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ORIGINAL RESEARCH

The Value of Dynamic of Alkaline Phosphatase and Neutrophil-to-Lymphocyte Ratio in Predicting the Efficacy of Neoadjuvant Immunochemotherapy in Patients with Non-Small Cell Lung Cancer

Li Gong[1,2,*], Qihang Yan[1,2,*], Dachuan Liang[1,2,*], Liang Li[1,2], Jie Yang[1,2], Wingshing Wong[1,2], Shuyue Zhang[1,2], Shuqin Dai[3], Wenyu Zhai[1,2], Junye Wang[1,2]

[1]Department of Thoracic Surgery, State Key Laboratory of Oncology in Southern China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong, People's Republic of China; [2]Lung Cancer Research Center, Sun Yat-Sen University, Guangzhou, Guangdong, People's Republic of China; [3]Department of Laboratory Medicine, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong, People's Republic of China

*These authors contributed equally to this work

Correspondence: Junye Wang; Wenyu Zhai, Department of Thoracic Surgery, State Key Laboratory of Oncology in Southern China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-Sen University Cancer Center, 651 Dongfeng Road East, Guangzhou, Guangdong, 510060, People's Republic of China, Email wangjy@sysucc.org.cn; diwy@sysucc.org.cn

Background: Currently, reliable and convenient markers for identifying patients with non-small cell lung cancer (NSCLC) who benefit from neoadjuvant immunochemotherapy remains elusive. Established static biomarkers, such as programmed cell death ligand 1 (PD-L1) expression and tumor mutational burden (TMB), exhibit limitations in capturing dynamic changes in the immune microenvironment and predicting treatment efficacy compared to dynamic biomarkers. This study aims to evaluate the value of dynamic peripheral blood markers in predicting pathological complete response (pCR) and survival outcomes in patients with NSCLC undergoing neoadjuvant immunochemotherapy.

Methods: In this retrospective analysis, clinicopathological data, along with baseline and post-treatment laboratory results, were examined from 113 patients with NSCLC who received neoadjuvant immunochemotherapy between October 2019 and April 2023. The least absolute shrinkage and selection operator (LASSO) algorithm was employed to identify candidate dynamic peripheral blood markers, which were then further refined using logistic regression. An integrated nomogram model incorporating the optimal biomarkers was developed to predict individual pCR. Event-free survival (EFS) was analyzed using the Kaplan-Meier method, with comparisons performed via the Log rank test.

Results: Dynamic alkaline phosphatase (dALP) and dynamic neutrophil-to-lymphocyte ratio (dNLR) emerged as independent predictors of pCR following neoadjuvant immunochemotherapy, as confirmed by LASSO and multivariate logistic regression. A predictive model, incorporating dNLR, dALP, degree of differentiation, and smoking history, demonstrated strong predictive capability (AUC = 0.745). Internal validation through bootstrapped resampling yielded a mean AUC of 0.728 (95% CI 0.686–0.749). Survival analysis revealed that patients who achieved pCR had significantly better EFS, and those with low dNLR and dALP values exhibited superior EFS compared to the high-value group.

Conclusion: The findings indicate that dNLR and dALP can serve as independent predictors of pCR and EFS in patients with NSCLC treated with neoadjuvant immunochemotherapy. The pCR prediction model incorporating these two markers showed excellent predictive performance, providing valuable clinical guidance for selecting patients who are most likely to benefit from neoadjuvant immunochemotherapy.

Keywords: NSCLC, neoadjuvant immunochemotherapy, peripheral blood dynamic indicators, pCR, survival benefit

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Introduction

Immune checkpoint inhibitors (ICIs) have markedly improved the prognosis for patients with NSCLC.[1,2] For resectable NSCLC, neoadjuvant ICIs combined with chemotherapy have demonstrated superior pathologic response rates and survival outcomes compared to neoadjuvant chemotherapy alone, as reflected in recent National Comprehensive Cancer Network guidelines. Phase III clinical trials have reported pCR rates ranging from 17.2% to 40.7% and major pathological response (MPR) rates from 30.2% to 56.2%. In terms of survival, studies such as Checkmate 816, AEGEAN, and KEYNOTE-671 have shown median event-free survival (EFS) of 43.8 months [30.6-NR] vs 18.4 months [14.0–26.7], HR = 0.66; NR [42.3-NR] vs 30.0 months [20.6-NR], HR = 0.69; and 47.2 months [32.9-NR] vs 18.3 months [14.8–22.1], HR = 0.59, respectively. Subgroup analyses further revealed that patients with high PD-L1 expression derived greater EFS benefit than PD-L1-negative patients, and those achieving pCR/MPR exhibited improved EFS.[3–5] These findings suggest that a subset of patients may experience a greater benefit from neoadjuvant immunotherapy. Consequently, the preoperative use of biomarkers to predict and identify populations likely to benefit from precision treatment is of significant clinical importance.

PD-L1 expression and TMB are commonly utilized to predict the efficacy of immunotherapy; however, both depend on tissue samples and are influenced by the technology and quality of clinical specimens. Their accuracy in predicting the outcomes of neoadjuvant immunotherapy, especially in patients with low PD-L1 expression, remains suboptimal.[6–9] In addition to these markers, circulating tumor cells (CTC)[10] and circulating tumor DNA (ctDNA)[11] have also been extensively studied as potential biomarkers for immunotherapy efficacy. The NADIM study demonstrated a significant association between ctDNA and overall survival (OS) and progression-free survival (PFS), with ctDNA showing superiority over radiologic assessments.[12] The Checkmate 816 study revealed that patients receiving nivolumab plus chemotherapy exhibited greater preoperative ctDNA clearance compared to those receiving chemotherapy alone, and this higher ctDNA clearance correlated with better prognosis.[13] However, routine ctDNA monitoring incurs a high economic burden, and its sensitivity and specificity are limited by low tumor burden and interference from clonal hematopoiesis. Thus, there is a pressing need to identify new, accurate, convenient, and cost-effective biomarkers to predict which patients will benefit from neoadjuvant immunochemotherapy.

