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ORIGINAL RESEARCH

Efficacy Assessment and Prognostic Value of Inflammatory Markers in Patients with Stage IV Acral and Cutaneous Melanoma Receiving PD-1 Inhibitors

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Background: Malignant melanoma (MM) is a highly aggressive cancer. Different subtypes have different sensitivities to immunotherapy and lack peripheral blood markers. Few studies have examined the role of inflammatory markers in predicting the overall survival (OS) in stage IV acral melanoma (AM) and cutaneous melanoma (CM) patients receiving immunotherapy.

Purpose: This study aimed to investigate the value of inflammatory markers in efficacy and prognosis for stage IV melanoma patients who underwent immunotherapy.

Patients and Methods: This multicenter study reviewed the clinicopathological characteristics and inflammatory markers of 94 stage IV AM and CM patients receiving PD-1 inhibitors therapy. Pearson's chi-squared test or Fisher's exact test was used to compare baseline characteristics. The optimal cut-off values for these markers were stratified using time-dependent receiver operating characteristic curves (t-ROC). Kaplan-Meier (KM) curves and Log rank test were used to explore the relationship between inflammatory markers and survival outcomes. Cox regression analysis was performed to screen for independent prognostic factors and a nomogram was constructed. The model ability was tested by the C-index, t-ROC, calibration curves, and decision curve analysis curves.

Results: High NLR level was significantly correlated with lymph node metastasis and 3 or above metastatic sites (P=0.009, P=0.012). High PNI level favored a better ECOG PS (P=0.023). According to the KM curves, patients with baseline NLR>2.37, PNI<=42.65, and RLR>11.08 had worse OS (P<0.001, P<0.001, P<0.001). Cox regression analysis based on P<0.05 showed that M1c/M1d (P<0.001), NLR (P=0.003), and PNI (P<0.001) were significantly correlated with OS, and were visualized in a nomogram. C-index, t-ROC, area under the curve (AUC), and calibration curves revealed promising discrimination and accuracy of the nomogram. Decision curve analysis curves showed good clinical utility.

Conclusion: We established a prognostic predictive model based on distant metastatic sites, NLR, and PNI, and verified its superior performance and potential for clinical application.

Keywords: melanoma, acral, inflammatory markers, immunotherapy, prognosis

Introduction

Melanoma is one of the most aggressive cancers. The proportion of melanoma subtypes in Asian populations differs from that in Western populations. Although acral melanoma (AM) appears rare in Europeans (1–9%), it accounts for most (58%) Asian individuals.[1] Cutaneous melanoma (CM) is the predominant subtype among Caucasian melanomas, accounting for the majority of overall cases (>90%),[2] with an estimated 331,647 new cases of CM and 58,645 deaths globally in 2022.[3] Previously, chemotherapy and interferon were the primary treatments for malignant melanoma (MM), and the mean median overall survival (mOS) of MM patients ranged from 6 to 12 months.[4] In recent years, immunotherapy has significantly improved overall survival (OS).

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Immune checkpoint inhibitors (ICIs) such as anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) and anti-programmed death protein-1 (anti-PD-1) have emerged as first-line treatment options for MM. Nivolumab and Pembrolizumab produce response rates of approximately 30–40% in patients with MM initially treated with Ipilimumab.[5] Among previously treated patients, the 5-year OS rate with Pembrolizumab alone was nearly 34% and was 41% among patients previously untreated.[6] In metastatic melanoma, PD-1 inhibitors has been shown to have superior outcomes with minimal toxicity and became standard care.[7] Besides, combined anti-CTLA-4 and anti-PD-1 immunotherapy seem to provide better efficacy than anti-PD-1 monotherapy, but with more significant toxicity.[8] However, CTLA-4 inhibitors are rarely used in China due to the Chinese Society of Clinical Oncology (CSCO) guidelines considering CTLA-4 inhibitors to have a lower level of evidence-based medicine (EBM) evidence for therapeutic applications than PD-1 inhibitors, as well as the drug's high price, poor accessibility, and side effects.[9] Therefore, most Chinese patients received only immunotherapy containing PD-1 inhibitors.

Despite tremendous progress in immunotherapy, most MM patients still suffer from poor clinical outcomes. It is worth noting that Asian and European-American populations vary in the subtypes of MM pathogenesis, where AM is prominent in Asians and CM is prominent in European and American populations.[1,2] AM is insensitive to immunotherapy and lacks immune-related peripheral blood markers. Therefore, reliable clinical biomarkers are urgently needed to predict prognosis. Previous studies have shown that BRAF V600 mutation,[10] low distribution of phenotypes of tumor-infiltrating lymphocytes (TILs),[11] and high levels of circulating extracellular vesicles (EVs) have a bad prognosis in MM.[12] In addition, most biomarkers rely on tumor tissue assays, which are complex to obtain and costly to test, and also increase the risk of invasive testing in patients, with repeated testing not readily available.[13] Consequently, selecting markers that are easy to examine and economical to use as tools for predicting prognosis in MM patients treated with PD-1 inhibitors is particularly crucial.

