Original Article

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Biochemical Predictors of Hepatocellular Carcinoma in Chronic Liver Disease: A Comparative Analysis of AFP and PIVKA-II

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) ranks among the deadliest cancers, and a sizeable proportion of tumors go undetected by alpha-fetoprotein (AFP). Protein induced by vitamin K absence or antagonist-II (PIVKA-II) has emerged as a possible substitute. We set out to compare how well PIVKA-II and AFP identify HCC in people with chronic liver disease (CLD).

Methodology: We conducted a case-control study across two tertiary care hospitals in Hyderabad, Pakistan, between August 2025 and August 2026, enrolling 335 adults with CLD, of whom 119 had HCC and 216 did not. ELISA was used to measure serum PIVKA-II and AFP. For each marker we calculated sensitivity, specificity, predictive values and the area under the ROC curve (AUC), and the two AUCs were then compared using the DeLong test.

Results: Cases had a median PIVKA-II of 4500 (IQR 6050) pg/mL against 40 (IQR 21) pg/mL in controls, and a median AFP of 40 (IQR 270) ng/mL against 10 (IQR 12) ng/mL ($p<0.001$ for both). Using a cut-off of 76.5 pg/mL, PIVKA-II reached an AUC of 0.98 (95% CI: 0.97–0.99), with 98.3% sensitivity and 96.8% specificity. AFP, at 47 ng/mL, gave an AUC of 0.96 (95% CI: 0.93–0.98), with 95.0% sensitivity and 90.3% specificity (DeLong $p=0.014$). When an HCC prevalence of 6.73% was assumed, the positive predictive value was 68.6% for PIVKA-II and 41.3% for AFP.

Conclusion: PIVKA-II separated HCC from non-malignant CLD a little more accurately than AFP, chiefly because it produced fewer false positives. Until prospective surveillance data are available, it is better used together with AFP than in place of it.

Keywords: *Alpha-Fetoproteins; Biomarkers, Carcinoma, Hepatocellular; Liver Diseases; Prothrombin; Tumor*

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INTRODUCTION

Worldwide, liver cancer is the sixth most frequently diagnosed malignancy and the third leading cause of cancer mortality; in 2022 it was responsible for approximately 7.8% of all deaths from cancer[1]. Most of these tumors are hepatocellular carcinoma (HCC), which usually arises on a background of chronic liver disease (CLD) due to hepatitis B or C, alcohol or metabolic dysfunction[1,2]. Outcome is largely decided by the stage at diagnosis. Early tumors can still be treated with curative intent by resection, ablation or transplantation, while patients who present late are largely limited to systemic or palliative options[2,3]. For this reason, international guidelines recommend surveillance every six months in high-risk groups, chiefly people with cirrhosis or chronic viral hepatitis[2,3]. Ultrasound remains the backbone of surveillance. Its yield for small lesions drops, though, when the patient is obese, when the cirrhotic liver is coarse, or when skilled operators are scarce, and a dependable blood test is needed alongside it[3,4]. For decades, alpha-fetoprotein (AFP) has served as the routine serum marker for HCC in high-risk patients. It performs poorly in early disease, however, and 25–40% of tumors are AFP-negative[5,6]. A raised AFP is also not always due to cancer. Regenerating hepatocytes secrete it during active hepatitis and in advanced cirrhosis, so its specificity is lowest in precisely the patients being monitored[7].

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The scale of the problem is clear from prospective work. Among 397 patients with cirrhosis who were followed for new HCC, AFP detected only 40.5% of tumors when specificity was fixed at 90%, whereas a single GALAD score, which combines sex, age, AFP-L3, AFP and PIVKA-II, detected 54.8%[8]. Gaps of this kind explain the ongoing interest in markers that add information, especially in patients whose AFP is normal.

PIVKA-II (protein induced by vitamin K absence or antagonist-II), also known as des-gamma-carboxy prothrombin (DCP), is a prothrombin variant in which gamma-carboxylation is incomplete. Tumor hepatocytes secrete large amounts of it, although modest elevations also occur in some benign liver diseases and with vitamin K deficiency[7]. The case for the marker has strengthened over time. Pooling 53 studies, one meta-analysis gave PIVKA-II an AUC of 0.89 compared with 0.78 for AFP, along with better sensitivity (0.71 vs 0.64) and specificity (0.90 vs 0.87)[9]. Another, based on 37 studies, reached a similar conclusion, including in early-stage HCC (summary AUROC 0.79 vs 0.74)[10]. In individual studies, the benefit is greatest for small tumors and for those that are AFP-negative[5,11]. PIVKA-II also rises with tumor burden, stage and vascular invasion[12], and the AFP and PIVKA-II combination has a higher AUC than either test used singly[12,13].