Recently, peripheral blood cellular and serum biomarkers have garnered attention due to their convenience and affordability. Inflammation plays a pivotal role in tumorigenesis and development, contributing to the formation of an immunosuppressive tumor microenvironment that impacts the efficacy of ICIs in malignant tumors.[14] Consequently, several peripheral blood inflammatory markers, including neutrophil-to-lymphocyte ratio (NLR), pan-immunoinflammatory value (PIV), serum amyloid A (SAA), lactate dehydrogenase (LDH), and C-reactive protein (CRP), have been investigated as predictors of immunotherapy efficacy in NSCLC.[15–19] Beyond inflammatory markers, other peripheral blood markers such as hemoglobin (Hb),[20] plasma extracellular vesicles (EVs),[21] and alkaline phosphatase (ALP),[22] have also been explored. Most previous studies have primarily focused on baseline peripheral blood marker values. However, the tumor immune microenvironment undergoes dynamic changes during immunotherapy, inflammation and peripheral blood markers also fluctuate dynamically during treatment, highlighting the advantage of dynamic biomarkers over static ones in capturing these temporal variations. Recently, emerging evidence suggests that these dynamic changes could predict the efficacy of NSCLC immunotherapy.[23–26] Michael Hwang et al demonstrated that dynamic NLR effectively predicts pathological response and long-term survival benefits in patients with NSCLC treated with immunotherapy, and complement the predictive value of ctDNA, particularly in early-stage patients with undetectable ctDNA.[24] However, these studies have not yet developed a clinical prediction model based on dynamic biomarkers. Therefore, this study investigated dynamic changes in peripheral blood indicators pre- and post - treatment in patients with NSCLC to identify potential predictive biomarkers for pCR following neoadjuvant immunochemotherapy and constructed a clinical prediction model.

Materials and Methods

Patient

This study adhered to the ethical principles outlined in the Declaration of Helsinki and received formal approval from the Institutional Review Board of Sun Yat-sen University Cancer Center (SYSUCC) (No. B2022-445-01). As a retrospective

study, the requirement for written informed consent was waived. All patient data were anonymized and managed in strict compliance with institutional confidentiality protocols to ensure participant privacy. No interventions or modifications to standard clinical practices were implemented during data collection or analysis.

This study retrospectively screened 151 patients with NSCLC who received neoadjuvant immunochemotherapy and underwent surgery at SYSUCC between October 2019 and April 2023 (Figure 1). The inclusion criteria were as follows: (1) pathologically confirmed NSCLC; (2) clinical stage IIA-IIIB (cT_{1-4}-N_{0-3}) according to the American Joint Committee on Cancer, Eighth Edition (AJCC, 2010) staging system; (3) completion of at least two cycles of neoadjuvant immunochemotherapy; (4) pathologic assessment of excised specimens following neoadjuvant immunochemotherapy. The exclusion criteria included: (1) patients with epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) gene rearrangements; (2) absence of laboratory data on peripheral blood within one week prior to neoadjuvant immunochemotherapy; (3) history of other malignancies; (4) presence of acute or chronic infections, ongoing hormone therapy, or diseases of the liver, gallbladder, or skeletal system during neoadjuvant immunochemotherapy; (5) incomplete follow-up or missing clinicopathologic data.

Definition of Peripheral Blood Indicators and Data Collection

Hematological indicators were systematically extracted from electronic medical records (EMR) at two critical time points: baseline assessment (≤ 1 week prior to initial neoadjuvant therapy) and preoperative assessment (≤ 1 week prior to surgical resection). The comprehensive set of peripheral blood indicators included: cellular components—neutrophil (N), monocyte (M), platelet (P), and lymphocyte (L) counts ($\times 10^9$/L); serum biomarkers—albumin (A, g/L, reference range: 35–52 g/L), C-reactive protein (CRP, mg/L, reference range: < 5.0 mg/L), lactate dehydrogenase (LDH, U/L, reference range: 135–225 U/L), serum amyloid A (SAA, mg/L, reference range: < 6.4 mg/L), and alkaline phosphatase (ALP, U/L, reference range: 45–125 U/L). All the above biochemical indicators were measured using the cobas® c 702 module from Roche Diagnostics. Derived inflammatory indices were calculated using the following formulas:

