV infection and alcohol, as opposed to much of the rest of the world, where hepatitis Bvirus (HBV) infection is the predominant cause of HCC. The study encouraged greater research into HBV infection-related HCC around the world, particularly in China and other Asian countries.

4. Diagnostic performance of Serum PIVKA-II in HCC Diagnosis

In this review, we looked at the latest developments regarding the diagnostic performance of PIVKA-II in HCC, incorporating a variety of studies from Asian countries. The studies discussed here were studies conducted in China, Korea, India, and Italy. A search was performed using Scopus, PubMed, and Web of Sciences database published from the year 2016 to 2021. Seven articles were incorporated into this review focused on the potential of PIVKA-II as a surveillance tool for early HCC diagnosis.

Studies from China and India tried to compare the diagnostic performance of PIVKA-II with AFP in the early diagnosis of HCC. Most of the studies were cross-sectional studies, with most HCC patients having hepatitis B. Those who were supplemented with vitamin K or warfarin, positive for other viruses such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), or human immunodeficiency virus (HIV), pregnant, or had other malignancies were excluded from the study because these were factors that affected the accuracy of the PIVKA-II level. (28) These studies showed similarities whereby PIVKA-II is comparable to AFP, with higher sensitivity but equal or lower specificity in HCC diagnosis. The researchers discovered that the pathway of PIVKA-II synthesis is unrelated to AFP and these two serum biomarkers performed better in combination, especially for early-stage HCC (18, 19, 20). Table 1 compares the diagnostic performance of these 3 studies. Based on these studies, PIVKA-II can be used as a promising tool for early HCC diagnosis in combination with AFP especially where HBV infection is the main cause of HCC (29).

Table 1: Comparison of sensitivity and specificity of PIVKA-II, AFP or in combination.

	AFP	**PIVKA II**	**AFP+PIVKA II**
Shujing et al., 2017	Sensitivity (68%) Specificity (91%) Cut-off value (20ng/ml) AUC (87%, CI 84-89.7%)	Sensitivity (80%) Specificity (89%) Cut-off value (40mAU/ml) AUC (90.5%, CI 87.8-92.7%)	Sensitivity (87%) Specificity (82%) AUC (90.6%, CI 88-92.9%)
Xiao Qiong et al., 2017	Sensitivity (73.9%) Specificity (82.9%) Cut-off value (12.3ng/ml) AUC (82.9% CI 78.6-87.1%)	Sensitivity (82.4%) Specificity (95.9%) Cut-off value (40.5mAu/ml) AUC (92.3% CI 89.4-95.3%)	Sensitivity (87.5%) Specificity (87%) AUC (92.6%, CI 89.9-95.4%)
Siraj et al., 2019	Sensitivity (77.14%) Specificity (95%) Cut-off value (12.3ng/ml) AUC (88.8%)	Sensitivity (85.71%) Specificity (95%) Cut-off value (6.715ng/ml) AUC (95.3%)	Sensitivity (94.29%) Specificity (90%)

AUC: area under the curve
CI: confidence interval

Furthermore, the four-year retrospective case-control research conducted in China in 2016 found that the levels of PIVKA-II and AFP were unaffected by liver function or cirrhosis and can accurately predict HCC before it is clinically evident. Ren Tao Yu et al. used a nested case control study to see if PIVKA-II could detect HCC before symptoms appeared. They organized their research into two stages: discovery and validation. The primary goal of the discovery stage is to determine whether liver function parameters (alanine transaminase, aspartate transaminase, alkaline phosphate) influence serum levels of PIVKA-II and AFP in Hep B-associated HCC patients, and thus whether liver function parameters should be considered as an influencing factor on the HCC biomarkers. Their findings revealed no differences in liver function between HCC and control patients, confirming that the liver's functional abilities have no impact on PIVKA-II and AFP serum levels in the presence or absence of HCC. The study showed that AFP has better predictive and diagnostic performance than PIVKA-II at all time points for HCC, but a combination of both serum markers offers higher accuracy for early HCC diagnosis (21). The study further supported that PIVKA-II when combined with AFP can be used as a valuable surveillance and diagnostic tool for HCC.