Several questions are still open. The pooled figures conceal considerable heterogeneity, there is no consensus PIVKA-II threshold, and cut-offs vary with the assay and the population[10]. Much of the evidence also originates in East Asia and Europe, with little from our region. Viral hepatitis underlies most HCC in Pakistan[14], and in a recent Peshawar hospital series approximately one in five patients with combined hepatitis B and C infection had HCC[15]. A Pakistani group has reported a population reference interval and a diagnostic cut-off for PIVKA-II, yet that work did not set the marker against AFP[16]. As a result, local clinicians have little evidence on whether PIVKA-II offers anything beyond the AFP test already in routine use. We therefore compared the diagnostic accuracy of PIVKA-II and AFP for HCC among patients with CLD attending two tertiary care hospitals in Pakistan.

METHODOLOGY

Study Design and Setting

We carried out this case-control study of diagnostic accuracy between August 2025 and August 2026 at two tertiary care hospitals in Hyderabad, Pakistan: Isra University Hospital and the Asian Institute of Medical Sciences (AIMS). Reporting follows the STARD 2015 checklist[17]. Participants were recruited consecutively using non-probability sampling.

Participants

We included adults above 18 years of age with CLD, defined as liver function tests that had remained abnormal for over 3 months plus ultrasound or histological evidence of chronic liver disease[18]. Each participant had abdominal B-mode ultrasound with color Doppler, performed by experienced radiologists who did not know the biomarker results. Nodules of uncertain nature (10–20 mm) were characterized further by four-phase contrast-enhanced CT or by MRI with a hepatobiliary agent, and the images were read using EASL criteria[2,4].

For each tumor we noted size, number, location and any vascular invasion. Following usual clinical practice, a patient with CLD was classed as a case when HCC was diagnosed on imaging and/or serum AFP exceeded 20 ng/mL; PIVKA-II had no role in this classification. Controls were patients with CLD in whom imaging showed no liver mass. We excluded patients with hepatic metastases from a primary tumor elsewhere, those on anticoagulants or vitamin K antagonists, pregnant or breastfeeding women, and anyone with a major comorbidity such as advanced kidney disease or active infection. Every participant provided written informed consent.

Sample Size

Buderer's formula for diagnostic accuracy studies was used to estimate the sample size[19]: $n = Z^2 \cdot P(1 - P)/d^2$, with P taken as the expected sensitivity for cases and the expected specificity for controls. Based on an anticipated PIVKA-II sensitivity of 85% and specificity of 80%[13], a 95% confidence level (Z = 1.96) and 7% precision (d), the minimum requirement was 100 cases and 126 controls, which rose to 111 and 139 after adding 10% for non-response. We eventually enrolled 335 participants (119 cases, 216 controls), more than the number required.

Data and Sample Collection

A structured case report form was used to record demographic details, duration of disease, cause of CLD and comorbid conditions from the medical records. Blood was drawn by trained phlebotomists using aseptic technique after the participant had fasted overnight for 8 hours or more, and collection was restricted to 08:00–10:00 to reduce diurnal effects. Serum

was separated no later than 2 hours after collection, divided into aliquots and kept at −80°C. Samples were assayed in batches within 6 months, and none was thawed and refrozen more than once. Both PIVKA-II and AFP remain stable at −80°C over this interval.

Laboratory Assays

PIVKA-II and AFP were quantified using sandwich ELISA kits (Bioassay Technology Laboratory [BT Lab], China) according to the kit instructions (PIVKA-II: catalogue no. E3246Hu, detection range 15–3000 pg/mL; AFP: catalogue no. E1630Hu, detection range 0.5–200 ng/mL). The PIVKA-II kit reports results in pg/mL, whereas published cut-offs for chemiluminescent platforms are in mAU/mL[16,20]. Those thresholds could not be used as they stood, so we derived our own cut-offs from the data. The usual AFP threshold of 20 ng/mL[21] served only as a point of reference.