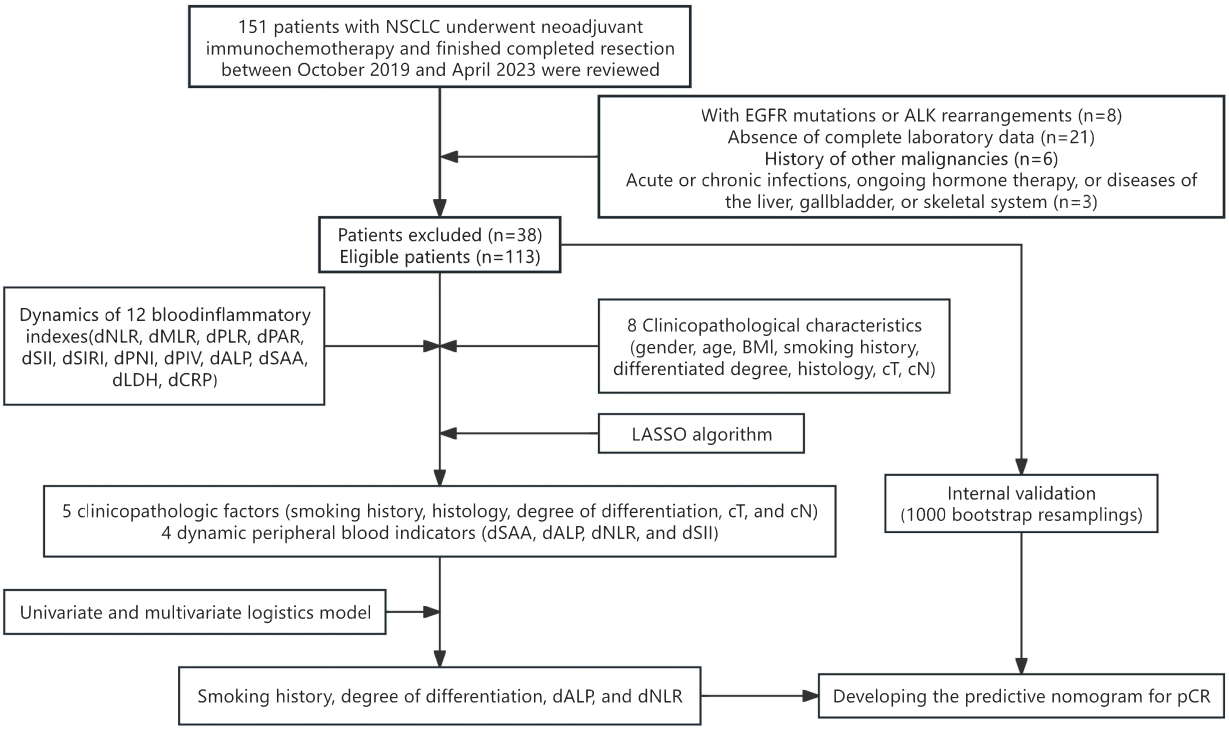

Figure 1 Flowchart of the study design.

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Neutrophil-to-lymphocyte ratio (NLR) = N/L; Systemic immune-inflammation index (SII) = P × NLR; Monocyte-to-lymphocyte ratio (MLR) = M/L; Systemic inflammation response index (SIRI) = N × MLR; Platelet-to-lymphocyte ratio (PLR) = P/L; Platelet-albumin ratio (PAR) = P/A; Prognostic nutritional index (PNI) = A + 5 × L; Pan-immune-inflammatory value (PIV) = (N × M × P)/L. Dynamic peripheral blood indicators, such as dNLR, dMLR, and dPLR, were computed as post-treatment values minus baseline values.

Clinicopathological variables were retrospectively collected through structured EMR reviews, including gender, age, smoking history, body mass index (BMI) (measured ≤ 1 week pre-treatment), cT stage, cN stage, degree of differentiation, histologic type, and post-treatment pathologic response.

Pathologic Evaluation After Neoadjuvant Immunotherapy

Neoadjuvant immunochemotherapy was administered at three-week intervals, with surgery performed four to six weeks after the final cycle of neoadjuvant therapy. Pathological evaluation of the samples was conducted by two specialized pathologists following the multidisciplinary recommendations of the International Association for the Study of Lung Cancer. pCR was defined as the absence of viable tumor cells in both the primary lesion and sampled lymph nodes upon histopathological examination after neoadjuvant therapy and surgery.[27]

Patient Follow-up and Study Endpoints

Patients were regularly followed up every three months via telephonic interviews or outpatient follow-up. The follow-up protocol included: (1) systematic physical examination; (2) hematological analysis; and (3) radiographic surveillance using thoracic and abdominal CT scans. If necessary, whole-body evaluation (18F-FDG PET/CT) or brain imaging (MRI with contrast enhancement) was performed. The primary endpoint of the study was pCR, while the secondary endpoint was EFS, defined as the time from the initiation of neoadjuvant immunotherapy to either radiologically confirmed disease recurrence or all-cause mortality.

Statistical Analysis

Continuous data are presented as median values with interquartile ranges (IQR), based on distribution characteristics. The normality of distributions was rigorously assessed using Shapiro–Wilk testing. Parametric analysis (Student's *t*-test) was applied to normally distributed variables, while non-parametric analysis (Mann–Whitney *U*-test) was employed for skewed distributions. Categorical variables were expressed as frequency counts with corresponding percentages, and between-group comparisons were made using the χ^2-test for expected counts ≥ 5, or Fisher's exact test for expected counts < 5.

To address multicollinearity and enhance model robustness, a two-stage feature selection strategy was used. Initially, the least absolute shrinkage and selection operator (LASSO) algorithm (via the "glmnet" R package) was applied to identify high-dimensional candidate variables. Variables selected by LASSO regression will subsequently undergo univariate logistic regression analysis. Variables for further multivariate logistic regression analysis will be selected based on both the results of the univariate analysis and their clinical relevance.

