

Correlation of Neutrophil-Lymphocyte Ratio, Alpha-Fetoprotein, and PIVKA-II with Staging of HCC Related HBV at Dr. Mohammad Hoesin Hospital, Palembang

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ABSTRACT

Background: The staging of Hepatocellular carcinoma (HCC) requires evaluating the tumor size and number, liver function, physical performance, tumor invasion, extrahepatic metastasis, and clinical symptoms. Biomarkers such as neutrophil-lymphocyte ratio (NLR), alpha-fetoprotein (AFP), and protein induced by the absence of vitamin K or antagonist-II (PIVKA-II) have been identified as valuable diagnostic and prognostic tools for HCC. This study aims to investigate the correlation between NLR, AFP, and PIVKA-II and HCC stages.

Methods: This observational study was conducted at the Department of Internal Medicine, Dr. Mohammad Hoesin Hospital, Palembang, from April to July 2024. HCC stages were classified using the Barcelona Clinic Liver Cancer (BCLC) system. NLR, AFP, and PIVKA-II levels were measured using an ELISA method. Data were analyzed using one-way ANOVA or the Kruskal-Wallis test.

Results: A total of 38 patients with HCC who met the inclusion criteria were included in the study. There was a weak positive correlation between NLR and HCC stage ($r = 0.334$; $p = 0.040$), a moderate positive correlation between AFP and HCC stage ($r = 0.499$; $p = 0.001$), and a strong positive correlation between PIVKA-II and HCC stage ($r = 0.797$; $p = 0.000$). PIVKA-II and AFP were found to have a significant sequential effect on the BCLC stage.

Conclusion: PIVKA-II and AFP are significant indicators for assessing BCLC stages, offering valuable support for the diagnosis and management of HCC.

Keywords: Hepatocellular Carcinoma, BCLC Staging, Neutrophil-to-lymphocyte Ratio, AFP, PIVKA-II

ABSTRAK

Latar belakang: Evaluasi stadium Karsinoma Sel Hati (KSH) melibatkan penilaian ukuran dan jumlah lesi tumor, fungsi hati, status fisik, invasi tumor, metastase ekstrahepatik, dan gejala klinis. Beberapa biomarker seperti neutrophil lymphocyte ratio (NLR), alfa fetoprotein (AFP), dan protein induced by the absence of vitamin k or antagonist-II (PIVKA-II) dapat digunakan sebagai penanda diagnostik dan prognostik untuk KSH. Penelitian ini bertujuan untuk menganalisis korelasi antara NLR, AFP, dan PIVKA-II dengan stadium KSH.

Metode: Studi observasional ini dilakukan di Departemen Ilmu Penyakit Dalam Rumah Sakit Dr. Mohammad Hoesin Palembang, dari April hingga Juli 2024. Stadium KSH diklasifikasikan menggunakan sistem Barcelona Clinic Liver Cancer (BCLC). Pengukuran NLR, AFP, dan PIVKA-II dilakukan melalui metode ELISA. Analisis data dilakukan menggunakan uji one-way ANOVA atau Kruskal-Wallis.

Hasil: Sebanyak 38 pasien KSH yang memenuhi kriteria inklusi diikutsertakan dalam penelitian. Analisis menunjukkan korelasi positif lemah bermakna antara NLR dengan stadium KSH ($r = 0,334$; $p = 0,040$), korelasi positif sedang bermakna antara AFP dengan stadium KSH ($r = 0,499$; $p = 0,001$), serta korelasi positif kuat bermakna antara PIVKA-II dengan stadium KSH ($r = 0,797$; $p = 0,000$). Pengaruh signifikan secara berurutan terhadap stadium BCLC adalah PIVKA-II dan AFP.

Kesimpulan: PIVKA-II dan AFP merupakan indikator yang signifikan untuk menilai stadium BCLC, sehingga dapat mendukung diagnosis dan manajemen KSH.

