

REVIEW ARTICLE

Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in hepatocellular carcinoma: From inflammation to clinical applications

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Abstract

Hepatocellular carcinoma (HCC) is the predominant type of primary liver cancer, with a rising global incidence and a high mortality rate. Chronic infections with hepatitis viruses, along with risk factors such as cirrhosis, metabolic dysfunction-associated steatohepatitis, and metabolic disorders, significantly contribute to HCC development. Systemic inflammation plays a key role in HCC pathogenesis, with inflammatory markers such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) emerging as significant prognostic indicators. Elevated NLR and PLR are associated with poor overall survival and progression-free survival in HCC patients, reflecting an imbalance in the immune response and tumor microenvironment (TME). Neutrophils and platelets contribute to tumor progression by promoting inflammation, angiogenesis, and immune evasion, while lymphocytes, particularly CD8⁺ T-cells, play essential roles in antitumor immunity. High levels of NLR and PLR are linked to adverse clinical outcomes, including shorter survival times and higher recurrence rates. These ratios provide valuable prognostic information and are increasingly utilized in clinical settings to guide treatment decisions. This review focuses on the roles of inflammatory markers, particularly NLR and PLR, in HCC progression and prognosis, examining their effects on the TME and their potential for improving diagnostic and prognostic strategies. The use of these inflammatory markers and emerging technologies holds promise for enhancing early detection and personalized treatment strategies, ultimately improving patient outcomes in HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for approximately 90% of all cases.¹ HCC poses a significant global health challenge, with an expectation of the estimated incidence to be 1 million cases by 2025.² HCC is associated with a high mortality rate, with 5-year survival rates of 25.9 – 41.7% for early-stage, 5.9% for intermediate-stage, and 0.2 – 0.4% for advanced-stage HCC.³ The primary risk factors for HCC include chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, cirrhosis, metabolic dysfunction-associated

steatotic liver disease (MASLD), metabolic dysfunction-associated steatohepatitis, alcohol abuse, obesity, and diabetes.⁴ HCC primarily affects older adults, with the 65 – 69 age groups having the highest prevalence.⁵ Over 80% of HCCs occur in developing countries, where chronic HBV and HCV infections are more prevalent. The development of HCC frequently results from the early viral transmission, particularly in regions where HBV and HCV are endemic.^{6,7}

The onset and progression of HCC are significantly influenced by systemic inflammation.⁸ Chronic activation of inflammatory signaling pathways leads to the generation of reactive oxygen species (ROS) and reactive nitrogen species, contributing to genetic alterations and mutagenesis in hepatocytes. This process initiates genomic instability, promotes oncogene activation, and impairs tumor suppressor pathways, ultimately facilitating hepatocarcinogenesis and HCC progression.⁹ Various cytokines, growth factors (GFs), chemokines, and proangiogenic factors produced by stromal and inflammatory cells promote the development and survival of tumors.¹⁰ Inflammation also impairs immune surveillance, allowing tumor cells to evade detection by the immune system. In addition, inflammatory mediators promote angiogenesis, invasion, and metastasis of HCC cells.¹¹ Systemic inflammation-based biomarkers, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), not only reflect the inflammatory and immune status of HCC patients but also correlate with the tumor-promoting effects of cytokines, GFs, and proangiogenic factors. These biomarkers are associated with similar oncogenic processes as those mediated by inflammatory mediators and have been extensively studied for their roles in predicting outcomes such as overall survival (OS) and recurrence-free survival (RFS).¹²

The NLR is calculated by dividing the number of neutrophils by the number of lymphocytes in a blood sample.¹³ As an indicator of inflammation, this ratio has been linked to a poor prognosis across several malignancies, including HCC. Patients with higher NLR (≥ 5) exhibit significantly shorter OS and progression-free survival (PFS) compared to those with lower NLR values.¹⁴ Targeting inflammation has emerged as a potentially effective therapeutic strategy, with various anti-inflammatory drugs currently being studied in clinical studies for HCC.⁹ A systematic review and meta-analysis of 24 studies encompassing 6318 patients determined that a high pre-treatment NLR is predictive of poor OS and RFS in HCC patients. Specifically, a high NLR was associated with an increased hazard ratio (HR) for both OS (HR: 1.54) and RFS (HR: 1.45).¹⁵ The underlying mechanism

involves neutrophils inhibiting lymphocyte activity, which promotes tumor growth, creating an imbalance in the immune response. This finding highlights NLR as a valuable, low-cost, and easily accessible predictive tool that can support clinical decision-making for HCC treatment.¹⁶ Moreover, NLR reflects the inflammatory microenvironment in HCC patients, leading to its use in predicting treatment outcomes, such as the likelihood of recurrence after surgical resection or response to immunotherapy.¹⁷

The PLR is another valuable and accessible biomarker for HCC, providing prognostic insights and guiding treatment decisions.¹⁸ Similar to NLR, PLR is calculated by dividing the total number of platelets by the total number of lymphocytes from a full blood count. Elevated PLR has been associated with adverse outcomes in HCC, including a lower OS (HR: 1.62) and a higher chance of an early recurrence (HR: 1.52).¹⁵ Both PLR and NLR are considered markers of the inflammatory microenvironment in tumors. An increase in these markers reflects the tumor's ability to modulate the immune system, creating an environment conducive to cancer progression and metastasis.¹⁹ This review examines the prognostic significance of inflammatory markers, particularly NLR and PLR in HCC. It aims to explore their effect on the tumor microenvironment (TME) and evaluate their potential to enhance diagnostic and prognostic strategies in HCC. Furthermore, integrating these markers into emerging technologies could facilitate personalized treatment approaches.