Based on multivariate logistic regression results, a pCR prediction model was developed using the "rms" package in R and presented as a nomogram. A web-based dynamic nomogram was developed using the "shiny" package in R. The predictive performance of the nomogram was evaluated using receiver operating characteristic (ROC) analysis, with the area under the curve (AUC) as the primary metric. Bootstrap resampling (1000 iterations) was performed for further validation of its predictive ability. Calibration performance was assessed by comparing predicted and actual pCR probabilities, where optimal alignment with the 45° reference line indicated good calibration. Decision curve analysis (DCA) was conducted to assess the net benefit of the nomogram for patients. In survival analysis, the optimal cutoff value for grouping was determined using Maximally Selected Rank Statistics. Kaplan-Meier survival curves for EFS were generated, and statistical comparisons were made using the Log rank test. Landmark analysis was performed using the "jskm" package in R. All statistical analyses were performed using R software (version 4.0.1, Vanderbilt University, Nashville, TN, USA), and a two-tailed P-value < 0.05 was considered statistically significant.

Results

Patient Clinical Characteristics

A total of 113 patients were included in this study, with their clinical characteristics summarized in Table 1. The cohort consisted of 104 male patients (92.0%) and 9 female patients (8.0%), with a median age of 61.0 years (IQR 57.0–66.0). A majority of the patients (87, 77.0%) had a history of smoking, and the median BMI was 22.6 (IQR 21.1–25.0). The distribution of patients by clinical T stage was as follows: cT1 (5, 4.4%), cT2 (54, 47.8%), cT3 (30, 26.5%), and cT4 (24, 21.2%). For clinical N stage, patients were categorized as: N0 (10, 8.8%), N1 (24, 21.2%), N2 (58, 51.3%), and N3 (21, 18.6%). Regarding histologic grade, 30 patients (26.5%) were diagnosed with moderately differentiated tumors, while 83 patients (73.5%) had poorly differentiated or undifferentiated tumors. Pathologically, 75 patients (66.4%) had squamous cell carcinoma, 24 patients (21.2%) had adenocarcinoma, and 14 patients (12.4%) had other histological types. Postoperative pCR was observed in 50 patients (44.2%).

Table 1 Patients' Characteristics

Characteristics	No. (%)
Gender	
Male	104(92.0)
Female	9 (8.0)
Age (year), median (IQR)	61.0 (57.0~66.0)
Smoking history	
No	26 (23.0)
Yes, or ever	87 (77.0)
BMI, median (IQR)	22.6 (21.1~25.0)
cT stage[a]	
T1	5 (4.4)
T2	54 (47.8)
T3	30 (26.5)
T4	24 (21.2)
cN stage[a]	
N0	10 (8.8)
N1	24 (21.2)
N2	58 (51.3)
N3	21 (18.6)
Differentiation degree	
Moderate	30 (26.5)
Poor or Undifferentiation	83 (73.5)
Histological type	
LUSC	75 (66.4)
LUAD	24 (21.2)
Others[b]	14 (12.4)
pCR	
Yes	50 (44.2)
No	63 (55.8)
dNLR, median (IQR)	−6.0 (−10.4~-4.2)
dMLR, median (IQR)	0.37 (−0.03~0.22
dPLR, median (IQR)	0.7 (−48.0~82.9)
dPAR, median (IQR)	−0.5 (−2.2~2.0)
dSII, median (IQR)	−149.7 (−500.0~-8.4)
dSIRI, median (IQR)	0.2 (−0.9~1.0)
dPNI, median (IQR)	−3.0 (−11.5~1.7)

(*Continued*)

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Table 1 (Continued).

Characteristics	No. (%)
dPIV, median (IQR)	−43.9 (−321.3~92.4)
dALP, median (IQR)	−4.0 (−19.9~11.5)
dSAA, median (IQR)	−4.5 (−84.1~0.4)
dLDH, median (IQR)	0.8 (−39.4~27.4)
dCRP, median (IQR)	−3.3 (−16.6~0.1)

Notes: [a]Diagnosed based on the AJCC criteria (8th edition). [b]Including lung lymphoepithelioma-like carcinoma, adenosquamous carcinoma, large cell neuroendocrine carcinoma.
Abbreviations: LUSC, lung squamous carcinoma; LUAD, lung adenocarcinoma; PIV, pan-immune-inflammatory value; NLR, neutrophil-lymphocyte ratio; MLR, monocyte-lymphocyte ratio; PLR, platelet-lymphocyte ratio; PAR, platelet- albumin ratio; SII, systemic immune inflammation index; SIRI, system inflammation response index; PNI, prognostic nutritional index; IQR, interquartile ranges.

Screening for Clinical Features and Peripheral Blood Markers

The LASSO regression algorithm was applied to identify the clinical features and peripheral blood markers most predictive of pCR, excluding indicators exhibiting multicollinearity. Among the 20 candidates, 9 indicators with non-zero coefficients were selected at the optimal -log(e)λ value of 3.57 (Figure S1A and B). These included five clinicopathologic factors: smoking history, histology, degree of differentiation, cT, and cN, as well as four dynamic peripheral blood indicators: dSAA, dALP, dNLR, and dSII. These indicators were then subjected to univariate and multivariate logistic regression analyses (Table 2). In the univariate analysis, significant predictors included smoking history ($P = 0.047$), histology_others ($P = 0.046$), degree of differentiation ($P = 0.027$), dALP ($P = 0.023$), and dNLR ($P = 0.025$). Owing to the small sample size in the others subgroup and the non-significant difference in the LUAD subgroup, we selected smoking history, degree of differentiation, dALP and dNLR for the further multivariate analysis. Multivariate analysis revealed that smoking history ($P = 0.015$, HR 3.692; 95% CI 1.285–10.611), degree of differentiation ($P = 0.036$, HR 2.959; 95% CI 1.071–8.174), dALP ($P = 0.006$, HR 0.976; 95% CI 0.959–0.993), and dNLR ($P = 0.029$, HR 0.975; 95% CI 0.953–0.997) were independent predictors of pCR following neoadjuvant immunochemotherapy.

