Journal of Hepatocellular Carcinoma

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ORIGINAL RESEARCH

Dynamic Changes of Neutrophil-to-Lymphocyte Ratio on Predicting Response of Immune Checkpoint Inhibitors Plus Targeted Therapies for Unresectable Hepatocellular Carcinoma

Jianming Yang[1,*], Yu Zhang[2,*], Yewu Chen[1,*], Yang Yang[1,3], Yinan Deng[1]

[1]Department of Hepatic Surgery and Liver Transplantation Center, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, People's Republic of China; [2]Department of Breast Surgery, Sun Yat-sen Memorial Hospital, Guangzhou, People's Republic of China; [3]Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yang Yang; Yinan Deng, Department of Hepatic Surgery and Liver Transplantation Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou, People's Republic of China, Tel +86 20 85253106, Fax +86 20 85252276, Email yysysu@163.com; dengyinan2010@163.com

Backgrounds and Aims: Multiple regimens of immune checkpoint inhibitors (ICIs) plus targeted therapies are commonly prescribed as first-line treatments for unresectable hepatocellular carcinoma (uHCC). Here, we aimed to investigate the correlation between dynamic changes of neutrophil-to-lymphocyte ratio (NLR) and tumor response to the combination of ICIs and targeted therapies for uHCC.

Methods: Sixty-one patients who received ICIs plus targeted therapies for uHCC were enrolled in this retrospective study. The NLR before and at 3–6 weeks after treatments were assessed to calculate the dynamic NLR changes (ΔNLR). Multivariate logistic regression and Cox regression models were used to explore the relationship between dynamic NLR changes and tumor response or progression-free survival (PFS), respectively. Furthermore, we assessed the predictive effect of alpha-fetoprotein (AFP) changes in combination with dynamic NLR changes compared to AFP changes alone.

Results: The NLR at 3–6 weeks and ΔNLR after treatments significantly increased in patients who underwent progressive disease (PD), while the baseline NLR showed no significant difference between different tumor responses. Increased NLR and AFP after treatments were both independent predictors of PD (For NLR increase: OR, 2.28; 95% CI, 1.47–3.88, $P < 0.001$; For AFP increase: OR, 1.46; 95% CI, 1.03–2.17, $P = 0.043$), and correlated with worse PFS (for NLR increase: HR, 4.08; 95% CI, 1.99–8.36, $P < 0.001$; for AFP increase: HR, 2.10; 95% CI, 1.04–4.24, $P = 0.039$). The receiver operating characteristic (ROC) curve and net reclassification index (NRI) showed that the combination of dynamic NLR and AFP changes was better than AFP changes alone on predicting PD (AUC: 0.83 vs 0.68, $P = 0.034$; NRI: 0.340, $P = 0.048$) and PFS (AUC: 0.80 vs 0.70, $P = 0.166$; NRI: 0.431, $P = 0.042$).

Conclusion: Dynamic changes of NLR might be an effective predictor of the therapeutic response to ICIs plus targeted therapies for uHCC.

Keywords: hepatocellular carcinoma, neutrophil-to-lymphocyte ratio, immune checkpoint inhibitors, targeted therapies

Introduction

Several regimens of immune checkpoint inhibitors (ICIs) plus targeted therapies are recommended as first-line treatments for unresectable hepatocellular carcinoma (uHCC).[1–3] However, due to the heterogeneity of HCC, not all tumors exhibit sensitivity to these treatments. For instance, some real-world Phase III clinical trials, including the LEAP-002 study designed to evaluate the efficacy of pembrolizumab combined with lenvatinib in uHCC, have not met their primary endpoints (NCT03713593). Consequently, identification of predictive and prognostic biomarkers in patients who received ICIs plus targeted therapies has aroused great interest.

Received: 14 March 2024
Accepted: 18 July 2024
Published: 5 August 2024

Recent studies have suggested that the expression of inflammatory molecules and gene signatures might predict tumor response to ICIs or targeted therapies. A sample analysis based on CheckMate 040, a prospective clinical trial, suggested that tumorous PD-1 and PD-L1 expression were associated with improved OS in HCC patients who receive Nivolumab treatment.[4] A prospective cohort study also reported that circulating tumor DNA was correlated with tumor response after targeted therapies.[5] However, the widespread clinical application of these biomarkers is hindered by the difficulties and high costs of continuous monitoring. Therefore, it is critical to identify a readily available predictive biomarker for the tumor response to ICIs and targeted therapies for uHCC.

The neutrophil-to-lymphocyte ratio (NLR) is recognized as an indicator of systemic inflammation and immune status, and has been established as a prognostic biomarker for HCC,[6–8] which is more readily available and has a lower cost compared to other biomarkers. Studies have reported that an elevated NLR is associated with poor outcomes and tumor response in HCC patients receiving transarterial chemoembolization (TACE),[9–11] radiofrequency ablation (RFA),[12–14] liver transplantation,[15–17] curative resection,[18–20] and ICIs treatments.[21] Despite these findings, the correlation between dynamic NLR changes and tumor response of ICIs plus targeted therapies for uHCC remains unclear. Here, we aim to explore the relationship between dynamic NLR changes and tumor response of ICIs plus targeted therapies for uHCC.