The cause of CLD was categorized as HCV, HBV, non-alcoholic fatty liver disease (NAFLD) or alcohol-related liver disease. On the day the sample was taken, hemoglobin and platelet count were measured on Sysmex automated hematology analyzers, and PT-INR on an automated coagulation analyzer. Values were judged against the reference intervals of the participating laboratories, which follow the manufacturers' specifications: hemoglobin 12–16 g/dL for women and 13.5–17.5 g/dL for men, platelets 150–400 × 10^9/L and INR 0.8–1.1.

Statistical Analysis

SPSS version 26 was used for the analysis. Because SPSS 26 cannot perform the DeLong comparison of AUCs, that test was run separately in Python 3.11 (SciPy). We assessed normality using the Shapiro–Wilk test. Continuous data with a normal distribution are given as mean ± SD and were compared between groups by the independent-samples t-test. Variables with a skewed distribution, PIVKA-II and AFP among them, are given as median (IQR) and were compared by the Mann–Whitney U test. Categorical data are presented as counts and percentages and were compared by the chi-square test, with Fisher's exact test used where any expected count was under 5.

We expressed diagnostic accuracy as sensitivity, specificity, PPV, NPV and AUC, each with a 95% confidence interval[22,23]. The optimal cut-off for each marker was the value giving the highest Youden index (sensitivity + specificity − 1)[24], and the DeLong method was applied to compare the AUCs of PIVKA-II and AFP[25]. In a case-control sample, predictive values are driven by the ratio of cases to controls, so we also recalculated them with Bayes' theorem for an HCC prevalence of 6.73%[14]. Statistical significance was set at $p<0.05$.

Ethical Approval

Ethical approval was granted by the Research Ethics Committee of the Faculty of Basic Medical Sciences, Isra University, Hyderabad (Ref. No. IU/CP.REC(FBMS)/2025/-037; 6 January 2025). Each participant was told about the purpose, procedures and potential risks of the study and gave written informed consent. The work was conducted in accordance with the Declaration of Helsinki[26].

RESULTS

Baseline Characteristics

The 335 participants comprised 119 patients with HCC and 216 with CLD but no HCC. The HCC group was older (66.05 ± 5.2 vs 62.54 ± 6.1 years, $p<0.001$), but the two groups had a similar share of men (73.9% vs 72.7%, $p=0.90$). Among cases, the cause of liver disease was HCV in 54.6%, HBV in 22.7%, NAFLD in 16.0% and alcohol in 6.7%; the corresponding figures among controls were 48.6%, 18.1%, 22.7% and 10.6% ($p = 0.225$).

Compared with controls, patients with HCC had lower hemoglobin, platelet count and albumin, and higher PT-INR, AST, ALT and bilirubin ($p<0.001$ for each). Child-Pugh class also differed between groups ($p<0.001$). None of the cases was in class A, 49 (41.2%) were in class B and 70 (58.8%) in class C; among controls, the numbers were 85 (39.4%), 121 (56.0%) and 10 (4.6%). Moderate or severe ascites was seen in 82.4% of cases against 19.4% of controls ($p<0.001$) (Table 1).

Table 1: Demographic, hematological and clinical characteristics of HCC cases and controls

Variables	HCC group (n=119)	Non-HCC control group (n=216)	p-value
Age (years), mean ± SD	66.05 ± 5.2	62.54 ± 6.1	<0.001[a]
Male, n (%)	88 (73.9)	157 (72.7)	0.90[b]
Aetiology, n (%)			
HCV	65 (54.6)	105 (48.6)	0.225[b]
HBV	27 (22.7)	39 (18.1)	
NAFLD	19 (16.0)	49 (22.7)	
Alcohol-related	8 (6.7)	23 (10.6)	
Hemoglobin (g/dL), mean ± SD	9.70 ± 0.60	11.98 ± 0.70	<0.001[a]
Platelet count (×10³/μL), mean ± SD	77.83 ± 15.73	154.10 ± 31.40	<0.001[a]
PT-INR, mean ± SD	1.57 ± 0.19	1.23 ± 0.15	<0.001[a]
AST (U/L), mean ± SD	124.03 ± 25.16	56.86 ± 15.05	<0.001[a]
ALT (U/L), mean ± SD	75.93 ± 15.49	44.64 ± 12.19	<0.001[a]
Albumin (g/dL), mean ± SD	2.60 ± 0.33	3.46 ± 0.38	<0.001[a]
Total bilirubin (mg/dL), mean ± SD	3.13 ± 0.78	1.36 ± 0.42	<0.001[a]
Child-Pugh class, n (%)			<0.001[b]
Class A	0 (0.0)	85 (39.4)	
Class B	49 (41.2)	121 (56.0)	
Class C	70 (58.8)	10 (4.6)	
Moderate/severe ascites, n (%)	98 (82.4)	42 (19.4)	<0.001[b]