There is growing evidence that systemic inflammation participates in tumorigenesis, progression, and metastasis.[14] Several studies have demonstrated the superiority of neutrophil-to-lymphocyte ratio (NLR) and prognostic nutritional index (PNI) as markers for the efficacy evaluation of immunotherapy in gastric cancer[15] and colorectal cancer.[16] Moreover, red blood cell distribution width-lymphocyte ratio (RLR) has been identified as a potential biomarker for prognosis monitoring in renal cell carcinoma receiving immunotherapy.[17] Therefore, they may serve as biomarkers for the efficacy and prognosis of immunotherapy in various neoplasms. However, the use of inflammatory markers in melanoma immunotherapy has rarely been reported. Recently, elevated NLR has been dramatically related to longer progression-free survival (PFS) in single-center local lymph node-infiltrating AM patients and high recurrence risk after immunotherapy in North American and European patients with metastatic melanoma,[18,19] and the lower PNI group is associated with OS in CM.[20] A small sample study showed that RLR has been identified as an independent prognostic factor for OS in CM patients.[21] Given the paucity of reports and lack of broad representation of these inflammatory markers in immunotherapy for different melanoma subtypes, we analyzed the relevance of several baseline inflammatory markers to survival in this retrospective study and probed the efficacy and prognostic value of inflammatory markers in stage IV AM and CM Chinese patients given PD-1 inhibitors therapy.

Specifically, we aimed to (1) assess the association between inflammatory markers and clinicopathological features; (2) compare the variations in inflammatory markers at baseline and progression stages, as well as the correlations between inflammatory markers and short-term outcomes; (3) analyze the association of inflammatory markers with PFS and OS in patients with stage IV AM and CM, and identify independent prognostic elements; (4) establish a prognostic prediction model for AM and CM with distant metastasis; and (5) assess the accuracy and clinical utility of the model.

Materials and Methods

Patients and Data Collection

We screened patients in the Department of Oncology of the First Affiliated Hospital of Anhui Medical University and the Second Affiliated Hospital of Anhui Medical University between April 2018 and February 2024, and included 94 patients with stage IV AM and CM treated with PD-1 inhibitors (Figure 1). Data collected retrospectively from the hospital received consent from all patients. This study was conducted in accordance with the Declaration of Helsinki (revised 2013). Clinical characteristics, treatment, serological and imaging data, and survival information of stage IV AM and CM

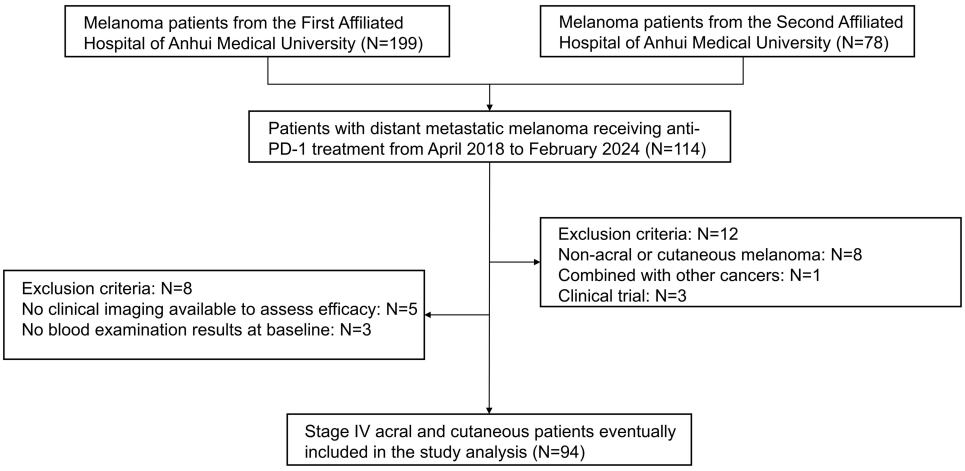

Figure 1 Flow chart.

patients receiving PD-1 therapy were obtained from the Hospital Information System, where clinical characteristic data included age, gender, height and weight at the initiation of anti-PD-1 therapy, Eastern Cooperative Oncology Group Performance Status (ECOG PS), treatment lines, and serum markers. Serum markers included neutrophil count (NC), lymphocyte count (LC), red blood cell distribution width-CV (RDW_CV) value, and albumin (ALB). Marker combinations were calculated as follows: NLR=NC/LC, PNI=ALB+5* LC, and RLR=RDW_CV/LC. Lymph node metastasis and in-transit metastasis were defined based on the American Joint Committee on Cancer (AJCC) 8th edition of Tumor Node Metastasis (TNM) classification. We classified the M stage as M1a/M1b (skin, soft tissue, and/or non-regional lymph node metastasis with/without lung metastasis) and M1c/M1d [visceral metastasis other than lung metastasis with/without central nervous system (CNS) metastasis].

Inclusion and Exclusion

Inclusion criteria: (1) patients diagnosed with acral and cutaneous melanoma by histopathology; (2) patients with stage IV cancers; (3) patients with physical conditions that could tolerate immunotherapy; and (4) patients with complete data on clinicopathological features, serological data, demographics, and follow-up information.