Table 2 Univariate and Multivariate Logistics Analysis

Factors	LASSO Coefficient	Univariate Analysis	Multivariate Analysis	
		P value	HR (95% CI)	*P* value
Smoking history	0.505	**0.047**	3.692 (1.285–10.611)	**0.015**
cT stage[a]	−0.098	0.613		
cN stage[a]	−0.053	0.281		
Histology	−0.425			
LUSC				
LUAD		0.115		
Others[b]		**0.046**		
Differentiation degree	0.862			
Moderate			Ref	
Poor or undifferentiated		**0.027**	2.959 (1.071–8.174)	**0.036**
dSAA	−0.0002	0.398		
dALP	−0.015	**0.023**	0.976 (0.959–0.993)	**0.006**
dNLR	−0.013	**0.025**	0.975 (0.953–0.997)	**0.029**
dSII	0.0002	0.125		

Notes: [a]Diagnosed based on the AJCC criteria (8th edition). [b]Including lung lymphoepithelioma-like carcinoma, adenosquamous carcinoma, large cell neuroendocrine carcinoma. Bold values mean statistical significance.
Abbreviations: LUSC, lung squamous carcinoma; LUAD, lung adenocarcinoma.

Development of a Nomogram for pCR Prediction

Based on multivariate logistic regression analysis results, four independent predictors—smoking history, degree of differentiation, dALP, and dNLR—were integrated to construct a nomogram for predicting pCR following neoadjuvant immunochemotherapy in patients with NSCLC (Figure 2A). The ROC curve demonstrated strong predictive performance for pCR, with an AUC of 0.745 (Figure 2B). Internal validation was performed using 1000 bootstrap resamplings. As presented in Figure 2C, the median AUC value was 0.730 (IQR 0.687–0.749), with AUC values predominantly ranging from 0.72 to 0.75. The calibration curve further confirmed the good agreement between predicted and actual pCR probabilities, indicating that the nomogram provides accurate prediction performance (Figure 2D). Clinical utility evaluation through DCA revealed significant net benefits when the nomogram was used to guide therapeutic decision-making for patients with NSCLC post-neoadjuvant immunotherapy (Figure 2E). To facilitate intuitive and convenient clinical application, we developed a web - based dynamic nomogram (Figure S2), which is publicly accessible at https://li-gong.shinyapps.io/DynNomogram/.

Prognostic Value of Peripheral Blood Markers and pCR in EFS

The median follow-up time for the 113 patients was 24.6 months. Prognostic analysis of pCR showed that the EFS of patients who achieved pCR was significantly better than that of those who did not (P = 0.008, HR 0.310; 95% CI 0.130–0.737) (Figure 3A). Using the Maximally Selected Rank Statistics method, the optimal cutoff for dALP was