Kata kunci: Karsinoma Hepatoseluler; Stadium BCLC, Rasio Neutrofil terhadap Limfosit, Alfa Fetoprotein, PIVKA-II

INTRODUCTION

Hepatocellular carcinoma (HCC) represents the most prevalent type of liver cancer, particularly in regions such as Asia and Sub-Saharan Africa, accounting for approximately 800,000 new cases annually.^{1,2} It ranks as the third leading cause of cancer-related deaths worldwide and ranks fourth in Indonesia. In Indonesia, the incidence of HCC is recorded at 21,392 cases (5,4%).³ HCC often remains asymptomatic in its early stages and is typically diagnosed when tumors exceed 10 cm or present at an advanced stage. Early detection is therefore crucial for improving patient outcomes.¹

The pathogenesis of HCC is closely associated with chronic liver inflammation and fibrosis, most commonly arising from persistent viral hepatitis, alcohol-related liver disease, or metabolic dysfunction. Chronic inflammation promotes hepatocyte injury, immune dysregulation, and tumor-supportive microenvironmental changes that drive hepatocarcinogenesis. These mechanisms influence both disease progression and prognosis. The Barcelona Clinic Liver Cancer (BCLC) system is commonly used for staging HCC, integrating tumor burden, liver function, and the patient's performance status. However, its reliance on imaging modalities such as CT or three-phase MRI limits its applicability in resource-constrained settings. In such contexts, serum biomarkers may offer a more accessible and cost-effective alternative for disease stratification.⁴⁻¹²

Among potential biomarkers, the neutrophil-to-lymphocyte ratio (NLR), alpha-fetoprotein (AFP), and protein induced by vitamin K absence or antagonist-II (PIVKA-II) have emerged as promising tools for diagnosis and treatment. NLR, derived from routine blood tests, reflects the balance between systemic

inflammation and immune surveillance.¹³ Notably, elevated NLR has been observed in advanced stages of HCC compared to earlier stages of the disease.⁶ A meta-analysis conducted by Lin et al. concluded that NLR is significantly associated with disease severity, particularly in East Asian populations with high HCC incidence.¹⁴

AFP remains the most established biomarker for the diagnosis and monitoring of HCC. Rahmadhini and Yao et al. have demonstrated that AFP correlates with HCC staging. However, AFP alone provides only weak to moderate correlation and limited sensitivity and specificity, especially for early-stage disease.¹⁵⁻¹⁸ PIVKA-II, or Des-gamma-carboxy-prothrombin (DCP), is an aberrant protein produced by malignant hepatocytes. Feng et al. reported that PIVKA-II levels increase significantly in advanced stages of HCC.¹⁹ Increased PIVKA-II levels are associated with aggressive tumor characteristics, including enhanced proliferation, metastasis, and invasion.^{20,21} Combining AFP with PIVKA-II may improve the detection of early-stage HCC; however, this approach still has limitations.²²⁻²⁴ While numerous studies have independently examined NLR, AFP, and PIVKA-II, few have explored their combined utility in relation to HCC staging. Moreover, the comparative predictive strength of these biomarkers across different stages of disease remains unclear. Therefore, this study aims to analyze the correlation between these biomarkers and HCC staging in patients treated at Dr. Mohammad Hoesin General Hospital, Palembang.

METHODS

This observational cross-sectional study was conducted from April to July 2024 at Dr. Mohammad Hoesin General Hospital, Palembang. Eligible

participants were adults (≥ 18 years) diagnosed with HCC associated with chronic hepatitis B infection (HBsAg reactive) who provided informed consent. Exclusion criteria included a history of non-HCC cancers within the previous five years, prior solid organ or bone marrow transplantation, receipt of local or systemic HCC therapy within the preceding 28 days, or presence of acute infection. Proportional consecutive sampling was employed to recruit eligible subjects until the target sample size of 29 was reached.

Serum NLR, AFP, and PIVKA-II levels were measured using ELISA kits (Eagle Biosciences, USA). The diagnostic criteria were adapted from the consensus of the Japan Society of Hepatology. HCC staging was classified according to the BCLC classification.

The study protocol was approved by the institutional ethics committee (No.DP.04.03/D.XVIII.6.8/ETIK/095/2024). Data analysis was performed using SPSS version 23. Correlations between each biomarker and HCC stage were assessed using Spearman's rho test, with statistical significance defined as $p < 0.05$. Differences in mean biomarker levels across stages were analyzed using one-way ANOVA or Kruskal-Wallis tests as appropriate. The combined association

of all biomarkers with HCC stage was evaluated through linear regression analysis.

RESULTS

A total of 38 patients with HCC met the inclusion criteria. Among them, 12 (31.6%) patients were classified as BCLC stage A, 14 (36.8%) as BCLC stage B, 8 (21.1%) as BCLC stage C, and 4 (10.5%) as BCLC stage D.