Exclusion criteria: (1) patients with combination of other neoplasms; (2) patients suffering acute or uncomplicated non-neoplastic diseases, especially autoimmune diseases; (3) patients who received leukocyte-boosting therapy when myelosuppression occurred with combination chemotherapy; (4) patients enrolled in a clinical trial (we were unable to determine whether the patient was receiving placebo or immunotherapy due to the treatment regimen being blinded); and (5) patients with incomplete clinical data, treatment interruptions, death due to other diseases or accidental deaths during the follow-up period.

Our primary study endpoint was OS, and the secondary one was PFS. OS was defined as the interval from the date of commencing ICIs treatment for stage IV AM and CM to death from any cause (event) or the final follow-up (review). PFS referred to the period from the initial date of anti-PD-1 therapy to disease progression, death, or last follow-up. Recurrence and metastasis were confirmed via pathological examination or imaging. Follow-up information for each patient was obtained from clinical records and telephone calls and terminated on April 15, 2024.

Statistical Analysis

According to the latest World Health Organization (WHO) classification criteria, patients were classified according to whether they were over 65 years old. In addition, we grouped BMI following the Chinese adult BMI grouping criteria, which is also consistent with previous studies on MM.[22] We defined the optimal cut-off value for inflammatory markers using time-dependent receiver operating characteristic curves (t-ROC) based on OS. Pearson's chi-squared test or Fisher's exact test was used to compare variables in different category groups. Kaplan-Meier (KM) curves and

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Log rank tests were used to compare survival differences between the high and low-risk groups. Independent prognostic variables were identified by univariate and stepwise Cox regression analysis. The hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Prior to modelling, we conducted multicollinearity tests on the variables to exclude covariates. Cox regression analysis was tested as satisfying the proportional hazard assumption. Of note, we applied stepwise Cox regression to eliminate any variables not fitting $P<0.05$ in multivariate analysis to adjust for potential confounders, further defining the final model. C-index, AUC, calibration plot, and decision curve analysis (DCA) curves assessed the nomogram. C-index and AUC were used to evaluate the validity. The t-ROC curves reflect the specificity and sensitivity.[23] The closer the t-ROC curve is to the upper left corner of the plot, the more accurate is the predictive model. The calibration curves were resampled using Bootstrap to determine the calibrated capacity of the column-line plots, allowing for agreement between the predicted and actual probabilities. We used DCA to appraise clinical utility, which derived the net benefit of the prediction model at different threshold probabilities.[24] Statistical significance was set at two-sided $P<0.05$. We utilized R studio version 4.2.3 for all statistical analysis, based on multiple R packages (including "survival", "survminer", "pROC", "timeROC", "regplot", "rms", "car", "ggDCA", "pec", and "ggplot2") with the help of (http://www.r-project.org/). For example, cut-off values for inflammatory markers were calculated using the survfit package, and a nomogram was created using the rms package.

Results

Baseline Characteristics

The study enrolled 94 stage IV AM and CM patients treated with ICIs. Among the entire cohort, 57 patients (60.64%) had AM, and 37 (39.36%) had CM. In terms of tumor metastasis, 15 patients (15.96%) developed in-transit metastasis, 64 patients (68.09%) had combined skin, soft tissue, and/or non-regional lymph node metastasis with/without lung metastasis, and 30 patients (31.91%) had visceral metastasis other than lung metastasis with/without CNS metastasis. In all cases, 78 patients were treated with ICIs in the first line, while 16 patients in the second or later lines. Detailed data are shown in Table 1. Furthermore, there were no remarkable differences in the clinicopathological characteristics and inflammatory markers between the acral and cutaneous groups (Supplementary Table 1).

The Optimal Cut-off Values for Inflammatory Markers

The t-ROC curves determined the optimal cut-off value for each inflammatory marker based on OS, which was 2.37, 42.65, and 11.08, respectively, for NLR, PNI, and RLR, and the patients were categorized into two groups (low group and high group) (Supplementary Figure 1).

Relationship Between Inflammatory Markers and Patients' Characteristics

We retrospectively analyzed patients in subgroups with high (H) and low (L) levels of inflammatory markers. In terms of clinicopathological characteristics, patients in the NLR-L group tended to harbor fewer lymph node metastasis and fewer number of metastatic sites than those in the NLR-H group ($P=0.009$, $P=0.012$), whereas the PNI-L group showed the opposite trend ($P=0.009$), and the PNI-H group additionally had better ECOG PS scores ($P=0.023$). Moreover, patients in the NLR-L group tended to favor immunotherapy as a first-line treatment option ($P=0.035$). The differences between the high- and low-level NLR, and PNI groups were not statistically significant with respect to other baseline characteristics such as age, gender, and BMI (Table 1). With regard to inflammatory markers, the NLR-L group had lower levels of RLR than the NLR-H group, and the RLR levels in the PNI-H group were significantly lower than those in the PNI-L group ($P<0.001$) (Table 1).