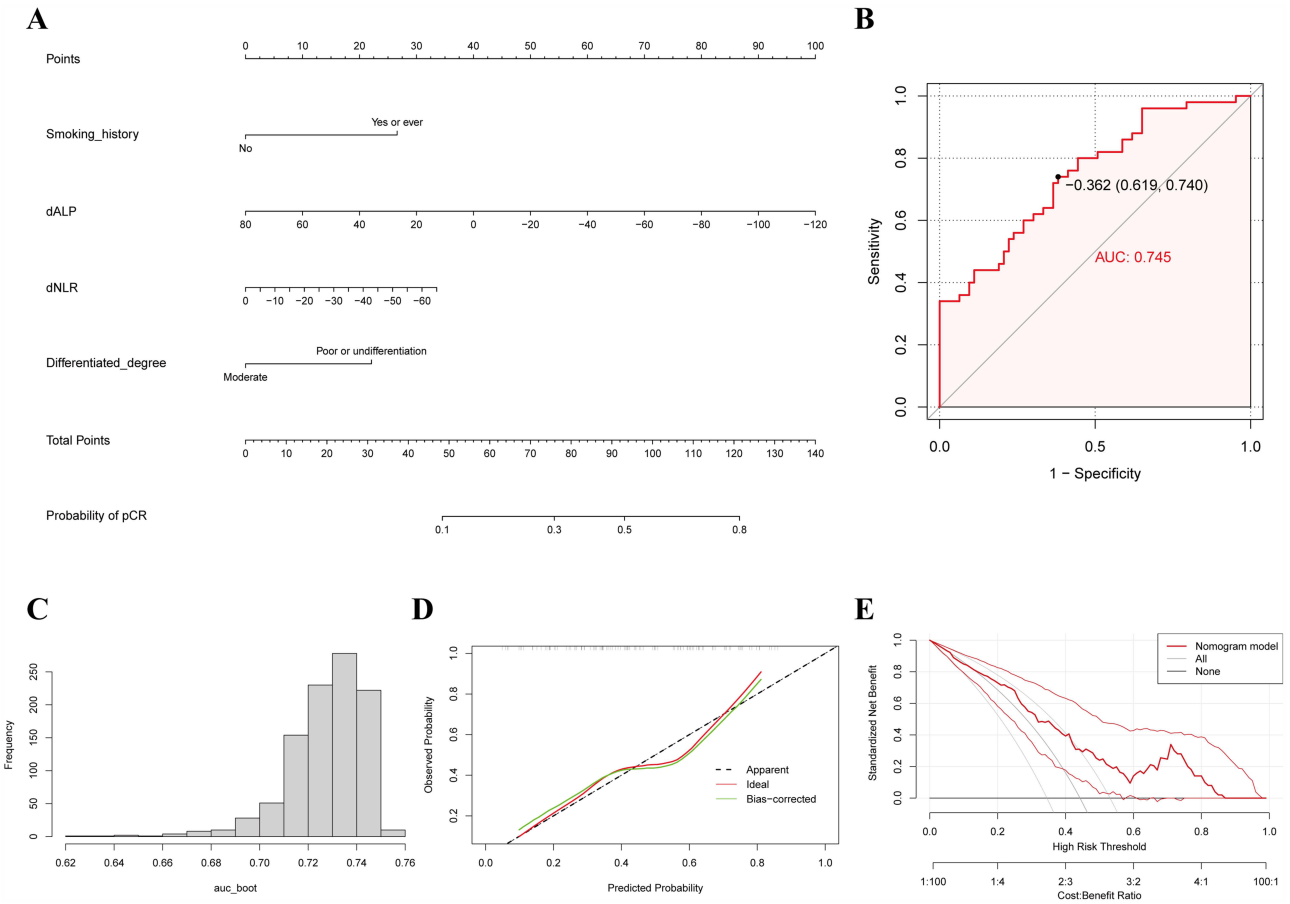

Figure 2 Development and validation of the nomogram. (**A**) The nomogram for predicting pCR in NSCLC patients after neoadjuvant immunochemotherapy, according to which each variable could be assigned a point on the point scale, and by summing up the points and locating it on the total point scale, we could draw a straight line downward to determine the corresponding pCR probability; (**B**) The receiver operating characteristic curve of the nomogram; (**C**) The Histogram of AUC distribution for 1000 times bootstrapped resampling; (**D**) Calibration plot of the nomogram. Predicted and actual pCR probability were plotted on the X and Y axis, respectively. The 45° dashed line through the origin of the coordinates represents the excellent calibration model; (**E**) Decision curve analysis of the clinical values of the nomogram showed that when the pCR probabilities are predicted by using the nomogram, the patients could obtain better clinical benefits within a wide range of probability threshold. The gray curve represents the hypothesis that all NSCLC patients receiving neoadjuvant immunochemotherapy. The black line represents the hypothesis that all NSCLC patients do not receive neoadjuvant immunochemotherapy.

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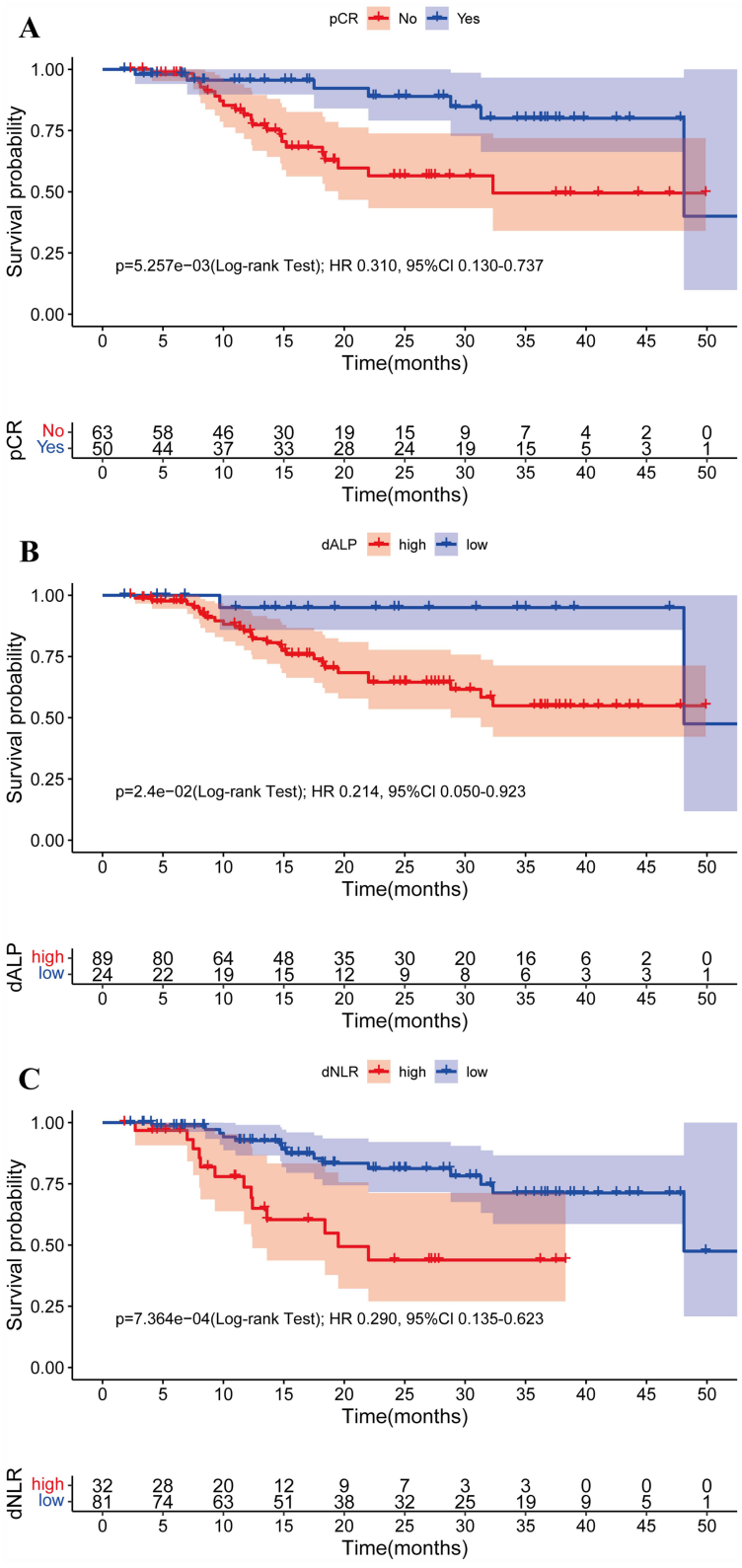