2. Biological basis of NLR and PLR

2.1. Neutrophils in tumor

Neutrophils are increasingly recognized as active contributors in cancer biology, exhibiting complex roles that can be categorized into both pro-tumor and antitumor effects. Neutrophils promote tumor growth and metastasis through several mechanisms, notably through persistent inflammation, frequently triggered by neutrophils themselves. The release of nitrogen species and ROS by neutrophils can lead to DNA alterations, thereby stimulating the development of tumors.²⁰ Tumor-associated neutrophils (TANs) contribute to an immunosuppressive TME by interacting with tumor cells and other immune cells. They release cytokines and GFs that promote the migration and proliferation of tumor cells. In addition, neutrophils produce neutrophil extracellular traps (NETs), which facilitate the spread of cancer by giving tumor cells a scaffold and releasing proteases that modify the extracellular matrix.²¹ Despite their pro-tumor capabilities, neutrophils also exhibit antitumor functions.

Neutrophils can kill tumor cells through the release of cytotoxic granules and ROS.²² Under certain conditions, neutrophils can be reprogrammed to present antigens, potentially activating T-cell responses against tumors.²³ This dual functionality highlights the significant plasticity of neutrophils, allowing them to adapt their functions based on the TME. This plasticity allows them to switch between pro- and antitumor roles depending on various stimuli, including cytokines and the presence of tumor cells.²⁰

Neutrophils are among the first responders to sites of inflammation and infection. Chronic inflammation, often associated with diseases such as cirrhosis and viral hepatitis, is a significant risk factor for the formation of tumors in the context of HCC. Neutrophils contribute to this inflammatory milieu by releasing many cytokines and chemokines that recruit other immune cells to the site, thereby perpetuating the inflammatory response. This persistent inflammation creates a supportive environment for cancer development, initiating a cycle of tissue regeneration and destruction.²⁴ Neutrophils release GFs such as vascular endothelial GFs (VEGFs), which promote angiogenesis essential for tumor growth and survival. This angiogenic activity facilitates the delivery of nutrients and oxygen to rapidly growing tumors.²⁵ TANs exhibit pro-tumorigenic effects by enhancing HCC cell growth and inhibiting apoptosis. Elevated TAN infiltration has been associated with poorer patient prognosis and increased tumor development.²⁶ In addition, neutrophils form NETs, which consist of networks of DNA and proteins that trap pathogens and tumor cells. This process facilitates metastasis by providing a scaffold for tumor cells to adhere to and invade other tissues.²⁷

Neutrophils facilitate tumor cells in evading immune surveillance by creating an immunosuppressive environment. This allows cancer cells to proliferate and disseminate without being targeted by the immune system.²⁵ The extracellular matrix-degrading enzymes secreted by neutrophils assist in tumor cell invasion and migration, enabling cancer cells to infiltrate surrounding tissues and enter the bloodstream, which is essential for metastasis.²⁶ The angiogenic factors released by neutrophils not only support primary tumor growth but also enhance the formation of new blood vessels at distant sites, promoting the establishment of metastases.²⁸

2.2. Platelets in tumor

Platelets shield circulating tumor cells (CTCs) from immune detection, enhancing their survival and facilitating metastasis. This interaction helps CTCs adhere to the endothelium and facilitates their extravasation

into other tissues. Platelets release various GFs, such as platelet-derived GFs (PDGF) and VEGF, which promote angiogenesis and tumor growth.²⁹ Platelets influence the immune response by interacting with immune cells, including neutrophils and macrophages, thereby shaping the TME. They stimulate an immunosuppressive milieu that supports the growth and survival of tumors. There is a strong connection between thrombosis and cancer, with tumor progression associated with elevated platelet activation. This activation results in hypercoagulability, which would facilitate the growth and metastasis of the tumor.²¹

Platelets help tumor cells evade the immune system by protecting them from natural killer (NK) cell lysis in the bloodstream.³⁰ Platelet activation and fibrin clotting create a physical barrier that hinders NK cell contact while exerting paracrine suppression of NK-mediated cytolytic activity. Transforming GFs-beta (TGF- β) from platelets reduces the release of interferon- γ , cytotoxicity, and the mobilization of NK granules. Other soluble mediators like prostaglandin E2 have similar immunosuppressive effects. Platelets facilitate the formation of hetero-aggregates with leukocytes, platelets, and tumor cells, a process that is crucial for determining the survival of tumor cells in the microvasculature.³¹ Platelets release factors such as TGF- β , PDGF, serotonin, and CXCL4 that activate hepatic stellate cells, turning them into extracellular matrix-producing myofibroblasts, which support tumor growth and promote metastasis. Platelets enhance tumor blood supply and initiate vascular invasion by interacting with endothelial cells and secreting pro-angiogenic substances, including VEGF and fibroblast GFs. Platelets contribute to HCC resistance to chemotherapy and other treatments by releasing GFs and cytokines that promote tumor cell survival and proliferation.³² Targeting platelet-tumor interactions can help overcome this therapeutic resistance. Combining antiplatelet therapy with chemotherapy or immunotherapy enhances drug delivery to the tumor and improves treatment outcomes.³³ Figure 1 presents the effect of high and low NLR and PLR on HCC, highlighting their association with inflammation, immune evasion, tumor progression, and prognosis.