Methods

Study Population

This study was approved by the institutional review board of the Third Affiliated Hospital of Sun Yat-sen University. Given the historical cohort nature of this study, the need for informed consent was therefore waived, and all the clinical data were collected and reviewed confidentially from the hospital's electronic database. A total of 406 consecutive patients received ICIs plus targeted therapies for uHCC from January 2019 to June 2022 at The Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) were enrolled in this retrospective study. The following exclusion criteria were adopted (Supplemental Material 1): age <18 years (n = 1), simultaneous treatments with hepatic arterial infusion chemotherapy (HAIC), TACE, RFA, portal vein embolization (PVE), or radiation therapy (n = 265), prior liver transplantation (n = 6), confirmed infection during treatments (n = 10), combined with cholangiocarcinoma (n = 1), follow-up time <1 month without any event (n = 37), and incomplete data (n = 25). The final population of 61 patients was analyzed, and NLRs before and 3–6 weeks after treatment were obtained to calculate the NLR dynamic changes (ΔNLR).

Treatments

All patients were treated with ICIs and targeted therapies. ICI treatments included Atezolizumab (1200 mg, every 3 weeks, intravenous infusion), Camrelizumab (200 mg, every 3 weeks, intravenous infusion), Sintilimab (200 mg, every 3 weeks, intravenous infusion), Tislelizumab (200 mg, every 3 weeks, intravenous infusion), Toripalimab (240 mg, every 3 weeks, intravenous infusion) and Pembrolizumab (200 mg every 3 weeks, intravenous infusion). The targeted therapies included sorafenib (400 mg, twice daily, oral), apatinib (250 mg, once daily, oral), lenvatinib (8 mg for <60 kg, 12 mg for ≥60 kg, once daily, oral), donafenib (200 mg, once daily, oral), regorafenib (160 mg, once daily, oral), and bevacizumab (15 mg/kg, every 3 weeks, intravenous infusion). Detailed regimens of ICIs plus targeted therapies are provided in Supplemental Material 2. All treatments were reduced or discontinued once poor drug-related adverse events occurred.

Outcomes and Follow-Up Assessment

Tumor responses were categorized as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), and were evaluated every 4 to 8 weeks via contrast-enhanced computed tomography (CT) or enhanced magnetic resonance imaging (MRI) according to the modified response evaluation criteria in solid tumors (mRECIST) 1.1 criteria. The objective response rate (ORR) was defined as the proportion of patients with CR or PR, and the disease control rate (DCR) was defined as the proportion of patients with CR, PR or SD. Serum alpha-fetoprotein (AFP) levels before and after treatments were collected to calculate dynamic AFP changes (ΔAFP), which serves as an additional indicator of tumor response. Progression-free survival (PFS) was calculated as the period from the initiation of ICIs plus

targeted therapies to the date of radiological progression, death from any cause, receiving other treatments after 1 month, or last follow-up (June 2022).

Statistical Methods

Continuous variables were described as median (interquartile ranges, IQR) and compared using the Mann–Whitney *U*-test. Categorical variables were described as exact numbers and proportions, and were compared using the chi-square and Fisher's tests. Progression-free survival (PFS) was assessed using the Kaplan–Meier method and was compared using the Log rank test. A multivariate logistic regression model was used to identify the risk factors for poor tumor response. A Cox proportional risk regression model was applied to estimate PFS, which were compared by using the Wald test. Variables with a P value <0.05 in the univariate analysis were eligible for multivariate logistic or Cox regression models. Receiver operating characteristic (ROC) curve and net reclassification index (NRI) analyses were utilized to assess the predictive effect of various factors on tumor response or PFS, which were compared by using Delong's test. Statistical significance was set at $P < 0.05$, and all statistical analyses were performed using R statistical software, version 4.1.0 (R Foundation Inc.; http://cran.r-project.org/).

Results

Baseline Characteristics and Follow-Up Status

Baseline characteristics of the 61 enrolled patients are detailed in Table 1. The majority of the patients were male (n = 57, 93.4%), and the predominant etiology of HCC was hepatitis B (n = 58, 95.0%), with a median age of 53 (IQR, 45–59). Patients were divided into groups based on baseline NLR and dynamic NLR changes, and comparative analysis revealed that most variables showed no significant differences between the two groups, except that the tumor size in the NLR-decreased group

Table 1 Baseline Characteristics of Patients with Decreasing or Increasing NLR After Treatments