BASELINE CHARACTERISTICS

Baseline characteristics are summarized in **Table 1**. The mean patient age was 53.61 ± 9.85 years, and most were male (84.2%). The mean body mass index (BMI) was 22.1 ± 4.60 kg/m², with the majority within the normal range. Positive hepatitis B envelope antigen (HBeAg) was detected in 34.2% of patients, and the mean HBV DNA level was $4.49 \times 10^6 \pm 1.31 \times 10^7$ IU/mL. Cirrhosis was present in 11 patients (28.9%). As shown in **Table 2**, most patients had multiple tumors (57.9%). Metastases were identified in the lungs and intra-abdominal sites in 5.3% of cases each, and vascular thrombus was observed in 8 patients (13.2%).

Table 1. Characteristics of HCC Patients Based on BCLC Staging HCC

Characteristics	Staging (BCLC)				Total	P value
	A	B	C	D		
Age (Years)						
Mean (SD)	52.3 (8.3)	54.8 (9.5)	57.3 (9.5)	46.3 (15)	53.6 (9.9)	0.306 ^a
Median	53	57	58.5	50	55.5	
Min-Max	38 - 63	32 - 64	44 - 69	25 - 60	25 - 69	
BMI (kg/m ²)						
Mean (SD)	21.9 (5.7)	22.3 (4.0)	23.5 (4.8)	19.0 (1.1)	22.1 (4.6)	0.194 ^a
Median	21	21.55	22.7	18.65	21.55	
Min-Max	16.4-38.3	17-30.8	16.5-33.3	18.1-20.6	16.4-38.3	
Gender, n (%)						
Male	9 (75)	13 (92.9)	6 (75)	4 (100)	32 (84.2)	0.421 ^b
Female	3 (25)	1 (7.1)	2 (25)	0 (0)	6 (15.8)	
HBV DNA						
Mean (SD)	4.2×10^6	9.3×10^5	4.1×10^6	1.87×10^7	4.49×10^6	0.738 ^a
Median	8.4×10^5	2.34×10^5	1.07×10^5	4.59×10^5	4.59×10^5	
Min-Max	$54 - 2.4 \times 10^7$	$1170 - 5.1 \times 10^6$	$0 - 3.0 \times 10^7$	$2210 - 7.4 \times 10^7$	$0 - 7.4 \times 10^7$	
HBeAg						
Positive	3 (25)	6 (42.9)	2 (25)	2 (50)	13 (34.2)	0.645 ^b
Negative	9 (75)	8 (57.1)	6 (75)	2 (50)	25 (65.8)	
Cirrhosis						
Yes	4 (33.3)	2 (14.3)	3 (37.5)	2 (50)	11 (28.9)	0.509 ^b
No	8 (66.7)	12 (85.7)	5 (62.5)	2 (50)	27 (71.1)	
Total (n)	12	14	8	4	38	

^aKruskal-Wallis Test (non-normally distributed data using median (min-max)), * $p < 0.05$

^bPearson Chi-Square Test, $p < 0,05$

Table 2. Characteristics of Tumors and Clinical Features of HCC Patients

Characteristics	Frequency	Percentage
Number of Tumors		
Single	16	42.1
Multiple	22	57.9
Metastasis		
Lung	2	5.3
Medulla Spinalis (MS)	1	2.6
Spleen	1	2.6
Lung and Bone	1	2.6
Lung, Pleura, and Medulla Spinalis	2	5.3
Intra Abdomen	1	2.6
No Metastasis	30	78.9
Thrombus		
Yes	8	21.1
No.	30	78.9
Child-Pugh Score		
A	16	42.1
B	12	31.6
C	10	26.3

CORRELATION OF NLR, AFP, PIVKA-II WITH HCC STAGE

The comparisons between NLR, AFP, and PIVKA-II based on BCLC staging are presented in **Table 3**. Significant differences were observed for NLR, AFP, and PIVKA-II across different BCLC stages ($p = 0.041$, $p = 0.026$, $p = 0.000$, respectively). As illustrated in **Figure 1**, patients with BCLC stage D exhibited the highest NLR, AFP, and PIVKA-II levels, whereas those patients with BCLC stage A had the lowest. Spearman’s correlation analysis demonstrated a positive correlation between NLR, AFP, PIVKA-II, and BCLC (**Table 4**). The strongest correlation was observed for PIVKA-II, followed by AFP and NLR ($r = 0.797$, $p = 0.000$; $r = 0.499$, $p = 0.001$; $r = 0.334$, $p = 0.040$, respectively). Linear regression analysis showed that both PIVKA-II and AFP significantly influence BCLC staging by 73.4% while the remaining 26.6% was attributed to other factors not assessed in this study (**Table 5**).