2.3. Lymphocytes in tumor

Lymphocytes, particularly T-cells, mediate antitumor responses in HCC. Their effectiveness is influenced by various factors, including their subsets, TME, and interactions with other immune cells. CD8⁺ T-cells are key effector cells that exert direct cytotoxic effects on tumor cells. They recognize tumor antigens presented by major histocompatibility complex class I molecules on cancer cells, leading to tumor cell apoptosis by releasing perforin

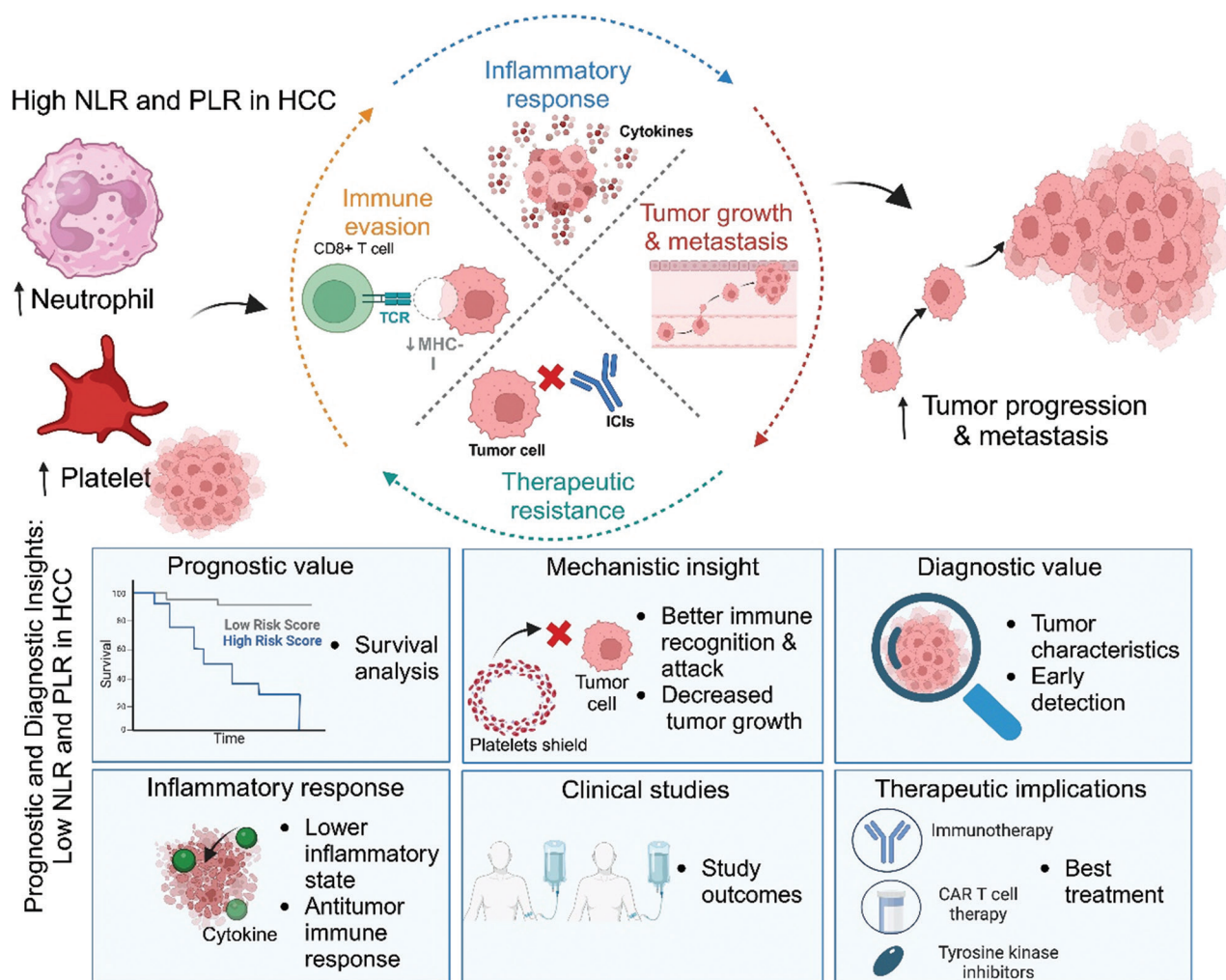


Figure 1. The effects of high and low neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) on hepatocellular carcinoma. (A): High NLR and PLR in tumor progression; High NLR and PLR are associated with increased inflammation, immune evasion, tumor growth, metastasis, and therapeutic resistance, all contributing to a poor prognosis. (B) Low NLR and PLR in prognostic and diagnostic; Low NLR and PLR are associated with better prognosis, enhanced immune response, and potential diagnostic and therapeutic benefits.

and granzymes.³⁴ Studies indicate that higher densities of CD8⁺ tumor-infiltrating lymphocytes (TILs) correlate with improved prognosis in HCC patients, as they are crucial for tumor surveillance and control.³⁵ CD8⁺ T-cells induce tumor cell death through several mechanisms, including the release of cytotoxic granules that perforate tumor cell membranes and trigger apoptosis. They also secrete pro-inflammatory cytokines such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha, which enhance the immune response.³⁵ CD4⁺ T-cells support the immune response by secreting cytokines that activate CD8⁺ T-cells and help coordinate the immune response against tumors.³⁶ They differentiate into various subsets, including T helper 1 cells, which are particularly effective in antitumor immunity.³⁷ Although the number of CD4⁺ TILs tends to decrease as HCC progresses – suggesting a

potential mechanism for tumor escape – the presence of these cells is associated with improved outcomes.³⁸ The recruitment of lymphocytes to the TME is critical for effective antitumor responses.³⁹ However, in HCC, there is often impaired infiltration of T-cells into the tumor, with higher concentrations observed in the peritumoral area. This suggests that while an immune response is initiated, its effectiveness is compromised by barriers preventing lymphocyte access to the tumor.³⁷

Natural killer cells contribute to the antitumor response by recognizing and killing tumor cells without prior sensitization. In HCC, the recruitment of NK cells to the tumor site is often impaired, despite their increased presence in the peripheral blood of patients.⁴⁰ The density and composition of lymphocyte populations within the

TME are significant prognostic indicators in HCC.⁴¹ Higher levels of CD3⁺ and CD8⁺ TILs are generally associated with improved OS and disease-free survival (DFS) rates in HCC patients.³⁵ However, the relationship between lymphocyte infiltration and patient outcomes is complex. Some studies suggest that the mere presence of TILs is not sufficient for effective antitumor activity; rather, the functional state of these cells is also critical.⁴²