Characteristics	NLR decrease(n=30)	NLR increase(n=31)	P
Demographic characteristics			
Male gender,(%)	28 (93.3)	29 (93.5)	>0.999
Age (median [IQR])	53.0 [45.2, 62.2]	53.0 [45.0, 58.0]	0.300
Diabetes (%)	6 (20.0)	3 (9.7)	0.438
Hypertension (%)	7 (23.3)	8 (25.8)	>0.999
HBV related (%)	27 (90.0)	31 (100.0)	0.225
Child Pugh B/C(%)	9 (30.0)	8 (25.8)	0.937
Tumor characteristics			
BCLC stage C,n(%)	28 (93.3)	23 (74.2)	0.094
ECOG score > 0,n(%)	15 (50.0)	13 (41.9)	0.708
Maximum tumor size (median [IQR]),cm	7.2 [4.2, 12.3]	4.2 [1.7, 9.2]	0.024
Number of lesion ≥ 3,n(%)	19 (63.3)	23 (74.2)	0.523
Macrovascular invasion,n(%)	11 (36.7)	12 (38.7)	>0.999
Macrovascular tumor thrombus,n(%)	13 (43.3)	10 (32.3)	0.530
Cirrhosis,n(%)	22 (73.3)	25 (80.6)	0.708
Metastasis,n(%)	16 (53.3)	13 (41.9)	0.526

(*Continued*)

Table 1 (Continued).

Characteristics	NLR decrease(n=30)	NLR increase(n=31)	P
Laboratory findings before treatments			
Baseline NLR (median [IQR])	2.7 [2.1, 4.2]	2.4 [1.9, 3.4]	0.189
Baseline AFP (median [IQR]),ng/mL	423.8 [8.1, 1201.0]	173.2 [30.7, 1201.0]	0.558
AST (median [IQR]),U/L	52.0 [35.2, 69.2]	48.0 [31.0, 77.0]	0.937
ALT (median [IQR]),U/L	39.5 [23.0, 58.5]	39.0 [30.5, 49.5]	0.806
ALB (median [IQR]),g/L	37.6 [35.0, 38.8]	36.9 [34.8, 40.4]	0.475
TB (median [IQR]),umol/L	15.4 [9.9, 30.2]	13.0 [7.8, 21.2]	0.121
Cr (median [IQR]),umol/L	66.0 [63.2, 77.4]	72.0 [64.0, 85.0]	0.225
PT (median [IQR]),sec	13.9 [13.2, 15.2]	14.1 [13.5, 14.8]	0.960
INR (median [IQR])	1.1 [1.0, 1.2]	1.1 [1.0, 1.1]	0.795
Theraputic response			
Iraes,n(%)	5 (16.7)	3 (9.7)	0.668
Tumor response,n(%)			
CR	1 (3.3)	0 (0.0)	<0.001
PR	13 (43.3)	1 (3.2)	
SD	7 (23.3)	6 (19.4)	
PD	9 (30.0)	24 (77.4)	

Abbreviations: NLR, Neutrophil-to-Lymphocyte Ratio; IQR, interquartile ranges; HBV, hepatitis B virus; BCLC stage, Barcelona Clinic Liver Cancer stage; ECOG score, Eastern Cooperative Oncology Group score; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; TB, total bilirubin; Cr, creatinine; PT, prothrombin time; INR, international normalized ratio; irAEs, immune-related adverse events; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

was larger than that in the NLR-increased group (7.2 cm vs 4.2 cm, P = 0.024). The median follow-up time of the entire cohort was 142 days. Before the cutoff date, one patient (1.6%) succumbed to liver failure and 43 (70.4%) experienced disease progression with a median progression time of 117 days.

Associations Between NLR Dynamic Changes and Tumor Responses

We investigated the association between dynamic NLR changes and therapeutic tumor response. Compared with the OR (CR/PR) or SD groups, the NLR at 3–6 weeks (Figure 1B) and ΔNLR (Figure 1C and D) after treatments significantly increased in patients who underwent PD ($P < 0.05$), while the baseline NLR showed no difference between different tumor responses (Figure 1A).

The proportions of various tumor responses at different baseline NLR levels and dynamic NLR changes are shown in Figure 1E and F. The cutoff of baseline NLR was estimated as 3.26 by using the X-tile plot software. In the analysis of baseline NLR subgroups, no significant difference was observed in ORR and DCR between the groups with baseline NLR <3.26 and ≥3.26 (ORR, 33.3% vs 9.1%, P = 0.072; DCR, 56.4% vs 27.3%, P = 0.054), whereas the ORR and DCR were significantly higher in the NLR-decreased group compared to the NLR-increased group (ORR, 46.7% vs 3.2%, P < 0.001; DCR, 70.0% vs 22.6%, P = 0.001). The NLR at 3–6 weeks after treatments showed decreasing, stable, and increasing patterns in the OR (P = 0.005), SD (P = 0.273), and PD (P = 0.046) groups, respectively (Figure 2). Multivariate logistic regression analysis showed that an increased NLR (OR, 2.28; 95% CI, 1.47–3.88, P < 0.001) and increased AFP (OR, 1.43; 95% CI, 1.03–2.17, P = 0.043) after treatments were both independent predictors of PD (Table 2).