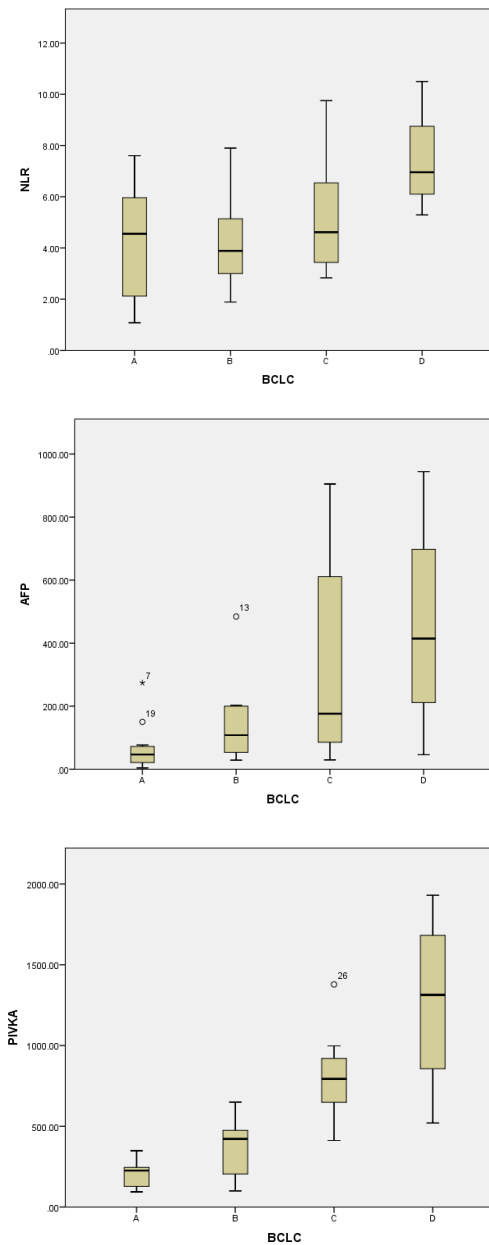


Figure 1. Levels of NLR, AFP, and PIVKA-II Based on BCLC Stages

Table 3. Comparison of NLR, AFP, and PIVKA-II Based on BCLC Stages

Characteristics	Staging (BCLC)				Total	P value
	A	B	C	D		
NLR						
Mean (SD)	4.19 (2.2)	4.25 (1.6)	5.22 (2.3)	7.43 (2.2)	4.77 (2.2)	0.041 ^a
Median	4.55	3.88	4.61	6.95	4.87	
Min-Max	1.08-7.6	1.89-7.9	2.83-9.75	5.29-10.5	1.08-10.5	
AFP						
Mean (SD)	67 (76)	139 (119)	334 (327)	454 (370)	190 (237)	0.026 ^b
Median	46.5	108.04	176.3	414.34	84.29	
Min-Max	4.04-274	28.7-484	29.6-905	45.9-943	4.04-943	
PIVKA-II						
Mean (SD)	200 (79)	377 (187)	814 (286)	1269 (586)	507 (421)	0.000 ^b
Median	225.6	421.7	793.2	1313.12	407.05	
Min-Max	93.6-348.6	99.6-649.6	412.4-1378	520.3-1930	93.6-1930	

^aOne-Way ANOVA Test (normally distributed data using mean±SD), $p < 0.05$

^bKruskall-Wallis Test (non-normally distributed data using median (min-max)), $p < 0.05$

Table 4. Correlation Between NLR, AFP, and PIVKA-II with BCLC Stages

Variable	Biomarker	R	p-value
Staging (BCLC)	NLR	0.334	0.040*
	AFP	0.499	0.001*
	PIVKA-II	0.797	0.000*

Spearman's Rho Test, * p < 0.05

Table 5. Linear Regression Analysis of NLR, AFP, and PIVKA-II on BCLC Stage

Variable	Biomarker	P value
Staging (BCLC)	NLR	0.999*
	AFP	0.003*
	PIVKA-II	0.000*