In HCC, chronic inflammation often precedes tumor development, primarily due to viral infections (such as HBV and HCV) or liver diseases such as cirrhosis. Inflammatory cells, including macrophages and neutrophils, contribute to the TME by secreting pro-inflammatory cytokines that promote tumor growth and immune evasion.⁴³ Tumor-associated macrophages (TAMs) adopt a pro-tumorigenic phenotype, enhancing tumor progression through the secretion of GFs and cytokines to promote angiogenesis and suppress effective immune responses.⁴⁴

CD8⁺ cytotoxic T lymphocytes are essential for targeting and killing tumor cells.⁴⁵ However, their effectiveness is hindered by the presence of regulatory T-cells (Tregs), which suppress immune responses and are often found in increased numbers within HCC tissues. A higher ratio of effector T-cells to Tregs is generally associated with better patient outcomes.⁴⁶ NK cells play a key role in recognizing and destroying tumor cells. However, their functionality is often impaired in HCC due to the TME, which leads to immune evasion.⁴⁷ The balance between activated NK cells and immunosuppressive cells, including Tregs, influences the overall antitumor response.³⁸ An imbalance favoring inflammatory cells and Tregs creates an immunosuppressive environment, allowing unchecked progression to HCC.⁴⁸ For instance, an increase in Tregs is associated with poor prognosis, as they inhibit the activity of effector T-cells and NK cells, thereby facilitating tumor growth and metastasis.³⁸ Conversely, a strong infiltration of activated CD8⁺ T-cells and NK cells correlates with improved survival rates, as these cells effectively target and eliminate tumor cells.⁴⁹ The presence of pro-inflammatory cytokines such as IFN- γ enhances the activity of these immune cells, promoting an enhanced antitumor response.⁵⁰

3. Prognostic value of NLR and PLR in HCC

High NLR and PLR have emerged as significant prognostic indicators in HCC, correlating to tumor progression, metastasis, and survival outcome. Elevated NLR is associated with poorer prognosis in HCC patients, indicating an increased likelihood of tumor progression and metastasis. High NLR is linked to greater vascular invasion and a higher rate of extrahepatic disease recurrence, particularly

in early-stage tumors. High neutrophil levels promote tumor progression through enhanced angiogenesis by VEGF and other proinflammatory cytokines.¹⁶ Similarly, elevated PLR correlates with poor OS and DFS in HCC patients. High PLR is associated with large tumor sizes and an increased risk of metastasis. This ratio is thought to reflect the inflammatory state of the TME, with platelets potentially facilitating tumor growth and dissemination through interactions with tumor cells and the immune system.⁵¹

Studies have demonstrated that patients with elevated NLR and PLR experience significantly shorter survival rates compared to those with lower ratios. For example, patients with an NLR-PLR score of 2 have a median OS of just 11.2 months, while those with a score of 0 have a median OS of 22.5 months. Similarly, PFS is notably shorter in patients with a high NLR-PLR score, with a median PFS of 5.2 months compared to 7.8 months for those with a score of 0. These findings highlight the strong prognostic value of the NLR-PLR score as an independent predictor of survival outcomes in cancer patients.⁵² The significance of these ratios is further highlighted in patients undergoing liver transplantation (LT), where high pre-operative NLR and PLR are associated with higher recurrence rates after the procedure.¹⁶ LT is considered the ideal treatment for HCC as it addresses both the tumor and underlying liver disease. Niu *et al.*⁵³ indicated that high pre-operative NLR significantly correlates with decreased OS (14 vs. 27 months) and DFS (8 vs. 23.5 months). In 110 HCC patients undergoing orthotopic LT, a combined NLR-PLR score improves prognostic accuracy, with score of 2 predicting significantly shorter OS (6 vs. 27 months) and DFS (6 vs. 24.5 months). The NLR-PLR score demonstrates greater predictive value compared to NLR and PLR alone.⁵³

A meta-analysis of nine studies involving 2017 patients showed that elevated PLR was associated with poor OS (HR = 1.63) and poor DFS/RFS (HR = 1.32). However, high PLR was not significantly associated with vascular invasion, tumor multifocality, poor tumor grade, or elevated serum alpha-fetoprotein (AFP) levels. Therefore, PLR is a reliable, cost-effective prognostic biomarker for HCC.⁵⁴ The Kyoto group proposed extending the selection criteria for living donor LT to include patients with up to 10 nodules, all <5 cm in diameter, and with des-gamma-carboxy prothrombin levels under 400 mAU/mL. In their study, patients within these criteria demonstrated a 5-year survival rate of 82%, significantly better than those beyond these criteria. Similarly, the Kyushu group suggested a maximum tumor diameter of <5 cm without limiting the number of nodules, achieving a 5-year RFS of 71% among those who met these extended criteria.⁵⁵ In addition, HCC

risk assessment and LT (HALT) is a tool used to predict the survival of patients with HCC. It evaluates various factors, including liver function, tumor features, and blood test results, to estimate the risk of cancer recurrence and OS. The HALT-HCC score helps doctors make informed treatment decisions and manage patients more effectively, ultimately improving outcomes for HCC patients.⁵³