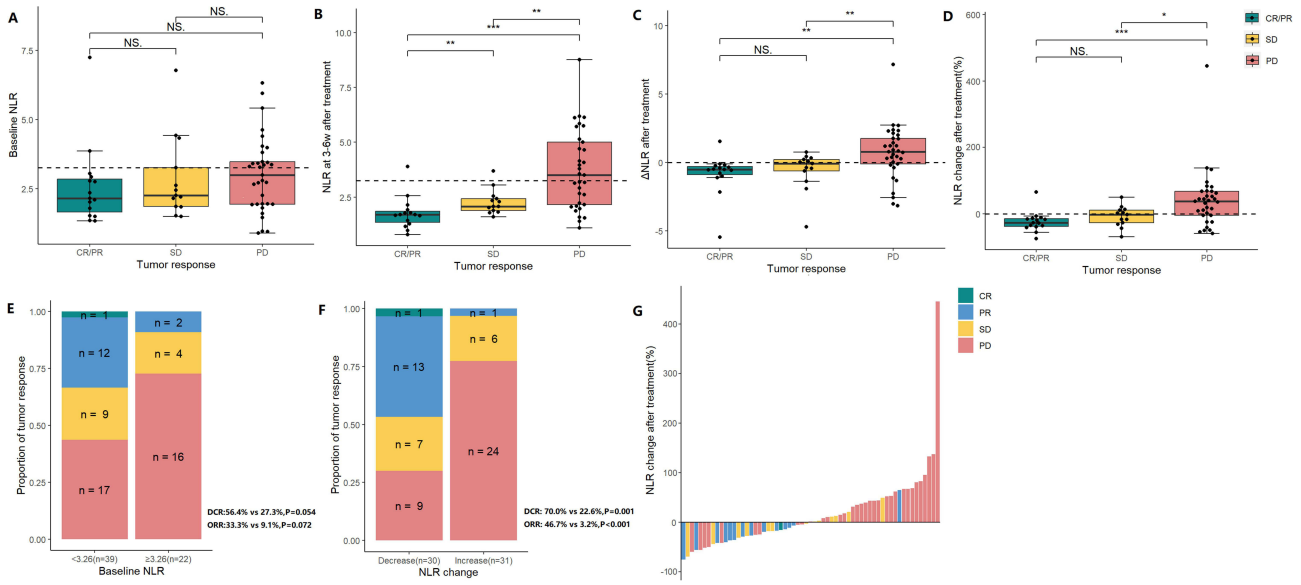

Figure 1 Association between tumor response and baseline NLR or dynamic NLR changes. (**A**) Comparison of baseline NLR level in patients with different tumor response. (**B**) Comparison of NLR at 3–6 weeks after treatment in patients with different tumor response. (**C**) Comparison of ΔNLR in patients with different tumor response. (**D**) Comparison of dynamic NLR change (%) in patients with different tumor response. (**E**) Comparison of DCR and ORR in patients with low or high level baseline NLR. (**F**) Comparison of DCR and ORR in patients with different NLR change. (**G**) Waterfall plot for NLR change of every patient.
Notes: A significant difference between the groups, ***P < 0.001, **P < 0.01, *P < 0.05.