Figure 3 Event-free survival of all patients. (**A**) Survival curves of patients with pCR and non-pCR (**B**) Survival curves of patients in the dALP low and high groups; (**C**) Survival curves of patients in the dNLR low and high groups.

determined to be −21.2 (Figure S3A), and the optimal cutoff for dNLR was −4.5 (Figure S3B). Based on these cutoff values, the patients were categorized into two groups: 89 patients in the dALP high group and 24 patients in the dALP low group. Survival analysis revealed that patients in the dALP low group had significantly better EFS than those in the dALP high group ($P = 0.039$, HR 0.214; 95% CI 0.050–0.923) (Figure 3B). Similarly, 32 patients were in the dNLR high group, and 81 patients were in the dNLR low group. Survival analysis indicated that the dNLR low group had significantly better EFS compared to the dNLR high group ($P = 0.001$, HR 0.290; 95% CI 0.135–0.623) (Figure 3C).

Landmark Analysis for EFS

Landmark analysis were performed at 6 and 12 months, with 100 and 75 patients included in the subsequent analyses, respectively. Landmark analysis showed significant differences in EFS between the pCR and non-pCR groups both at 6 months ($P = 0.006$, HR 0.271; 95% CI 0.108–0.684) and at 12 months ($P = 0.049$, HR 0.342; 95% CI 0.117–0.997) (Figure S4A and B). Similarly, significant differences in EFS were observed between the dALP low group and high group at both 6 and 12 months. However, in the univariate Cox proportional hazards model, the effect of dALP was not statistically significant at 12 months (6 months: $P = 0.048$, HR 0.228; 95% CI 0.053–0.988; 12 months: $P = 0.081$, HR 0.160; 95% CI 0.020–1.256) (Figure S1A and D). For the dNLR groups, significant differences in EFS were also observed at 6 months ($P = 0.002$, HR 0.283; 95% CI 0.128–0.625) and at 12 months ($P = 0.038$, HR 0.333; 95% CI 0.117–0.943) (Figure S4E and F).

Discussion

This study incorporated 12 dynamic peripheral blood indicators, which were statistically analyzed to identify and confirm that dALP and dNLR were independent, negative predictors of pCR following neoadjuvant immunochemotherapy in patients with NSCLC. These indicators, along with two clinical features, were integrated into a nomogram model for predicting post-treatment pCR, which demonstrated good predictive performance upon validation. Survival analysis further revealed that patients achieving pCR with low dALP and dNLR levels exhibited improved EFS.

ALP plays a pivotal role in various physiological processes, including bone formation and liver function.[28] Elevated ALP levels are typically associated with primary tumors in the liver and bone or with liver and bone metastases. However, increased ALP levels have also been observed in patients with pancreatic cancer[29] and lung cancer[30,31] without bone metastases. Koch N et al found that in patients with stage IIIA-IV lung cancer, ALP levels significantly decreased from pre-treatment baseline after chemotherapy, radiotherapy, or chemoradiotherapy.[32] While there is limited research on the relationship between serum ALP levels and the efficacy of ICIs in tumor individuals, Hu J et al reported that high serum ALP levels were linked to poorer PFS in patients with HER-2 negative gastric cancer receiving immunotherapy.[33] Yang T et al identified that pre-treatment high ALP levels, independent of bone or liver metastases, were a prognostic factor in patients with NSCLC treated with ICIs, correlating with shorter median PFS.[34] This study suggests that elevated ALP, even in the absence of bone or liver metastases, may impact immunotherapy efficacy, though the underlying mechanism warrants further investigation. To our knowledge, no direct studies have investigated the underlying mechanism by which ALP predicts the efficacy of immunotherapy, although its role in inflammation and immunity has been explored. As an unconventional immune protein, ALP modulates inflammatory responses by hydrolyzing extracellular ATP/ADP into AMP and adenosine.[35] Adenosine is known to suppress antitumor T cell responses.[36,37] We therefore hypothesize that ALP may indirectly influence antitumor T cell function by regulating adenosine levels, thereby serving as a potential predictor of immunotherapy efficacy. In the present study, dALP was defined as the difference between post-treatment ALP and pre-treatment ALP levels. Our findings indicated that dALP served as an independent predictor of pCR following neoadjuvant immunochemotherapy. Additionally, survival analysis revealed that patients with high dALP levels had significantly poorer EFS compared to those with low dALP levels. In further landmark analysis, high dALP was associated with poor EFS. However, univariate Cox analysis indicated that the effect of dALP was not statistically significant at 12 months, which may be attributable to the limited sample size.