Another study was done in Korea by Joon Park et al. 2017 added AFP-L3 (Lens culinaris-agglutinin-reactive fraction of AFP) to PIVKA-II and AFP, with the objective to establish the best tumour marker for early HCC diagnosis. AFP-L3 isoform is a subtype of AFP that is more specific for detecting HCC (26). The serum levels of PIVKA-II, AFP, and AFP-L3 in 79 newly diagnosed HCC patients (mixed aetiologies) and 77 non-HCC controls with liver cirrhosis were evaluated. The results showed that PIVKA-II had the best sensitivity (70.89%) of all three markers, while AFP had the highest specificity (81.82%). The combination of PIVKA-II and AFP yielded the highest accuracy with AUC (0.765, 95%, CI 0.708-0.823), sensitivity of 55.7%, and specificity of 97.4%. They also reported that AFP-L3 did not improve the diagnostic performance of HCC when combined with PIVKA-II and AFP (22). Hence, the combination of PIVKA-II and AFP remains the best group of serum biomarkers for HCC to date.

Most recently, Feng et al. in 2021 studied on evaluating PIVKA-II in detecting HBV-associated HCC. The best cut-off value they found for distinguishing HCC from healthy controls was 35.60mAU/ml for PIVKA-II, which had a sensitivity of 83.93 percent and a specificity of 91.50 percent. With a sensitivity of 64.29 percent and specificity of 90.20 percent, the optimum cut-off value for AFP was 17.76 ng/ml. The optimum cut-off value for PIVKA-II to identify HCC from benign liver disorders was 43.47mAU/ml, with sensitivity and specificity of 81.55 and 86.00 percent, respectively. The best cut-off value for AFP was 21.47 ng/ml, with 63.10 and 84.67 percent sensitivity and specificity, respectively. Serum PIVKA-II concentrations were found to have a strong relationship with tumour size, TNM stage, metastases, cell differentiation, and HCC consequences. These associations suggest that PIVKA-II may have a role in predicting HCC prognosis. PIVKA-II shows a greater difference between pre- and post-HCC resection, making it more sensitive than AFP in measuring HCC resection therapeutic response.

Table 2: Summary on studies of diagnostic performance of PIVKA-II among HCC patients

No.	Author	Objective	Type of Study	Conclusion	Limitations
1.	Rentao Yu et al., 2016	To determine if PIVKA-II can identify preclinical HCC	Retrospective case control study Discovery stage to confirm liver function does not affect PIVKA-II and AFP levels (15 HCC cases, 30 at-risk controls) Validation stage, where the actual study was done (36 HCC cases, 108 at-risk controls)	Both AFP and PIVKA-II can be used for HCC prediction. A combination of both AFP and PIVKA-II can be used for HCC diagnosis, but it is insufficient. AFP levels were the same in early and advanced HCC, while PIVKA-II is increased in advanced HCC compared against early HCC.	Retrospective study, Missing or unregistered data.
2.	Famei et al., 2019	To investigate the role of PIVKA-II, AFP, AFP-L3, CEA and combinations of these biomarkers in the diagnosis of primary and metastatic HCC	Retrospective case control study. Subjects: 120 primary HCC cases 115 metastatic HCC cases 89 chronic liver disease (CLD) 116 healthy subjects	PIVKA-II, AFP, AFP-L3 were increased in primary HCC compared against CLD, metastatic liver disease and healthy subjects. PIVKA-II is the best in discriminating CLD and primary HCC.	
3.	Viggiani et al., 2016	To measure AFP and PIVKA-II levels in Italian HCC patients against benign liver disease patients	Cross-sectional cohort study. Subjects: 60 HCC patients 60 benign liver disease patients 60 healthy subjects	Increases in AFP were not statistically different between HCC and benign liver disease. The PIVKA-II diagnostic performance is more superior to AFP. The combination of AFP and PIVKA-II is the best in discriminating between HCC and non-HCC.	Does not differentiate AFP and PIVKA-II levels between early and advanced stage HCC (no tumour staging). Most HCC subjects are secondary to HBV infection