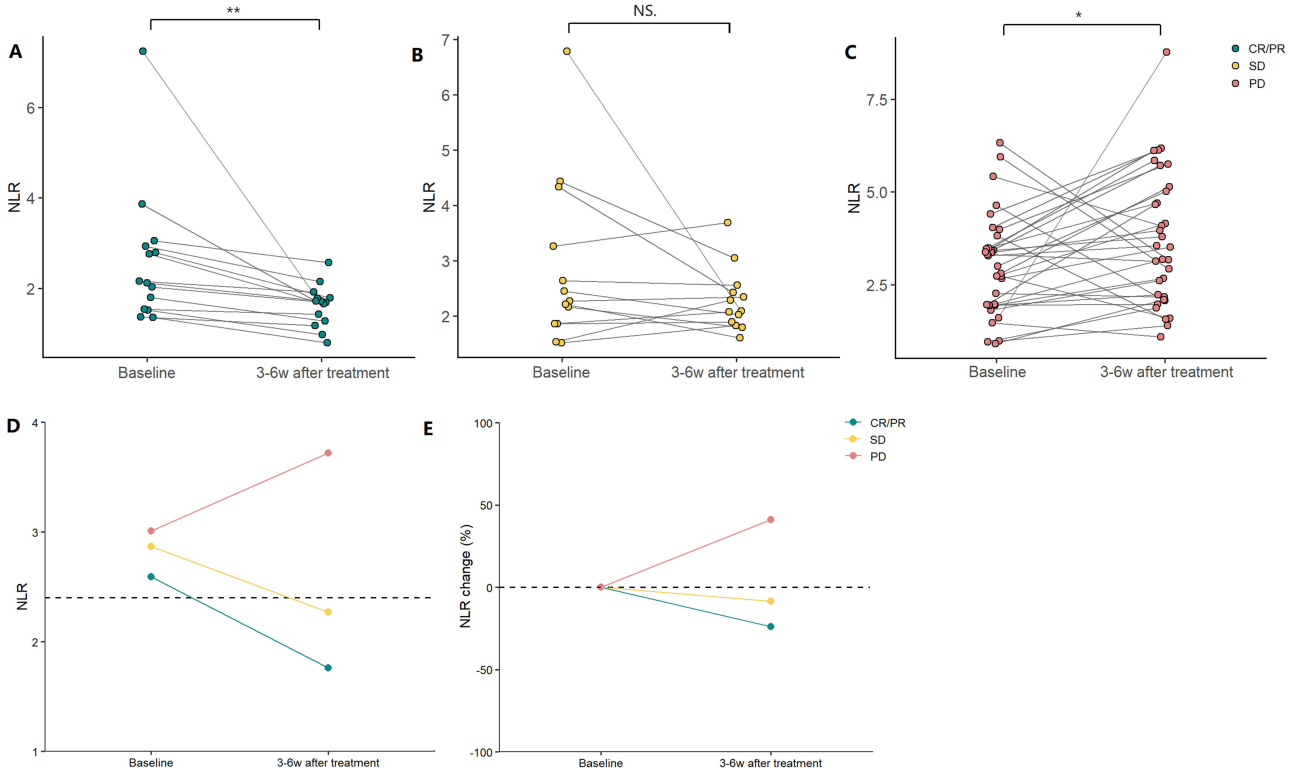

Figure 2 Patterns of NLR change after treatments in patients with different tumor response. (**A**) NLR before and after treatments in patients who underwent CR or PR. (**B**) NLR before and after treatments in patients who underwent SD. (**C**) NLR before and after treatments in patients who underwent PD. (**D**) Average NLR level before and after treatments in patients with different tumor response. (**E**) Average NLR change in patients with different tumor response.
Notes: A significant difference between the groups, **P < 0.01, *P < 0.05.

Table 2 Univariable and Multivariable Logistic Regression Analysis for PD

Characteristics	Univariate		Multivariate	
	OR(95% CI)	P	OR(95% CI)	P
Age,per 1 year	0.95(0.90–1.01)	0.081		
ECOG score > 0	2.20(0.77–6.07)	0.144		
BCLC stage C	0.76(0.47–1.24)	0.278		
Child Pugh B/C	0.94(0.31–2.88)	0.910		
HBV related	2.50(0.21–28.7)	0.472		
Baseline NLR,per 1	1.20(0.80–1.70)	0.420		
NLR change after treatments, per 30% increase	2.30(1.50–3.68)	<0.001	2.28(1.47–3.88)	<0.001
Number of lesion ≥ 3	1.50(0.50–4.40)	0.479		
Maximum tumor size ≥ 5cm	0.61(0.22–1.69)	0.341		
Macrovascular invasion	0.67(0.24–1.89)	0.445		
Macrovascular tumor thrombus	0.88(0.31–2.49)	0.815		
Metastasis	0.83(0.30–2.29)	0.723		
Cirrosis	0.85(0.26–2.84)	0.795		
Baseline AFP ≥ 400ng/mL	1.30(0.46–3.46)	0.660		
AFP change after treatments, per 30% increase	1.50(1.10–2.03)	0.019	1.46(1.03–2.17)	0.043
AST ≥ 40U/L	1.20(0.41–3.21)	0.784		
ALT ≥ 40U/L	0.88(0.31–2.45)	0.804		

Abbreviations: PD, progressive disease.; OR, odds ratio; CI, confidence interval; ECOG score, Eastern Cooperative Oncology Group score; BCLC stage, Barcelona Clinic Liver Cancer stage; HBV, hepatitis B virus; NLR, Neutrophil-to-Lymphocyte Ratio; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Associations Between Dynamic NLR Changes and Progression-Free Survival

Survival analysis revealed that both groups of NLR-decreased and baseline NLR < 3.26 had a significantly prolonged PFS compared to the NLR-increased (P = 0.001, Figure 3A) and baseline NLR ≥ 3.26 groups (P = 0.009, Figure 3B), respectively. These results were corroborated by a subsequent multivariate Cox regression analysis (For NLR increase, hazard ratio [HR], 4.08; 95% confidence interval [CI], 1.99–8.36; P < 0.001; for baseline NLR ≥ 3.26, HR, 2.23; 95% CI, 1.13–4.42; P = 0.021) (Table 3). The other independent risk factors associated with disease progression included an ECOG score >0 (HR, 2.64; 95% CI, 1.32–5.32; P = 0.006) and an increasing AFP (HR, 2.10; 95% CI, 1.04–4.24; P = 0.039). We further conducted a subgroup analysis according to the baseline NLR. In the subgroup with a baseline NLR < 3.26, a decreasing NLR was again associated with better PFS than an increasing NLR (P = 0.003, Figure 3C). However, no significant difference between the two groups was observed in the subgroup with a baseline NLR ≥ 3.26 (P = 0.097, Figure 3D).