Patients with baseline NLR of <5 and a moderate decrease in NLR during treatment exhibited significantly longer OS, with a median OS of 27.8 months. In contrast, patients with a baseline NLR of 5 or higher and those experiencing a significant increase in NLR had the shortest OS, with a median of 5.0 months. Patients with a significant decrease in NLR also had poorer outcomes, with a median OS of 11.4 months. These findings highlight the non-linear relationship between NLR and survival, demonstrating that moderate decreases in NLR during treatment with immune checkpoint inhibitors (ICIs) are associated with the most favorable outcomes.⁵⁶ A systematic review of 123 studies indicated that higher pre-treatment NLR levels correlated with poorer OS and DFS in HCC patients. For instance, patients with an $\text{NLR} \geq 3.37$ had a median OS of 14 months, compared to 27 months for those with lower NLR.^{53,57} A meta-analysis encompassing 16 studies with a total of 4654 patients found that high NLR is significantly associated with poor prognosis, demonstrating a pooled sensitivity of 68% and specificity of 73% for predicting unfavorable outcomes.⁵⁸ In a cohort study, patients with a score of 2 (indicating both high NLR and high PLR) had a median OS of only 6 months, significantly lower than those with scores of 0 or 1, with median OS of 27 and 26.5 months, respectively. Multivariate analysis confirmed that the NLR-PLR score is an independent risk factor for prognosis and survival. These findings suggest that NLR and PLR, when assessed individually, have limited predictive capability.⁵³

Table 1 shows the studies examining the prognostic values of the NLR and PLR in patients undergoing transarterial chemoembolization (TACE) and receiving treatment medications for HCC.

4. Diagnostic value of neutrophil-to-lymphocyte and PLR in HCC

Recent studies have highlighted the potential of NLR and PLR as diagnostic tools for identifying HCC at early stages, particularly in patients with underlying liver conditions such as cirrhosis and hepatitis.¹⁷ Both NLR and PLR have been associated with survival outcomes in HCC patients. A meta-analysis revealed that a high baseline NLR is significantly linked to poor prognosis, with pooled sensitivity and specificity values of 0.68 and 0.73, respectively.

This suggests that while NLR and PLR provide valuable information about a patient's prognosis, their effectiveness in the early diagnosis is not fully established.⁶⁹ In addition, a retrospective study found that PLR is associated with tumor size and survival, indicating its potential use for screening patients with hepatitis or cirrhosis to detect small, potentially curable tumors. This highlights the value of these ratios for the early detection, particularly when traditional biomarkers may not be as effective.⁷⁰

Both NLR and PLR can serve as supplementary tools alongside imaging techniques such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI).⁷¹ While imaging is crucial for identifying tumor characteristics, these ratios provide additional context regarding the tumor's inflammatory environment.⁷² Elevated NLR and PLR are often associated with malignancy and aid in differentiating HCC from benign liver conditions, particularly in patients with chronic liver disease. It has been demonstrated that tumor size and aggressiveness are correlated with both NLR and PLR.⁷³ For instance, studies showed a substantial correlation between PLR and maximum tumor diameter, which is useful in determining the stage of HCC and assessing the need for surgical intervention. Utilizing NLR and PLR as non-invasive biomarkers allows for ongoing monitoring of disease progression and treatment response.⁷⁴ Regular blood tests are performed to track changes in these ratios, providing real-time insights into the patient's inflammatory status and potential tumor activity without the need for repeated invasive procedures.⁷⁵

5. Neutrophil-to-lymphocyte and platelet-to-lymphocyte in HCC: Mechanisms and therapeutic implications

High NLR and PLR indicate an inflammatory TME in HCC through several key mechanisms. An elevated NLR reflects increased neutrophil infiltration into TME. TANs release cytokines, chemokines, and enzymes that contribute to tumor growth, invasion, and angiogenesis.¹⁶ Neutrophils produce ROS and proteolytic enzymes, inducing oxidative stress within the TME, which results in genetic instability, promoting tumor progression.⁷⁶ Similarly, high PLR indicates increased platelet activation in response to the inflammatory TME. Activated platelets release GFs such as VEGF, enhancing angiogenesis and tumor growth.⁷⁷ Platelets also interact with immune cells such as neutrophils and lymphocytes, which modulate the inflammatory responses, potentially suppressing antitumor immunity and creating a conducive environment for tumor progression.⁷⁸ In HCC, the inflammatory TME suppresses lymphocyte function and reduces their numbers, leading

Table 1. The prognostic significance of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in hepatocellular carcinoma

Study design	Markers	Sample size	Treatment	Cutoff value	P-value	Endpoint	Statistical method	Reference
Meta-analysis	NLR	302	TACE	NA	<0.0001	OS	Fixed effect	59
Retrospective	NLR	495	TACE	3	<0.001	OS	Cox (ROC)	60
Retrospective	NLR	931	TACE	<5	0.021	OS	Multivariable Cox proportional hazards model, logistic regression	61
Retrospective	NLR	216	TACE	1.77	<0.0001	OS	Log-rank	61
Retrospective	PLR	291	TACE	150	0.002	OS	Multivariate Cox	62
Retrospective	PLR	216	TACE	94.62	0.0022	OS	Log-rank	61
Retrospective	PLR	204	TACE RFA	95.65	<0.0001	OS	Cox (ROC) logistic regression	63
Retrospective	PLR	283	Lenvatinib	150	0.033	OS, PFS	Multivariate Cox	64
Retrospective	PLR	134	TACE+apatinib	150	0.014	OS	Cox	65
Retrospective	PLR	128	TACE	92	<0.05	PFS	Cox (ROC)	66
Retrospective	PLR	48	Atezolizuma+Bevacizumab	230	0.001	PFS	Multivariate cox model	67
Retrospective	PLR	46	DEB-TACE	NA 113.1	0.004 <0.001	PFS	Linear regression Cox (mean)	68

Abbreviations: DEB-TACE: Drug-eluting bead transarterial chemoembolization; NA: Not available; NLR: Neutrophil-to-lymphocyte ratio; OS: Overall survival; PFS: Progression-free survival; PLR: Platelet-to-lymphocyte ratio; RFA: Radiofrequency ablation; ROC: Receiver operating characteristic; TACE: Transarterial chemoembolization.

to a relative increase in neutrophils and platelets compared to lymphocytes. This imbalance results in elevated NLR and PLR. Since lymphocytes are crucial for antitumor immunity, their depletion or dysfunction, as indicated by elevated NLR and PLR, impairs the body's ability to mount an effective immune response against the tumor¹⁶ (Figure 2).