Relationship Between Inflammatory Markers and Efficacy

We assessed the short-term outcomes of anti-PD-1 therapy in all patients. All subjects underwent a minimum of two therapeutic courses when the outcome was reached by the ICIs. According to RECIST 1.1, the numbers of cases of CR,

Table 1 Baseline Characteristics of Stage IV AM and CM Patients

	Total (n=94) N(%)	NLR<=2.37 (n=46) N(%)	NLR>2.37 (n=48) N(%)	*P*	PNI<=42.65 (n=21) N(%)	PNI>42.65 (n=73) N(%)	*P*
Age (years)				0.882			0.197
<=65	62 (65.96)	30 (65.22)	32 (66.67)		12 (54.55)	50 (69.44)	
>65	32 (34.04)	16 (34.78)	16 (33.33)		10 (45.45)	22 (30.56)	
Gender				0.818			0.934
Male	42 (44.68)	20 (43.48)	22 (45.83)		10 (45.45)	32 (44.44)	
Female	52 (55.32)	26 (56.52)	26 (54.17)		12 (54.55)	40 (55.56)	
BMI (kg/m^2)				0.749			0.056
<18.5	6 (6.38)	2 (4.35)	4 (8.33)		4 (18.18)	2 (2.78)	
18.5–23.9	60 (63.83)	31 (67.39)	44 (60.42)		12 (54.45)	48 (66.67)	
≥24	28 (29.78)	13 (28.26)	15 (31.25)		6 (27.27)	22 (30.55)	
ECOG PS (points)				0.056			0.023
<=1	74 (78.72)	40 (86.96)	34 (70.83)		13 (59.09)	61 (84.72)	
≥2	20 (21.28)	6 (13.04)	14 (29.17)		9 (40.91)	11 (15.28)	
Lymph node metastasis				0.009			0.678
Negative	29 (30.85)	20 (43.48)	9 (18.75)		6 (27.27)	23 (31.94)	
Positive	65 (69.15)	26 (56.52)	39 (81.25)		16 (72.73)	49 (68.06)	
In-transit metastasis				0.187			0.510
Negative	79 (84.04)	41 (89.13)	38 (79.17)		16 (76.19)	63 (86.30)	
Positive	15 (15.96)	5 (10.87)	10 (20.83)		5 (23.81)	10 (13.70)	
M stage				0.235			0.120
M1a/M1b	64 (68.09)	34 (73.91)	30 (62.50)		12 (54.55)	52 (72.22)	
M1c/M1d	30 (31.91)	12 (26.09)	18 (37.50)		10 (45.45)	20 (27.78)	
Number of metastatic sites				0.012			0.009
<=2	71 (75.53)	40 (86.96)	31 (64.58)		12 (54.55)	59 (81.94)	
≥3	23 (24.47)	6 (13.04)	17 (35.42)		10 (45.45)	13 (18.06)	
Treatment lines				0.035			0.255
1	78 (82.98)	42 (91.30)	36 (75.00)		16 (72.73)	62 (86.11)	
≥2	16 (17.02)	4 (8.70)	12 (25.00)		6 (27.27)	10 (13.89)	
RLR				<0.001			<0.001
<=11.08	66 (70.21)	42 (91.30)	24 (50.00)		5 (22.73)	61 (84.72)	
>11.08	28 (29.79)	4 (8.70)	24 (50.00)		17 (77.27)	11 (15.28)	

Abbreviations: AM, acral melanoma; CM, cutaneous melanoma.

PR, SD, and PD were 0, 7, 55, and 32, respectively. Based on the paired-sample *t*-test, PNI decreased (P=0.044) and RLR increased (P<0.001) in the SD group (Figure 2E and 2F). Similarly, this trend of elevated RLR was observed in the DCR group (P<0.001) (Figure 2f). There were no notable shifts in inflammatory markers among the other short-term outcome groups (Figure 2A–D, 2G–I, and 2a–e). NLR and RLR were significantly higher after anti-PD-1 treatment failure (P=0.001, P<0.001) (Figure 2g and 2i). PNI levels were reduced (P<0.001) in comparison with baseline (Figure 2h).

Survival Analysis

Figure 3 shows the survival analysis of OS. Of these patients, 60.6% died during the follow-up period, and the mOS was 5.22–27.42 months. In terms of clinicopathological characteristics, OS was longer in low ECOG PS points patients than in high points (mOS=19.52 months, 95% CI=16.600–36.197 vs 8.77 months, 95% CI=7.741–18.670, P=0.002) (Figure 3A). The M1c/M1d group had terrible OS versus the M1a/1b group (mOS=9.48 months, 95% CI=7.654–16.483 vs not reached, P<0.001) (Figure 3B). Moreover, we found a shorter OS among the group with 3 or above metastatic sites (mOS=8.77 months, 95% CI=6.317–16.595 vs 22.03 months, 95% CI=17.294–36.204, P=0.002) (Figure 3C). Unfortunately, lymph node metastasis, in-transit metastasis, and treatment lines did not differ significantly in terms of OS (Figure 3D, 3E, and 3F). In terms of inflammatory markers, patients with baseline NLR>2.37, PNI<=42.65, and RLR>11.08 had shorter OS than