Comparison between the combination of dynamic NLR with AFP changes and mono-predictor of AFP change on predictive effect of PD and PFS

Patients with ΔAFP within the range of −20 to 20 ng/mL were defined as the AFP-stable group. In this group, we again observed that a decreasing NLR was significantly associated with better PFS (P = 0.011, Figure 4A). We also performed ROC curve and NRI analysis to assess the predictive effect of the combination of dynamic NLR and AFP changes, which indicated that the combination of the two predictors was better than the mono-predictor of AFP changes in predicting tumor response

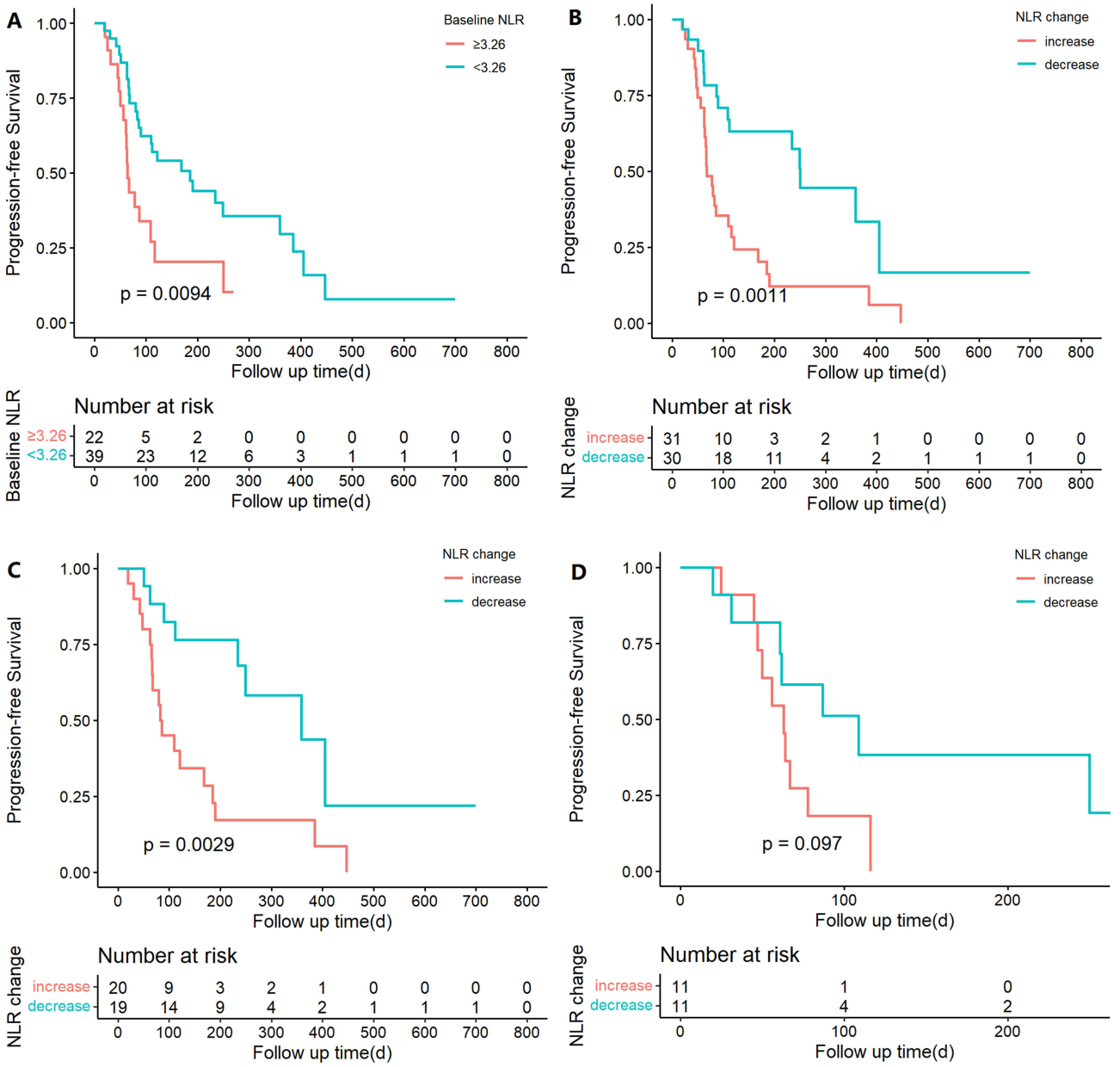

Figure 3 Progression-free survival of patients with different baseline NLR level or dynamic NLR change. (**A**) Comparison of progression-free survival between patients with low and high baseline NLR. (**B**) Comparison of progression-free survival between patients with decreasing and increasing NLR after treatment. (**C**) Comparison of progression-free survival between patients with decreasing and increasing NLR after treatment in the low-baseline NLR (<3.26) subgroup. (**D**) Comparison of progression-free survival between patients with decreasing and increasing NLR after treatment in the high-baseline NLR (≥3.26) subgroup.