In recent years, immunotherapy has significantly influenced the treatment of advanced HCC (aHCC), making the identification of predictive biomarkers crucial for improving patient outcomes. Jia *et al.*⁷⁹ conducted a study involving 117 aHCC patients treated with anti-programmed cell death protein 1 antibodies. Their findings identified peripheral blood biomarkers, including hemoglobin, NLR, and AFP, as significant predictors of PFS and OS. The median PFS was reported as 7.0 months, while the median OS was reported as 18.7 months. The study also highlighted the development of a prognostic nomogram model that accurately predicts PFS and OS, aiding in the selection of patients who are most likely to benefit from immunotherapy. These findings revealed the importance of peripheral blood biomarkers in guiding personalized treatment strategies for aHCC.⁷⁹ In addition, Liu *et al.*⁸⁰ demonstrated that lower baseline NLR and PLR are significantly associated with improved OS and PFS in patients undergoing immunotherapy for HCC. Furthermore, reductions in these ratios during

early treatment cycles correlated to improved disease control rates, indicating their role as dynamic predictors of treatment efficacy. These markers not only reflect the inflammatory status of the TME but also provide actionable insights into optimizing therapy, highlighting the potential of NLR and PLR for patient stratification and monitoring response during treatment.⁸⁰ Dharmapuri *et al.*⁸¹ studied 361 HCC patients treated with ICIs and found that those with lower baseline NLR and PLR were at higher risk of developing severe immune-related adverse events (irAEs). Their results showed that patients with a PLR of more than 300 had a significantly lower incidence of grade ≥ 2 irAEs, and a similar trend was observed for an NLR >5 . These findings suggest that higher NLR and PLR indicate a less reactive immune environment, thereby reducing the risk of irAEs. Monitoring baseline NLR and PLR can help in identifying patients likely to experience irAEs, facilitating personalized treatment planning.⁸¹

The inflamed TME attracts myeloid-derived suppressor cells, Tregs, and TAMs, all of which contribute to the suppression of antitumor immune responses.⁸² Chronic inflammations lead to the upregulation of immune checkpoint molecules such as programmed death ligand 1, and cytotoxic T-lymphocyte-associated protein 4 on both tumor and immune cells, diminishing their responsiveness to immunotherapy.⁸³ In addition, inflammatory cytokines and metabolites within the TME inhibit the function and

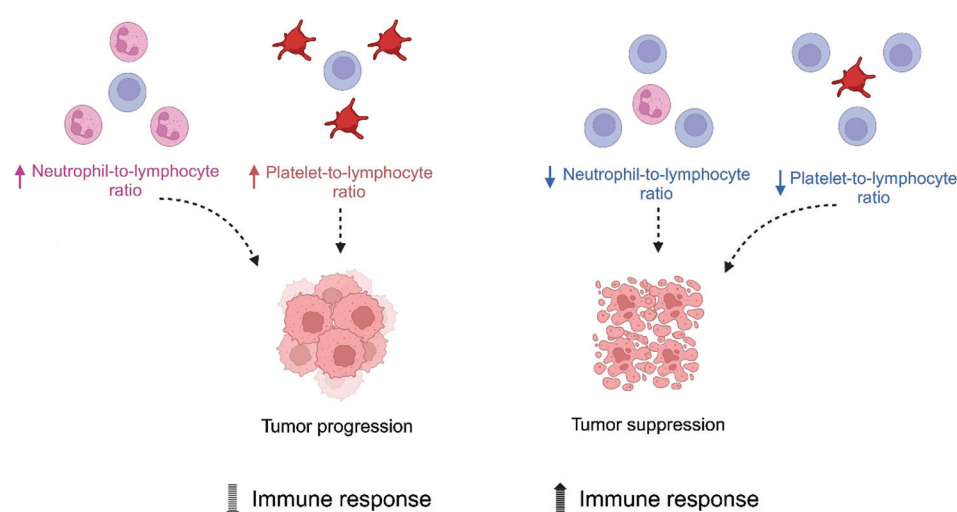


Figure 2. The effect of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) on tumor progression and immune response. On the left, elevated NLR and PLR are associated with tumor progression and a reduced immune response. On the right, lower NLR and PLR are associated with tumor suppression and higher immune response.

proliferation of effector T-cells, impairing their ability to eliminate tumor cells.⁸⁴ The inflammatory response also promotes the formation of new blood vessels, which supports tumor growth and metastasis while creating barriers that hinder immune cell infiltration into the tumor.⁸⁵

High NLR and PLR often indicate more aggressive tumor biology, suggesting that patients could benefit from ICIs or combination therapies targeting inflammatory pathways.⁸⁶ Conversely, patients undergoing systemic treatment, such as sorafenib, who exhibit lower NLR and PLR values tend to demonstrate better OS and PFS. This suggests that lower inflammatory profiles are associated with better responses to targeted therapies, assisting clinicians in choosing the most appropriate treatment regimens.⁸⁷ Monitoring changes in NLR and PLR during treatment provides information on therapy effectiveness. An increase in these ratios post-treatment may signal a poor prognosis, prompting clinicians to reconsider or modify the treatment strategy.⁸⁸ Since NLR and PLR are derived from routine blood tests, they are easily accessible and non-invasive. Incorporating these measures into clinical practice enables regular monitoring of patients' inflammatory status, allowing timely adjustments to treatment plans based on individual responses.⁸⁹