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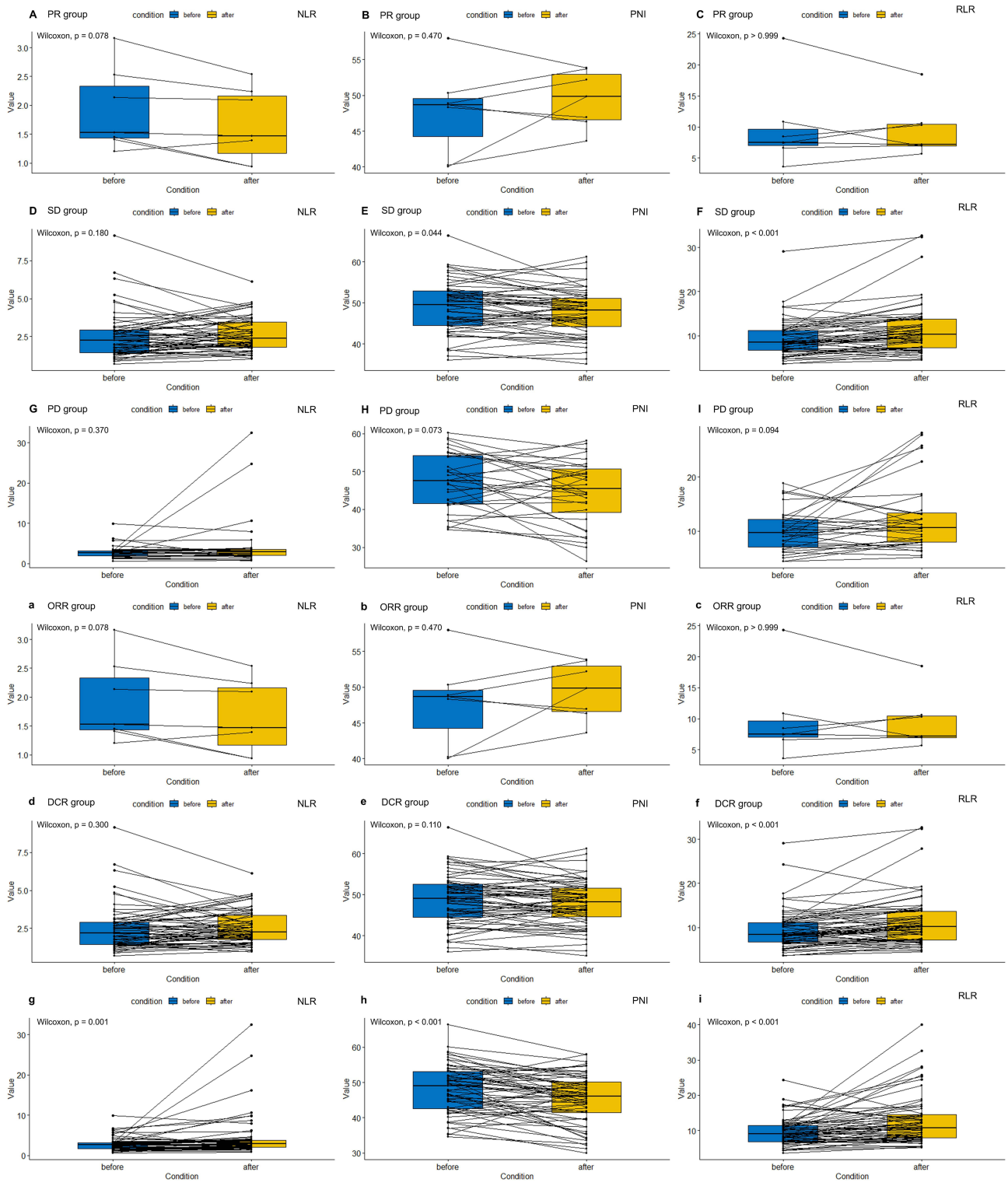

Figure 2 Efficacy of anti-PD-1 therapy according to the changes in inflammatory markers among stage IV acral melanoma and cutaneous melanoma patients in the PR group (**A–C**), SD group (**D-F**), PD group (**G-I**), ORR group (**a–c**), DCR group (**d-f**), and subgroups with disease progression at follow-up deadline (**g-i**).

those with NLR<=2.37 (mOS=8.77 months, 95% CI=7.739–14.366 vs not reached, P<0.001) (Figure 3G); PNI>42.65 (mOS=6.18 months, 95% CI=5.125–8.995 vs 23.70 months, 95% CI=17.296–36.197, P<0.001) (Figure 3H); and RLR<=11.08 (mOS=8.11 months, 95% CI=5.932–9.765 vs not reached, P<0.001) (Figure 3I).