(AUC: 0.83 vs 0.68; P = 0.034; NRI, 0.340; P = 0.048; Figure 4B) and PFS (AUC: 0.80 vs 0.70, P = 0.166; NRI: 0.431, P = 0.042).

Discussion

The advent of various regimens of ICIs combined with targeted therapies as first-line treatments has marked a significant shift in the management of HCC. However, owing to the diverse therapeutic responses and scarcity of easily obtainable predictive biomarkers, there is an urgent need to identify a readily available predictive biomarker to stratify patients who would benefit from ICIs plus targeted therapies for uHCC.

NLR is a readily accessible peripheral blood marker that reflects the systemic immune status and has been established as a prognostic factor in various solid tumors, including HCC.[22] The increase of neutrophils, a numerator for NLR, could

Table 3 Univariable and Multivariable Cox Regression Analysis for PFS

Characteristics	Univariate		Multivariate	
	HR(95% CI)	P	HR(95% CI)	P
Age,per 1 year	0.97(0.94–1.00)	0.07		
ECOG score > 0	2.27(1.21–4.25)	0.011	2.64(1.32–5.32)	0.006
BCLC stage C	0.96(0.75–1.25)	0.782		
Child Pugh B/C	1.19(0.60–2.36)	0.613		
HBV related	1.11(0.33–3.69)	0.868		
Baseline NLR ≥ 3.26	2.29(1.20–4.37)	0.012	2.23(1.13–4.42)	0.021
NLR increased after treatments	2.80(1.47–5.33)	0.002	4.08(1.99–8.36)	<0.001
Number of lesion ≥ 3	1.21(0.63–2.34)	0.569		
Maximum tumor size ≥ 5cm	0.80(0.44–1.47)	0.473		
Macrovascular invasion	1.09(0.59–2.04)	0.778		
Macrovascular tumor thrombus	0.94(0.49–1.79)	0.850		
Metastasis	0.98(0.53–1.80)	0.938		
Cirrhosis	0.65(0.32–1.31)	0.226		
Baseline AFP ≥ 400ng/mL	0.99(0.53–1.84)	0.972		
AFP increased after treatments	2.27(1.14–4.53)	0.020	2.10(1.04–4.24)	0.039
AST ≥ 40U/L	1.31(0.70–2.44)	0.393		
ALT ≥ 40U/L	0.88(0.46–1.66)	0.683		

Abbreviations: PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ECOG score, Eastern Cooperative Oncology Group score; BCLC stage, Barcelona Clinic Liver Cancer stage; HBV, hepatitis B virus; NLR, Neutrophil-to-Lymphocyte Ratio; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

induce tumor immune escape and progression via cytokine secretion (eg, vascular endothelial growth factor [VEGF] and platelet-derived growth factor [PDGF]), neutrophil extracellular traps formation (NETs), and HCC stem-like cell stimulation.[23–25] In contrast, the increase of lymphocytes, a denominator for NLR, might enhance anti-tumor immunosurveillance and immunoediting to reduce tumor progression.[26] Notably, neutrophil-derived NETs might induce T cell exhaustion during immunotherapies,[27] which promotes a positive feedback of higher NLR, and then mediates immunosuppression and tumor progression. Therefore, in the era of immuno-targeted therapies, NLR emerges as a composite indicator is highly valuable for predicting the therapeutic response to ICI plus targeted therapies.

In this retrospective study, we focused on the predictive effect of dynamic NLR changes after ICIs plus targeted therapies on uHCC tumor responses. We identified a strong link between dynamic NLR changes and tumor outcomes. The NLR at 3–6 weeks after treatments significantly increased in patients who underwent PD, while those with a decreasing NLR showed higher ORR and DCR. Further survival analysis suggested that decreasing NLR was significantly associated with improved PFS. Moreover, ROC curve and NRI analysis showed that the combination of dynamic NLR with AFP changes was better than the mono-predictor of AFP changes in predicting tumor response and PFS.

Several studies have demonstrated the predictive value of NLR in ICI-based treatments for HCC.[21,28,29] Yuji et al[28] reported that a lower pre-treatment NLR was associated with better tumor response and PFS in patients who were treated with Atezolizumab plus Bevacizumab. However, studies focusing on mono-ICIs therapy of nivolumab have reported that dynamic NLR changes or post-treatment NLR, but not pre-treatment NLR, were correlated with tumor response.[21,29]