Besides ALP, other hematological markers have been extensively studied. CRP, a widely used inflammatory marker, was evaluated by Klümper et al for predicting response to immunotherapy in NSCLC.[18] They reported that a CRP flare could predict treatment efficacy and survival in patients receiving anti-PD-1 monotherapy.However, early CRP kinetics

showed limited predictive power in patients treated with immunochemotherapy and corticosteroids. In current clinical practice, most NSCLC patients undergoing neoadjuvant therapy receive immunochemotherapy combined with steroids. Thus, the predictive value of CRP in this context remains uncertain.

NLR is a well-established indicator of peripheral blood inflammation and was also included in our research. Tumor-associated neutrophils (TANs) are key mediators in the promotion of malignant transformation, growth, angiogenesis, and modulation of anti-tumor immunity.[38] Tumor-infiltrating lymphocytes (TILs) are associated with better clinical outcomes in patients with malignant tumors.[39,40] NLR reflects both TANs and TILs to some extent, and previous studies have demonstrated that elevated NLR levels correlate with poorer survival outcomes in patients with NSCLC.[41,42] Nakaya et al showed that NLR levels measured at 2 and 4 weeks after the initiation of nivolumab treatment were independent predictors of PFS.[43] Sun et al further observed that high NLR levels were associated with poorer pathological response and shorter DFS. They found that baseline NLR levels before neoadjuvant immunochemotherapy and preoperative NLR levels after neoadjuvant immunochemotherapy independently predicted pathological response and DFS in the neoadjuvant immunochemotherapy group, but not in the neoadjuvant chemotherapy alone group.[44] In the multivariate analysis by Sun et al, baseline NLR independently predicted pCR after neoadjuvant immunochemotherapy in NSCLC patients, while preoperative NLR was not an independent predictor. The authors suggested that this may be due to an inherent correlation between baseline and preoperative NLR. Therefore, we introduced dynamic NLR by calculating the difference between preoperative and baseline NLR. In the present study, dNLR was identified as an independent predictor of pCR following neoadjuvant immunochemotherapy, and patients with high dNLR levels exhibited poorer EFS, demonstrating the value of dynamic biomarkers in predicting short-term treatment response and long-term prognosis.

In conclusion, our study identified two dynamic peripheral blood markers, dALP and dNLR, that predict pCR following neoadjuvant immunochemotherapy in NSCLC patients. Univariate and multivariate logistic regression analyses indicated that dALP and dNLR exhibited comparable predictive value for pCR. Survival and landmark analyses further supported their predictive value for prognosis. Furthermore, we developed a clinical prediction model based on dALP and dNLR and created a web-based dynamic nomogram. The use of readily measurable indicators and an intuitive, user-friendly prediction model enhances the clinical practicality and translational value of our study. In terms of biological interpretation, we speculate that low dALP may reflect enhanced antitumor immune cell responses, while low dNLR may may be associated with a more robust immune response and enhanced T-cell clonal expansion.[24] Although we have obtained encouraging results, the potential interaction between ALP and NLR, as well as their clinical reliability, require further validation through large-scale prospective studies.

In real-world clinical settings, ALP and NLR offer several advantages over commonly used biomarkers such as PD-L1 expression and TMB, including lower cost, minimal invasiveness, and ease of repeated measurement. PD-L1 and TMB are static biomarkers assessed before treatment, while ALP and NLR can be dynamically monitored during therapy. Although ctDNA is a promising dynamic biomarker, as shown in the AEGEAN trial where ctDNA clearance predicted pCR with high NPV but modest PPV (~50%).[45] Moreover, the detection of ctDNA is associated with high costs, and its predictive value and cost-effectiveness need to be further evaluated. Unfortunately, due to the lack of PD-L1, TMB, and ctDNA data in our cohort, we were unable to to perform an in-depth analysis of these biomarkers. The antitumor immune response is complex and highly variable, making it difficult for any single factor to reliably predict immunotherapy outcomes. We propose that integrating the dynamic biomarkers identified in this study with established static biomarkers may enable the construction of a predictive model with superior performance. This represents a promising direction for future research.

This study has several limitations. Firstly, as a single-center, retrospective study, the sample size was relatively small, leading to potential selection bias and overfitting in model construction. Additionally, the overrepresentation of male patients and squamous cell carcinoma may limit the generalizability to female patients and non-squamous subtypes. Secondly, most of the pretreatment biopsy samples were obtained from primary hospitals where initial diagnoses were made, and critical data on indicators related to ICI efficacy, such as PD-L1 expression, TMB, and MSI, were missing from the patient specimens. Future prospective multicenter studies with larger cohorts and more comprehensive clinical data are needed for further analysis and external validation.

Conclusion

This study explored the predictive value of dynamic peripheral blood indicators for the pathological response in patients with NSCLC receiving neoadjuvant immunochemotherapy. A nomogram was developed incorporating dNLR, dALP, degree of differentiation, and smoking history to predict pCR in patients with NSCLC. Our analysis revealed that patients with high dALP and high dNLR levels had a lower probability of achieving pCR and experienced poorer EFS. The nomogram based on dALP and dNLR demonstrated good predictive performance, underscoring its potential for clinical application.

Data Sharing Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author Dr. Wenyu Zhai on reasonable request.

Ethics Approval and Informed Consent

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Board of Sun Yat-sen University Cancer Center (SYSUCC) (No. B2022-445-01).

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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.

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Disclosure

The authors report no conflicts of interest in this work.

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