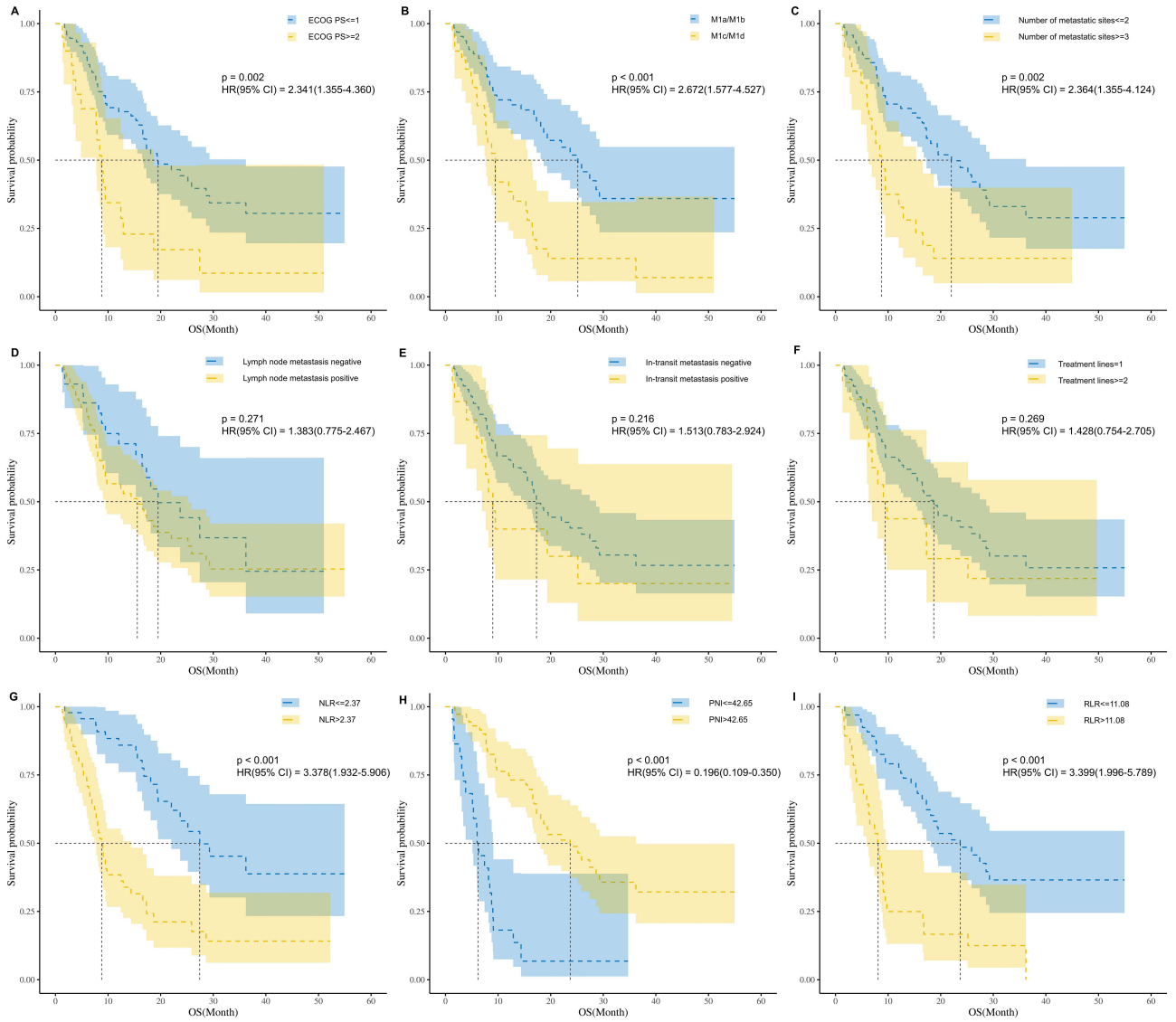

Figure 3 Kaplan–Meier curves of overall survival (OS) according to ECOG PS (**A**), M stage (**B**), Number of metastatic sites (**C**), Lymph node metastasis (**D**), In-transit metastasis (**E**), Treatment lines (**F**), NLR (**G**), PNI (**H**), and RLR (**I**) at baseline. The P values were calculated using the Log rank test (two-sided).

Prognostic Value of Inflammatory Markers and Clinicopathological Characteristics in Stage IV AM and CM Patients

Univariate Cox regression analysis showed that BMI, ECOG PS, M stage, number of metastatic sites, NLR, PNI, and RLR were significantly associated with OS (P<0.05). Furthermore, Cox proportional risk models, based on stepwise regression, suggested that independent risk factors for OS include M stage (HR=2.478, 95% CI=1.450–4.235, P<0.001), NLR (HR=2.463, 95% CI=1.344–4.514, P=0.004), and PNI (HR=0.260, 95% CI=0.137–0.493, P=0.003) in stage IV AM and CM patients (Table 2). There was no significant interaction between variables (P>0.05).

Nomogram and Model Performance

The nomogram was constructed according to the stepwise regression Cox proportional risk model (P<0.05) to quantitatively assess the OS rate of stage IV AM and CM patients treated with PD-1 inhibitors, and we finally integrated a total of 3 risk factors: M stage, NLR, and PNI. Each patient was assigned a total score by adding the score for each prognosis-

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Table 2 Univariate and Stepwise Regression Multivariate Cox Analysis of Factors According to OS in Stage IV AM and CM Patients Treated with Anti-PD-1 Therapy