[a] Independent-samples t-test; [b] chi-square test. HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; PT-INR, prothrombin time–international normalized ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Comparison of Biomarkers

Levels of both markers were substantially higher in the HCC group (Table 2). The median PIVKA-II concentration was 4500 pg/mL among cases and 40 pg/mL among controls, while median AFP was 40 and 10 ng/mL (p<0.001 for both). On average, PIVKA-II was about 65 times higher in cases than in controls; for AFP the difference was about 22-fold.

Table 2: Serum PIVKA-II and AFP levels in cases and controls (N=335)

Biomarker	HCC group (n=119)	Control group (n=216)	p-value*
PIVKA-II (pg/mL)			
Median (IQR)	4500 (6050)	40 (21)	<0.001
Mean ± SD	5652.13 ± 4409.05	87.33 ± 287.07	
AFP (ng/mL)			
Median (IQR)	40 (270)	10 (12)	<0.001
Mean ± SD	691.59 ± 1548.78	31.67 ± 80.20	

**Mann–Whitney U test. Because both markers had right-skewed distributions, means are included only to allow comparison with earlier studies. PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP, alpha-fetoprotein; IQR, interquartile range.*

Diagnostic Performance

Table 3 lists the accuracy measures, and Figure 1A compares them visually. The AUC for PIVKA-II was 0.98 (95% CI: 0.97–0.99). With the Youden-optimal cut-off of 76.5 pg/mL, PIVKA-II classified 117 of the 119 cases and 209 of the 216 controls correctly, which corresponds to a sensitivity of 98.3%, specificity of 96.8%, PPV of 94.4% and NPV of 99.1%. For AFP, the AUC was 0.96 (95% CI: 0.93–0.98); at 47 ng/mL it correctly classified 113 of 119 cases and 195 of 216 controls (sensitivity 95.0%, specificity 90.3%, PPV 84.3%, NPV 97.0%). The AUC of PIVKA-II was significantly greater than that of AFP (DeLong p = 0.014). Assuming an HCC prevalence of 6.73%, the PPV dropped to 68.6% for PIVKA-II and 41.3%

for AFP, whereas the NPV remained over 99% for both markers (Figure 1B).

Table 3: Diagnostic accuracy of PIVKA-II and AFP for HCC

Measure	PIVKA-II	AFP
AUC (95% CI)	0.98 (0.97–0.99)	0.96 (0.93–0.98)
DeLong test (PIVKA-II vs AFP)	p = 0.014	
Optimal cut-off (Youden index)	76.5 pg/mL	47 ng/mL
TP / FN / TN / FP	117 / 2 / 209 / 7	113 / 6 / 195 / 21
Sensitivity, % (95% CI)	98.3 (94.1–99.5)	95.0 (89.4–97.7)
Specificity, % (95% CI)	96.8 (93.5–98.4)	90.3 (85.6–93.6)
PPV, % (95% CI)	94.4 (88.8–97.2)	84.3 (77.2–89.5)
NPV, % (95% CI)	99.1 (96.6–99.7)	97.0 (93.6–98.6)
PPV at 6.73% prevalence, %	68.6	41.3
NPV at 6.73% prevalence, %	99.9	99.6

Cut-offs were chosen to maximize the Youden index (sensitivity + specificity − 1), and Wilson's method was used for the 95% CIs of proportions. The PPV and NPV shown in the upper rows depend on the case-to-control ratio of this sample (119:216). TP, true positive; FN, false negative; TN, true negative; FP, false positive; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ROC curve.