*Linear Regression Test, p < 0.05

DISCUSSION

HCC or hepatoma is the most common primary liver malignancy and typically arises in the setting of chronic liver disease or cirrhosis. Depending on the area and clinical trials, HCC is more prevalent in males than in females.^{25,26} In this study, 84.2% of HCC patients were reported to be male, with a male-to-female ratio of 5.3:1. Furthermore, Chen et al. reported a similar result, stating that out of 3,486 HCC patients, 76.9% were male.²⁷ Another study by Liou et al. in 2023, involving 1,716 HCC patients, also reported that the majority were male (80%) with a ratio of 4:1.²⁸ The causes of this gender gap are multifaceted and could include variations in tumor biology, metabolic variables, behavioral risk factors, and therapeutic outcomes.²⁹ Estrogen appears to exert a protective role in the development of HCC, and the expression of specific estrogen receptor subtypes differs between sexes, which may impact the progression of HCC.³⁰

In most populations, the incidence of HCC is directly correlated with age.³¹ The average diagnosis age is 60–64 years for men and 65–69 years for women.³² In this study, the mean age of patients was 53.61 years. Similarly, Le et al. reported a median age of 58 years (19–87 years), and Ali et al. (2023) observed a median age of 57 years (28–83 years).³³ Cellular aging is an ever-evolving process that results in the gradual deterioration of physiological capabilities. Telomere shortening during cell division, which prevents somatic cell proliferation, is a key cause of cellular aging. Oxidative stress, mitochondrial dysfunction, DNA damage, epigenetic modifications, and changes in metabolic pathways all lead to cellular and tissue aging, which in turn raises the risk of liver cancer.³⁴

A higher risk and worse oncological outcomes are linked to obesity. In addition to having a higher frequency of microvascular invasion and a propensity for higher recurrence rates, HCC patients with an obese BMI are twice as likely to die. This implies that elevated vascular endothelial growth factor (VEGF) expression, triggered by adipose tissue, may promote tumor formation.³⁵ The mean BMI of patients in this cohort was 22.09 kg/m², which aligns with the findings by Cha et al. (2020), who reported a mean BMI of 22.4 kg/m² for HCC patients.³⁶ Cirrhosis was identified in 28.9% of patients, indicating that hepatitis B virus (HBV) infection can lead to hepatocarcinogenesis both through fibrosis and direct viral oncogenic mechanisms.³⁷ HBV DNA integration into the host genome induces chromosomal instability, while viral proteins, such as HBx, promote cell proliferation, inhibit apoptosis, and activate oncogenic pathways, including p53, Wnt/β-catenin, and MAPK.³⁸

No significant differences were observed in age, sex, BMI, HBV DNA level, HBeAg status, or cirrhosis across BCLC stages, suggesting these factors were not associated with disease stage. The majority of patients were classified as Child-Pugh Class A (CPA, 42.1%), with multiple tumors occurring in 57.9% of cases, and the most common metastases occurring at the lungs and intra-abdominal regions. Aly et al. also reported similar results with 42.6% of patients having CPA.³⁹ CPA is often associated with better prognostic outcomes and eligibility for a broader range of therapeutic options, including surgical interventions and curative treatments.⁴⁰

Systemic inflammation plays a crucial role in the pathogenesis and progression of HCC, particularly in the context of chronic liver disease. Persistent liver damage caused by viral hepatitis (HBV/HCV), alcohol use, or non-alcoholic fatty liver disease triggers a systemic inflammatory response that promotes hepatocyte death, compensatory proliferation, and genomic instability, which are key processes in hepatocarcinogenesis. When an injury occurs, innate immune cells (e.g., neutrophils and monocytes) infiltrate the liver and secrete reactive oxygen species (ROS), proteases, and proinflammatory cytokines such as IL-6 and TNF-α, which can stimulate angiogenesis and inhibit tumor-specific immune surveillance. This chronic inflammatory milieu leads to activation of hepatic stellate cells, resulting in fibrosis and remodeling of the extracellular matrix, both of which contribute to tumor initiation and progression. At the same time, the adaptive immune response becomes

dysregulated. CD8⁺ cytotoxic T cells and natural killer (NK) cells, which are crucial for tumor elimination, are often functionally impaired in patients with HCC. This facilitates immune evasion by malignant hepatocytes and accelerates tumor development.^{41–50}