4.	Shujing et al., 2017	To compare the diagnostic performance of AFP and PIVKA-II alone or in combination in early HCC detection.	Cross-sectional cohort study. Subjects: 132 HCC. 250 non-HCC liver disease (50 cirrhosis, 100 viral hepatitis, 50 metastatic hepatitis, 50 benign liver disease) 200 healthy subjects	PIVKA-II has the same diagnostic performance as AFP in the diagnosis of HCC. In the early-stage of HCC, the combination PIVKA-II and AFP performed better diagnostically. In HBsAg positive HCC patients, PIVKA-II shows better performance than AFP.	Small sample sized study
5.	Xiao Qiong et al., 2017	To evaluate PIVKA-II in detecting HBV-associated HCC.	Cross-sectional cohort study. Subjects: 176 Hep B-HCC 98 Hep B liver cirrhosis 92 non-cirrhotic chronic Hep B patients	PIVKA-II more accurate than AFP in differentiating HCC from cirrhosis and chronic Hep B infection. AFP is insufficient as a tumour marker in discriminating HCC from non-HCC. There was no difference in PIVKA-II between cirrhosis and chronic Hep B infection, while AFP was higher in cirrhosis patients compared to chronic Hep B patients.	Results not applicable to non-Hep B aetiology HCC A retrospective study was done in one centre only.
6.	Siraj et al., 2019	To evaluate the role of PIVKA-II in HCC diagnosis and the ability to distinguish HCC from cirrhosis	Cross-sectional cohort study Subjects. 35 HCC patients 35 cirrhotic patients	PIVKA-II is more sensitive than AFP in detecting HCC. There is a positive correlation between tumour size and serum PIVKA-II level.	Small sample size.
7.	Feng et al., 2021	To evaluate the diagnostic value of prothrombin induced by vitamin K deficiency or antagonist- II (PIVKA-II) as a potential	Prospective cohort study 168 HCC patients 150 benign liver disease patients 153 healthy controls	PIVKA-II in HCC patients is more than benign liver disease and healthy controls. PIVKA-II can be used as a potential biomarker for HCC. PIVKA-II similar in benign liver disease patients and healthy controls. PIVKA-II is equally with AFP in distinguishing HCC	Did not assess the relationship between PIVKA-II and survival of HCC.

		biomarker that complements α-fetoprotein (AFP) in HCC		from benign liver disease. PIVKA-II is more accurate than AFP in distinguishing HCC from healthy controls. PIVKA-II and AFP in combination have a better sensitivity and specificity in distinguishing HCC from BLD and/or healthy controls.	

5. Conclusion and Suggestions for The Future

PIVKA-II is not only comparable but even superior to AFP for HCC detection in terms of sensitivity and specificity. Almost all studies demonstrate that combining bothtumours markers will result in more accurate early detection of HCC. With this, we believed that PIVKA-II and AFP might be one of the standard surveillance tools for HCC diagnosis bringing new hope for HCC patients. However, this is not a universal outcome from all studies and there are enough conflicting results to warrant further research in identifying and eliminating confounding factors to serum PIVKA-II and AFP levels.

Several limitations were identified from all the studies mentioned above. Most of the studies we include in this review were from Asian countries such as China and India, where HBV infection is the main aetiology for HCC. Hence, the results obtained from these studies cannot be applied to all, as other non-viral aetiology of HCC such as non-fatty liver disease, alcoholic liver disease and aflatoxin exposure were not addressed. Furthermore, the studies above were single-centre studies with small number of patients and might not be representative of our population. Most of the studies retrospectively urging for more prospective research in this field.

Perhaps larger sample sized, multi-centered studies, with definite tumour staging, should be conducted in the future. More advanced studies are also required to determine the best cut-off value of PIVKA-II for standardized HCC diagnosis as PIVKA-II values vary by population around the world. The lack of adequate scientific evidence or guidelines to support PIVKA-II for the local clinical practice warrants more integrated PIVKA-II studies in the future. There is an urge for studies in the future, to study the prognostic performance of PIVKA-II in relation to treatment response as most of the current studies did not address the effect of PIVKA-II on the post-treatment survival rate of HCC patients.

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