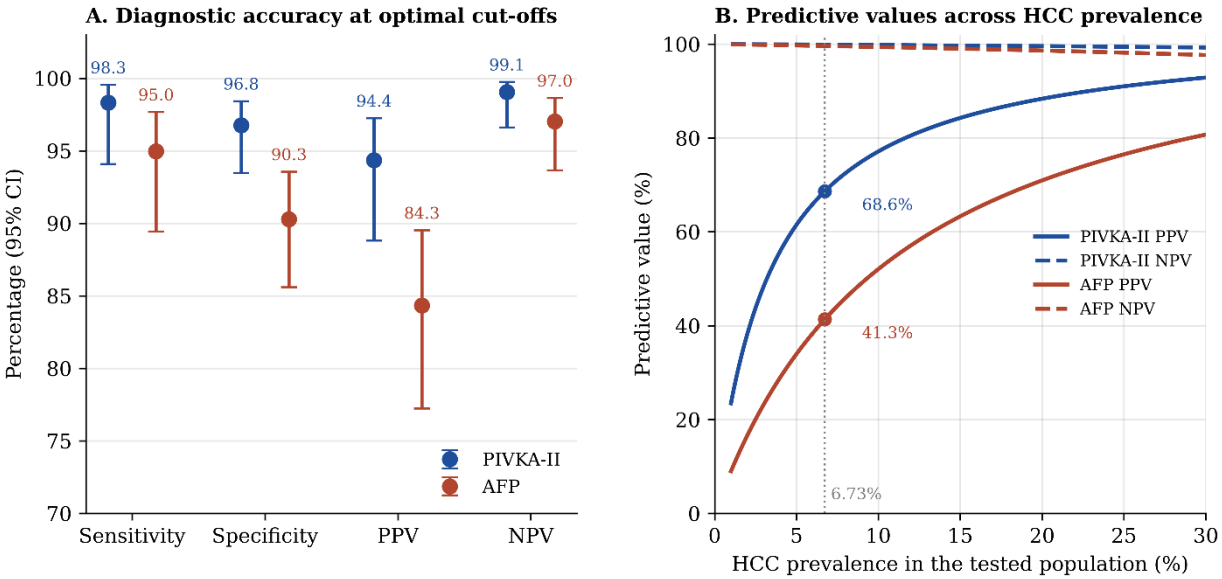


Figure 1: Accuracy of PIVKA-II and AFP for HCC. (A) Sensitivity, specificity, PPV and NPV of each marker at its Youden-optimal cut-off (PIVKA-II 76.5 pg/mL; AFP 47 ng/mL); error bars show Wilson 95% confidence intervals. (B) PPV (solid lines) and NPV (dashed lines) across HCC prevalences of 1–30%, calculated from the observed sensitivity and specificity; the dotted line indicates the 6.73% prevalence applied in the analysis.

DISCUSSION

In this study from two centers, PIVKA-II distinguished HCC from non-malignant CLD with an AUC of 0.98, 98.3% sensitivity and 96.8% specificity; the corresponding values for AFP were 0.96, 95.0% and 90.3%. Both markers showed high accuracy, and the advantage of PIVKA-II arose mainly from its lower false-positive rate, with 7 controls misclassified against 21 for AFP. This is in keeping with Kudo's recent argument that PIVKA-II should be used more widely for the surveillance and management of HCC[27].

There is a reasonable biological explanation for the gap in specificity. AFP comes from regenerating hepatocytes as well as from tumor cells, so active hepatitis and advanced cirrhosis can elevate it in the absence of HCC[7]. Over half of our control group was in Child-Pugh class B,

and 21 controls had an AFP above the optimal cut-off, against only seven above the PIVKA-II cut-off. PIVKA-II, on the other hand, results from faulty vitamin K-dependent carboxylation inside malignant cells and is less influenced by liver inflammation. It is still not perfectly specific, as levels also rise with vitamin K deficiency, vitamin K antagonist treatment and obstructive jaundice[7], which is why we excluded patients on vitamin K antagonists.

These findings are consistent with a growing literature. Liu et al., analyzing HCV-related HCC across several centers, reported that PIVKA-II performed better than AFP and that the pair together was more accurate again[11]. PIVKA-II has similarly shown superior performance in high-risk Thai patients[28] and in work spanning diagnosis, treatment monitoring and prognosis[21]. Hadi et al. found it particularly helpful in separating HCC from benign liver disease in high-risk patients[7], the same distinction we examined, and Chan et al. confirmed that the two markers are clinically useful and complement each other[20]. Studies from Italy and the Asia-Pacific region report comparable findings[29–31]. Raised PIVKA-II has also been associated with larger tumor burden, more advanced stage and vascular invasion[6,21,32], although prognosis was not evaluated in the present study.