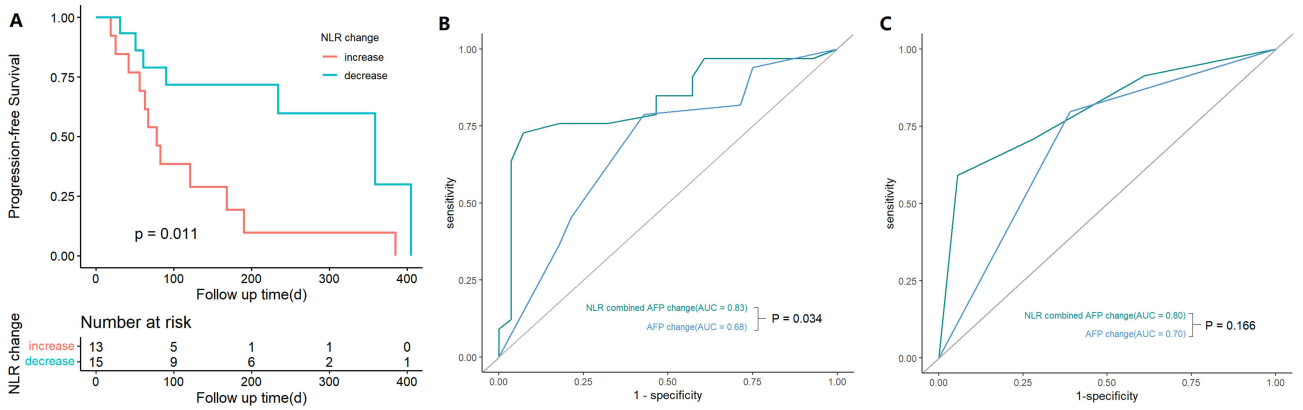

Figure 4 Predictive effect analyses of dynamic NLR change in combination with AFP change. (**A**) Comparison of progression-free survival between patients with decreasing and increasing NLR after treatment in AFP-stable subgroup. (**B**) ROC curve of AFP change with or without NLR dynamic change on predicting PD. (**C**) Time-dependent ROC curve of AFP change with or without dynamic NLR changes in predicting PFS at 180 d.

Given that previous studies have suggested that pre-treatment NLR has a great predictive effect on the efficacy of Sorafenib or Lenvatinib,[30,31] the predictive effect of pre-treatment NLR should be interpreted with caution in the case of ICIs plus targeted therapies. Although pre-treatment NLR reflects the long-term and relative stable systemic inflammatory status, combining with ICIs might alter this inflammatory status, thereby enhancing the predictive effect of dynamic NLR changes on tumor response and prognosis.

Our results also support this hypothesis. In our study, the baseline NLR independently correlated with PFS (HR, 2.23; 95% CI, 1.13–4.42; P = 0.021), but not with the tumor response (OR, 1.20; 95% CI, 0.80–1.70, P = 0.420). This discrepancy might be ascribed to immune status alterations induced by ICI treatments, which might weaken the predictive effect of baseline NLR. A initially low NLR might elevate after treatment, indicating a poor prognosis (Figure 3C). These results indicate that the efficacy of immuno-targeted therapies in systemic or tumor immune environments is a dynamic process. Therefore, it is more precise to apply dynamic NLR changes, rather than a static indicator of pre-treatment NLR, to predict tumor response.

Furthermore, to the best of our knowledge, this study is the first to evaluate the predictive effect of the dynamic NLR in combination with AFP changes on tumor response. Although AFP is widely recognized as a biomarker of HCC, we found that a proportion of patients (36.1%) receiving ICI plus targeted therapies had a significant radiographic response but little AFP fluctuation. In patients with stable AFP levels, decreasing NLR was still associated with better PFS than increasing NLR (Figure 4A), and the ROC and NRI analyses revealed that the introduction of dynamic NLR changes greatly improved the predictive effect on tumor response and patient prognosis, indicating that dynamic NLR changes might be a potent supplement to the traditional AFP in predicting tumor outcomes.

The major limitation of our study is that, as a retrospective study based on single-center, observational data with a small sample size, it may be subject to selection bias and confounding. Additionally, the heterogeneity of immuno-targeted therapies (Supplemental Material 2) may further contribute to potential confounding. Although most clinical trials have demonstrated the efficacy of these regimens in intermediate-advanced HCC, differences between regimens could introduce heterogeneity in treatment responses, which would result in bias in our analyses. Finally, owing to the short observational period, we did not consider overall survival (OS) as one of the study endpoints, and the short follow-up period might result in an underestimation of PD events, which would lead to bias.

Conclusion

Our study showed that the dynamic changes of NLR, not baseline NLR, could predict the tumor response as accurately as AFP. This might facilitate better selection of treatment strategies for uHCC and timely adjustment of ICIs plus targeted therapies based on NLR dynamic changes. Multicenter clinical trials with larger scale is mandatory to validate this result.

Data Sharing Statement

The data generated in this study are available upon request from the corresponding author: Yang Yang, Department of Hepatic Surgery and Liver Transplantation Center, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board of The Third Affiliated Hospital of Sun-Yet Sen University, Guangzhou, China. The need for informed consent was waived, and all methods were performed in accordance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The original study described in this review was supported by the National Natural Science Foundation of China (81702393).

Disclosure

The authors declare that they have no conflicts of interest.

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