	Univariate Analysis		P	Multivariate Analysis		P
	HR	95% CI		HR	95% CI	
Age(years)						
>65 vs <=65	1.317	0.764–2.271	0.322			
Gender						
Female vs Male	0.770	0.455–1.303	0.330			
BMI						
18.5–23.9 vs <18.5	0.305	0.106–0.876	0.027	0.302	0.091–1.001	0.050
≥24 vs <18.5	0.395	0.131–1.191	0.099	0.366	0.097–1.376	0.137
ECOG PS						
≥2 vs <=1	2.425	1.352–4.349	0.003	1.575	0.733–3.211	0.211
Lymph node metastasis						
Positive vs Negative	1.382	0.775–2.466	0.273			
In-transit metastasis						
Positive vs Negative	1.511	0.782–2.920	0.219			
M stage						
M1c/M1d vs M1a/M1b	2.661	1.571–4.509	<0.001	2.478	1.450–4.235	<0.001
Number of metastatic sites						
≥3 vs<=2	2.357	1.351–4.112	0.003	1.152	0.568–2.336	0.694
Treatment lines						
≥2 vs 1	1.430	0.755–2.709	0.272			
NLR						
>2.37 vs <=2.37	3.372	1.929–5.894	<0.001	2.463	1.344–4.514	0.004
PNI						
>42.65 vs <=42.65	0.195	0.109–0.350	<0.001	0.260	0.137–0.493	0.003
RLR						
>11.08 vs <=11.08	3.396	1.994–5.783	<0.001	1.825	0.963–3.456	0.065

Notes: HR: Hazard Ratio, CI: Confidence Interval.
Abbreviations: OS, overall survival; AM, acral melanoma; CM, cutaneous melanoma.

specific parameter. In particular, patients in the M1c/M1d stage received a score of 68.1; those with NLR>2.37 received 67.5 scores; and PNI<=42.65 corresponded to 100 scores. Higher total indicated implied poorer clinical outcomes. As shown in Figure 4, the model visually predicted the patients' 6-month, 12-month, 18-month, and 24-month OS rates in the study cohort.

We performed t-ROC curves to evaluate the susceptibility and specificity of the prognostic characteristics. The AUC values of the 6-month, 12-month, 18-month, and 24-month OS rates were 0.820, 0.851, 0.884, and 0.791 (Figure 5A), demonstrating positive predictive values. As an AUC curve for multiple-time survival information, Supplementary Figure 2A assessed the predictive performance of the survival model at different time points. The C-index was 0.769, which varied over time and was similar to the AUC value. We plotted a time-dependent C-index (Supplementary Figure 2B) and found that the AUC and C-index were essentially the same numerically. In addition, the calibration curves are shown in Figure 5. The curves at 6-, 12-, and 18-month were close to the ideal performance (45° line), which showed the high accuracy of the nomogram in this cohort (Figure 5C–E). However, the model was poorly calibrated when predicting 24-month OS rates (Figure 5F). DCA curves indicated the clinical benefit of predicting the 6-month OS rate in the range of 6–56% threshold probability (Figure 5B-a). Similarly, the threshold probabilities for predicting 12-month, 18-month, and 24-month OS rates were 12%-82%, 22%-99%, and 31–99% (Figure 5B-b, B-c, B-d).

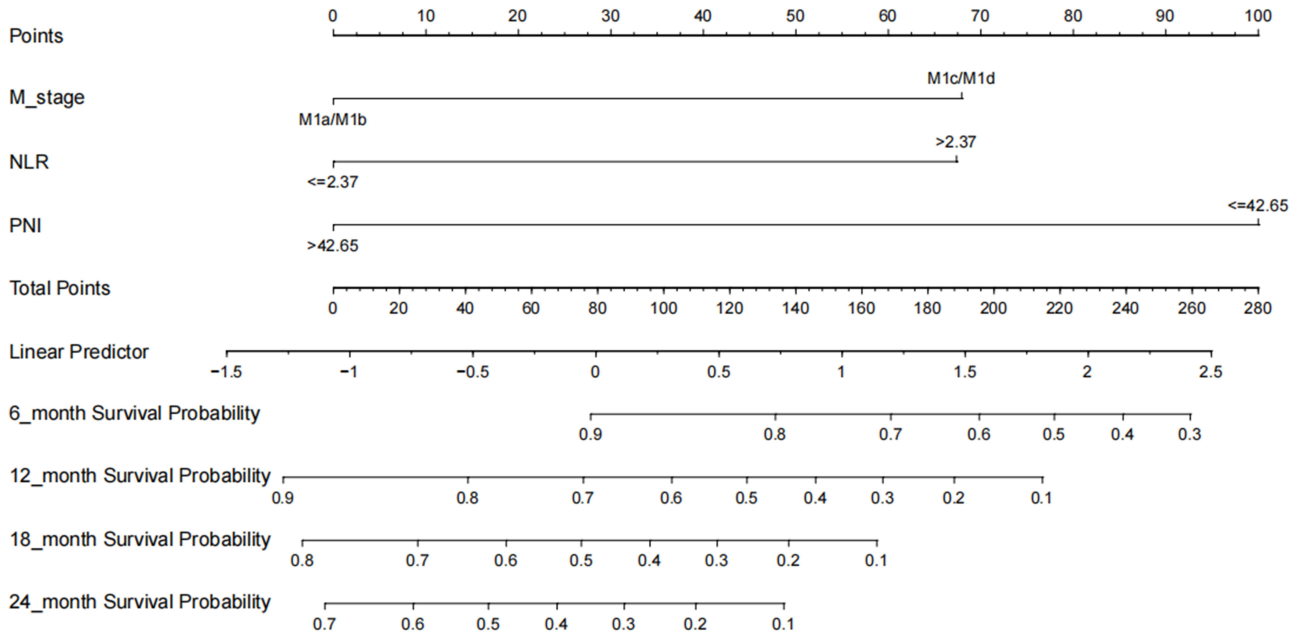

Figure 4 Predictive nomogram of 6-, 12-, 18-, and 24-month OS rate for stage IV acral melanoma (AM) and cutaneous melanoma (CM) patients treated with anti-PD-1 therapy, in which the total score corresponds to a survival probability at the bottom, summing each value of the variable.