The innate immune response, primarily mediated by neutrophils, and the adaptive immunological response, facilitated by lymphocytes, are two components of the immune system that are integrated by NLR.⁵¹ Poor patient outcomes are linked to a higher NLR.⁵² High NLR levels are strongly correlated with vascular invasion, tumor multifocality, and a higher incidence of AFP (≥ 400 ng/mL).⁵² In this study, it was found that NLR levels differed based on BCLC stages, where patients with HCC in stage D had higher NLR values compared to those in other stages, while patients in stage A had the lowest NLR values among all stages. Additionally, a weak but significant positive correlation was found between NLR and HCC stages, indicating that patients with higher HCC stages tend to have higher NLR values. Patients in the high NLR group had greater tumor diameters than those in the low NLR group.⁵³

As the fetus's primary serum-binding protein, alpha-fetoprotein (AFP) plays a crucial role in transporting various ligands, including bilirubin, hormones, fatty acids, and minerals.⁵⁴ When liver carcinogenesis occurs in adults, elevated AFP levels are frequently seen. Evidence suggests that inhibiting AFP can induce the death of cancer cells. AFP can also promote cell motility, proliferation, and the invasive characteristics of HCC cell lines.^{55,56} Therefore, AFP is a non-specific marker for HCC. Additionally, cytoplasmic AFP can enhance the occurrence of HCC by promoting cancer cell proliferation, inhibiting apoptosis, and inducing immunosuppression. Additionally, AFP stimulates invasion, metastasis, and angiogenesis of HCC cells while increasing proliferation and preventing apoptosis.⁵⁷

AFP levels in this study varied according to the stage of BCLC; patients with HCC in stage D had higher AFP values than those in other stages, while those in stage A had the lowest AFP levels. Furthermore, a moderately significant positive correlation was found between AFP levels and HCC stages, indicating that patients' AFP levels increased as their HCC stage progressed. Ramadhini et al. demonstrated a substantial and positive relationship between BCLC stages and AFP levels.¹⁶

PIVKA-II is widely used as a predictive and diagnostic marker for HCC, particularly in Asia.⁵⁸ The Japan Society of Hepatology (JSH) identifies PIVKA-II as a specific and reliable serum biomarker for HCC, recommending it as a surveillance tool for at-risk populations.⁵⁹ Since vitamin K is essential for the catalytic activity of γ -glutamylcarboxylase, its deficiency contributes to elevated PIVKA-II levels in individuals with HCC. Studies have shown that vitamin K1 supplementation significantly lowers plasma PIVKA-II levels in HCC patients, further supporting its role as a phenotypic biomarker for HCC.⁶⁰ In this study, it was found that PIVKA-II levels varied based on BCLC stages. PIVKA-II levels increase progressively with advancing stages of disease. Wang et al. found a positive relationship between PIVKA-II levels and the largest tumor mass. PIVKA-II levels were higher in individuals with infiltrative HCC, portal vein thrombosis, and advanced-stage HCC.⁵⁵

The findings of this study have significant clinical implications, particularly in the context of hepatology practice. Elevated levels of PIVKA-II and AFP were shown to be significantly associated with more advanced BCLC stages, highlighting their potential utility as non-invasive biomarkers in clinical staging of HCC. In resource-limited settings where access to imaging modalities may be restricted, these serum markers could aid clinicians in estimating disease severity and inform early treatment strategies. Furthermore, the strong predictive value of PIVKA-II, in particular, suggests its role not only in diagnosis but also in disease monitoring and potentially in evaluating therapeutic response.

This study has several limitations that should be acknowledged. First, the cross-sectional design precludes any causal inference between biomarker levels and BCLC stage. Second, although the sample size is adequate for initial analysis, it may not be representative of the broader HCC population in other geographic or clinical settings. Third, the study was conducted in a single center, which may introduce selection bias and limit generalizability. Additionally, some potential confounding variables—such as liver function status, comorbidities, or genetic factors—were not included in the regression model, which may account for the unexplained variance. Despite these limitations, the findings provide important insight into the relationship between NLR, AFP, PIVKA-II, and HCC staging, and suggest directions for future research with larger and more diverse populations.

CONCLUSION

The study results showed a positive correlation between NLR, AFP, and PIVKA-II levels with HCC stages. Among these biomarkers, PIVKA-II exhibited the strongest correlation with HCC stages, indicating its greater potential as an indicator for evaluating disease progression.

Conflict of Interest

The authors declare there is no conflict of interest.

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Author Contribution

F.R. was responsible for the conception, study design, data collection, analysis, and drafting of the manuscript. S. and L. supervised the study, provided critical feedback, and contributed to the final revision of the manuscript. All authors have read and approved the final version of the manuscript.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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