6. Other biomarkers in the diagnosis and management of HCC

Other important biomarkers in the diagnosis and management of HCC include AFP, AFP-L3, and circulating tumor DNA (ctDNA). Each of these

biomarkers demonstrates the presence and progression of liver cancer, guiding clinicians to make informed decisions regarding patient care.⁹⁰ AFP is a glycoprotein whose levels are typically very low in healthy individuals but rise significantly in liver diseases and cancers. In healthy adults, the levels are generally below 4.7 ng/mL, while levels exceeding 200 ng/mL are highly suggestive of HCC, although elevations can also occur in other liver diseases.⁹¹ AFP is commonly used as a tumor marker for HCC. Elevated levels indicate the presence of liver cancer, especially in patients with underlying liver disease such as hepatitis or cirrhosis.⁹² Monitoring AFP levels over time is crucial in assessing treatment response and detecting recurrence, as a significant rise in AFP indicates cancer progression or recurrence after treatment.⁹³ AFP testing is often conducted alongside imaging studies such as ultrasound, CT, MRI, contrast-enhanced ultrasound, and positron emission tomography to improve diagnostic accuracy.⁹⁴ A phase III clinical trial demonstrated the efficacy of ramucirumab as a second-line treatment for patients with aHCC and elevated AFP levels. The trial results revealed that ramucirumab significantly improved OS and PFS in patients with baseline AFP levels of 400 ng/mL or higher. In an analysis involving 155 Chinese patients, those treated with ramucirumab had a median OS of 7.1 months compared to 4.7 months for those receiving a placebo, along with improved PFS and disease control rates. A phase III clinical trial (REACH/REACH-2) studied the use of ramucirumab as a second-line treatment for aHCC in patients with high AFP levels (≥ 400 ng/mL). The analysis of 542 patients showed that ramucirumab improved OS and PFS in patients with different liver

disease causes, including HBV, HCV, and others. Median OS (ramucirumab vs. placebo) was 7.7 vs. 4.5 months for HBV, 8.2 vs. 5.5 months for HCV, and 8.5 vs. 5.4 months for other causes. Patients with active HBV had the worst outcomes, but using antiviral therapy improved survival and liver health. These results show that ramucirumab is effective and safe for aHCC patients with high AFP levels.⁹⁵ Recent studies suggest that combining immunotherapy with AFP monitoring can improve treatment outcomes in HCC. ICIs may help reduce tumor burden and monitor AFP levels to assess treatment response. Rising AFP levels during therapy can signal disease progression, allowing for timely treatment adjustments.⁹⁶ Locoregional therapy (LRT) techniques such as TACE and radiofrequency ablation are used for localized liver tumors. Evaluating AFP levels before and after these treatments helps assess their effectiveness. A significant drop in AFP levels usually indicates a positive response, while continual elevation suggests incomplete treatment.⁹⁷

AFP L3 is a specific glycoform of AFP produced by malignant hepatocytes. It can be distinguished from other forms of AFP by its ability to bind to the lectin lens culinaris agglutinin.⁹⁸ An AFP-L3 percentage >10% is indicative of HCC and has shown potential in detecting early-stage HCC, especially when total AFP levels are low (<200 ng/mL).⁹⁹ AFP-L3 is detected in approximately 35% of patients with small HCC lesions (<2 cm) and provides a lead time of 9 – 12 months over traditional imaging techniques for early diagnosis.⁹³ In addition, high levels of AFP-L3 are associated with more aggressive tumor characteristics, including early vascular invasion and metastasis. While AFP-L3 is primarily useful for monitoring recurrence after treatment, using it alongside total AFP and imaging techniques enhances diagnostic accuracy and guides in monitoring high-risk populations.¹⁰⁰ A meta-analysis evaluated the diagnostic value of AFP-L3 percentage for the early HCC using six studies involving 2,447 patients. AFP-L3 percentage showed high specificity (92%) but low sensitivity (34%), indicating it is more effective for ruling out HCC in cases with elevated AFP rather than diagnosing early HCC. Improving the detection method could enhance its diagnostic accuracy.¹⁰¹ Monitoring AFP-L3 levels during immunotherapy can provide insights into treatment efficacy, allowing clinicians to make informed decisions regarding therapy adjustments based on biomarker responses.¹⁰² Moreover, pre-transplant LRT significantly lowers AFP-L3 levels, which correlates with improved post-transplant outcomes. The combination of LRT followed by careful monitoring of AFP-L3 ensures that patients are optimally prepared for transplantation, thereby minimizing the risk of recurrence.¹⁰³

ctDNA refers to small fragments of DNA that are released into the bloodstream from tumor cells and serve as a non-invasive biomarker for cancer detection and monitoring.¹⁰⁴ ctDNA analysis demonstrates tumor dynamics, including genetic mutations and treatment responses, making it useful for personalized medicine.¹⁰⁵ Early studies have suggested that ctDNA reveals prognostic information regarding disease progression and recurrence risk in HCC patients. It has the potential to detect small amounts of residual cancer following treatment.¹⁰⁶ ctDNA levels are monitored before, during, and after treatment to evaluate the effectiveness of treatments such as targeted treatments or immunotherapy. A decrease in ctDNA levels typically indicates a favorable response to treatment, while an increase suggests disease progression or treatment failure.¹⁰⁷ In a study involving 47 HCC patients post-resection, ctDNA was detectable in 87.2% of patients, with 45.7% exhibiting detectable tumor mutational burden (TMB). Notably, high TMB was associated with reduced RFS and proved to be a better predictor of recurrence than AFP levels. A TMB cutoff of 4.8 mutations per megabase effectively predicted recurrence and poorer RFS, with TMB-high status emerging as an independent risk factor for worse RFS. These results suggest that post-operative ctDNA, particularly TMB detection, is a valuable tool for monitoring recurrence and predicting outcomes in HCC patients, highlighting the need for further research.¹⁰⁸ Moreover, integrating ctDNA analysis with immunotherapy strategies offers potential benefits in managing HCC by measuring tumor dynamics during immunotherapy, demonstrating treatment efficacy, and guiding necessary adjustments. For example, changes in ctDNA levels can indicate tumor response or resistance to ICIs, allowing for timely modifications in therapy. Studies have shown that monitoring ctDNA levels before and after LRT helps assess treatment effectiveness. For instance, one study indicated that changes in ctDNA levels following TACE correlated with disease progression, allowing clinicians to stratify patients into high- or low-risk groups for HCC progression.¹⁰⁹

Table 2 summarizes biomarkers for HCC, describing their defining characteristics, prognostic implications, and roles in clinical practice for diagnosing, guiding treatment approaches, and monitoring disease progression.