Our accuracy estimates exceed those pooled in recent meta-analyses, where the AUC ranged from 0.85 to 0.89 for PIVKA-II and from 0.78 to 0.81 for AFP[9,10]. This difference is likely explained, at least in part, by the characteristics of the study population. Cases were drawn from everyday hospital practice, none was in Child-Pugh class A, and the markedly elevated median PIVKA-II suggests a large tumor load in many of them. Large tumors are readily detected by most markers, and setting them against controls with no liver mass on imaging widens the separation further. The meta-analyses included more early tumors and a broader mix of control groups. Our figures should therefore be read as a best-case estimate rather than the accuracy a surveillance clinic could expect.

PIVKA-II also had a higher PPV than AFP (94.4% vs 84.3%), meaning a raised value is more likely to indicate genuine HCC and may reduce unnecessary further investigation, and its NPV of 99.1% supports a role in excluding the disease. Some caution is needed in interpreting these numbers. In a case-control study, predictive values are set by the 119:216 ratio of cases to controls and not by the real prevalence of HCC in CLD. Once a prevalence of 6.73% is applied, the PPV falls to approximately 69% for PIVKA-II and 41% for AFP (Figure 1B), while the NPV stays above 99%. In practice, then, PIVKA-II is most useful for ruling HCC out and for adding to the information from AFP, not for confirming the diagnosis by itself.

The results are relevant to settings where imaging is in short supply. A blood marker taken during the same visit as routine liver tests could help identify which patients should be prioritized for contrast-enhanced CT or MRI. PIVKA-II is also a component of multi-marker scores. In a prospective cohort with cirrhosis, the GALAD score, of which PIVKA-II is a part, detected newer HCC than AFP alone[8], and data from the Asia-Pacific region favor using PIVKA-II and AFP together for surveillance and follow-up[30]. Whichever strategy is chosen, cut-offs specific to the local assay must first be validated. The 76.5 pg/mL threshold found here came from a research ELISA and does not translate to the mAU/mL scale of chemiluminescent analysers[16,20]. Common units and calibration across platforms would considerably facilitate comparison of results across centers.

Our study has several strengths. Recruitment took place at two centers, reporting adheres to STARD 2015[17], the radiologists did not know the biomarker results, and PIVKA-II was not used to assign participants to groups. We compared the AUCs formally using the DeLong test and recalculated predictive values for a realistic HCC prevalence, giving a better sense of how the markers might perform beyond a case-control setting.

The study also has limitations. First, this was a hospital-based case-control study from two centers in a single region of Pakistan, and the results may not hold where the causes of HCC or the genetic background differ. Second, contrasting confirmed HCC with definitively non-malignant CLD tends to inflate accuracy relative to an unselected surveillance population. Third, because a raised AFP was part of the case definition, incorporation bias is present and is likely to have overestimated the accuracy of AFP in particular. Keeping PIVKA-II out of the classification and blinding the radiologists to marker results reduces this bias but cannot eliminate it. Fourth, cut-offs were derived and evaluated in the same sample, with no internal or external validation, so performance may be overestimated. Fifth, although we recorded tumor size, number and vascular invasion, we did not analyze accuracy

by stage, and with no Child-Pugh class A cases we could not assess performance in early HCC. Sixth, we did not test AFP and PIVKA-II as a combined panel, even though earlier work suggests the combination outperforms either marker alone[13,30]. Finally, the research ELISA used here has not been standardized against commercial chemiluminescent assays, and we did not assess cost-effectiveness, an important issue where resources are limited.

CONCLUSION

Among patients with chronic liver disease, PIVKA-II was slightly better than AFP at distinguishing HCC from non-malignant CLD, showing higher specificity and positive predictive value in this case-control sample. Its main advantages were a lower false-positive rate and a high negative predictive value. PIVKA-II is therefore better viewed as a complement to AFP than as a substitute. Before it can be recommended for routine use in Pakistan, prospective multicenter studies in surveillance populations are needed, with analysis by tumor stage and validated cut-offs for each assay.

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