7. Challenges and future directions

The NLR and PLR are increasingly recognized as potential biomarkers in the management of HCC. However, their clinical use is limited by several factors, including variability in results, confounding factors, and the need for standardization. Different studies have used various cutoff values for NLR and PLR, leading to inconsistent results

Table 2. Biomarkers for hepatocellular carcinoma with their characteristics, prognostic significance, and clinical applications in diagnosis, treatment decisions, and monitoring

Biomarker	Biomarker characteristics	Prognostic value	Clinical use	References
NLR	The ratio of neutrophils to lymphocytes. Reflects systemic inflammation and immune response.	High NLR is associated with poorer OS and RFS. It indicates a pro-tumor inflammatory environment.	Used to stratify patients for treatment aggressiveness; simple and cost-effective.	110
PLR	The ratio of platelets to lymphocytes. Indicates inflammatory status and immune response balance.	Elevated PLR correlates with worse OS and earlier recurrence in HCC patients. It suggests a pro-tumor state influenced by platelet activity.	Helps in treatment decision-making; indicates the need for closer monitoring post-surgery.	111
AFP	A protein produced by the liver; elevated levels are often seen in liver diseases, including HCC.	AFP levels >400 ng/mL are associated with poor prognosis; however, not all HCC cases present elevated AFP levels, leading to false negatives.	Commonly used for diagnosis and monitoring; however, limited specificity as it can be elevated in other liver conditions.	112
AFP-L3	A specific isoform of AFP that is more closely associated with HCC than total AFP.	Higher AFP-L3 levels suggest a greater likelihood of malignancy and are correlated with a worse prognosis.	Provides additional specificity for HCC diagnosis when total AFP is elevated; useful in monitoring disease progression.	113
ctDNA	Fragments of circulating tumor DNA shed from cancer cells into the bloodstream.	Emerging evidence suggests that ctDNA can provide insights into tumor dynamics, treatment response, and recurrence risk. Early studies indicate its potential as a prognostic marker in HCC.	Non-invasive way to monitor tumor burden and genetic mutations; still under investigation for standard clinical use but shows promise for personalized medicine approaches.	114

Abbreviations: AFP: Alpha-fetoprotein; ctDNA: Circulating tumor DNA; HCC: Hepatocellular carcinoma; NLR: Neutrophil-to-lymphocyte ratio; OS: Overall survival; PLR: Platelet-to-lymphocyte ratio; RFS: Recurrence-free survival.

regarding their prognostic significance. For instance, while some studies suggest an NLR cutoff of 3.82 or PLR of 140 for predicting poor outcomes, others use different thresholds, complicating the interpretation of results across populations.¹¹⁵ The prognostic value of NLR and PLR also varies based on patient demographics, including ethnicity and gender. Studies have shown that inflammatory responses differ significantly among populations, which can influence the effectiveness of these biomarkers in different cohorts.⁵⁷ Moreover, prior therapies may affect inflammatory markers, potentially skewing results and making it difficult to ascertain the true prognostic value of these ratios in treatment-naïve populations. For NLR and PLR to be effectively used in clinical practice, their roles must be clearly defined within existing clinical guidelines for HCC management. This includes establishing standardized cutoff values and integrating these markers into prognostic models alongside other clinical parameters. Advances in diagnostic technologies, including imaging techniques and blood-based biomarkers, are enhancing early detection and prognosis. The use of these biomarkers and emerging technologies such as spatial transcriptomics and single-cell RNA sequencing holds potential for improving HCC management and patient outcomes, while advanced tools, including CIBERSORTx, mass cytometry, artificial intelligence, and machine learning, further enhance diagnostic precision and prognostic accuracy by uncovering intricate immune and molecular profiles.

8. Conclusion

HCC is a major global health challenge, being the most common primary liver cancer and contributing significantly to cancer-related deaths. The high mortality rate of HCC is driven by its late diagnosis and the limited effectiveness of current treatments. The prognosis for HCC patients is generally poor, with a 5-year survival rate of <5% for those not undergoing LT or resection. Chronic liver conditions, particularly HBV and HCV infections, are primary risk factors for the majority of HCC cases. Cirrhosis from any cause further increases HCC risk. Emerging risk factors, such as MASLD, linked to obesity and metabolic syndrome are also becoming increasingly significant. These conditions contribute to the accumulation of liver damage and oncogenic mutations. Systemic inflammation plays an important role in HCC progression. Chronic inflammatory states lead to the release of cytokines and GFs that promote hepatocellular proliferation and suppress immune responses. The NLR and PLR have been identified as valuable biomarkers in the management of HCC. Elevated NLR and PLR correlate with advanced stages of HCC and poorer patient outcomes, providing prognostic insights and assisting in patient management and treatment planning. As crucial biomarkers for HCC diagnosis and prognosis, NLR and PLR reflect the systemic inflammatory response and are strongly associated with tumor progression, patient survival, and recurrence. This makes them valuable tools for guiding clinical decisions and personalizing treatment strategies in patients with HCC.

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Conflict of interest

The authors declare that they have no competing interests.

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