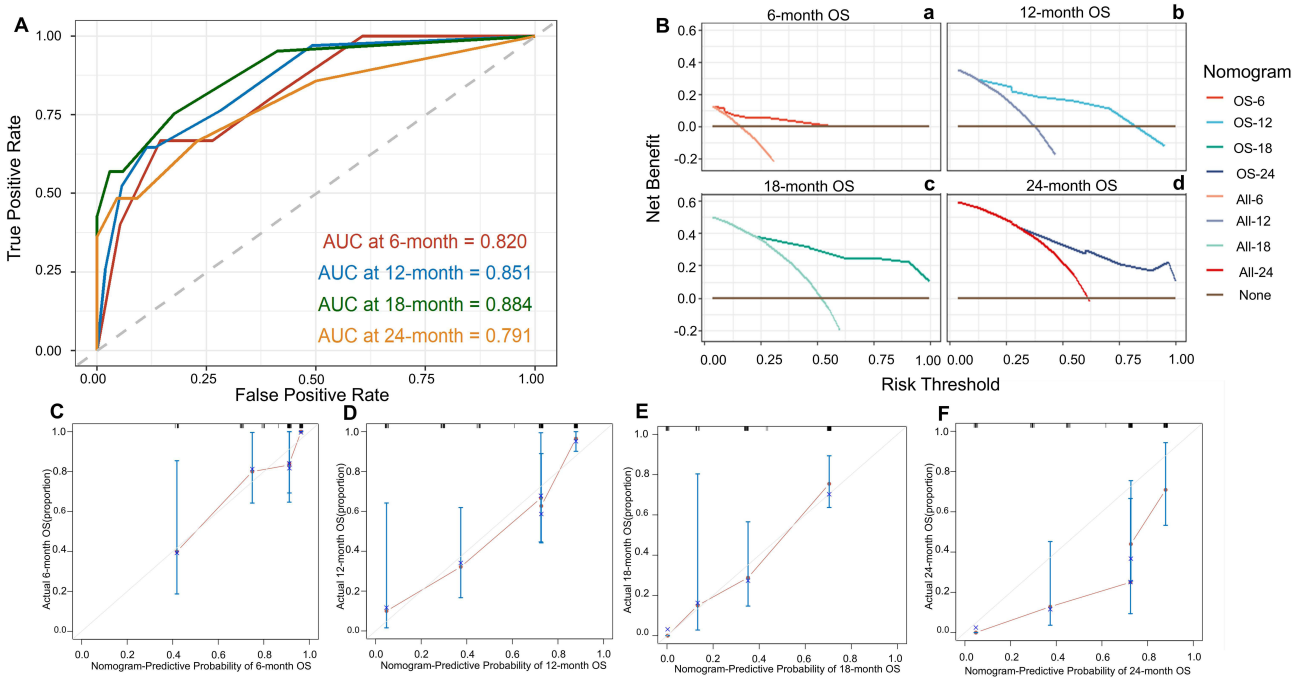

Figure 5 Performance of the nomogram. (**A**) Time-dependent receiver operating characteristic curve (T-ROC) curves. The ability of the nomograms at 6-, 12-, 18-, and 24-month was measured according to the area under the curve (AUC) values for this cohort. (**B**) Decision curve analysis (DCA) curves of the nomogram for the survival prediction of patients with stage IV acral melanoma (AM) and cutaneous melanoma (CM). (**a**) 6-month survival benefit. (**b**) 12-month survival benefit. (**c**) 18-month survival benefit. (**d**) 24-month survival benefit. (**C-F**) Calibration curves for stage IV AM and CM patients. (**C**) 6-month overall survival (OS). (**D**) 12-month OS. (**E**) 18-month OS. (**F**) 24-month OS.

Discussion

Melanoma is an aggressive cancer with high metastatic potential.[25] Although combination therapy with multiple drugs has greatly improved the prognosis of MM patients, some of them still have poor outcomes in clinical applications. This

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may be due to different sensitivities of various MM subtypes to drugs and distinct mutational landscapes. So far, there have been mutations and pathogenic pathways widely used in clinical treatment, commonly including the BRAF gene, the NRAS gene, the KIT gene, the MEK/MAPK pathway,[26] and so on. Substantial inflammatory factors have been reported to trigger cascade responses and tissue shrinkage, thereby facilitating neoplasm hyperplasia and metastasis.[27] Therefore, the efficacy and prognostic role of inflammatory markers in immunotherapy for various malignancies, including gastric cancer,[15] colorectal cancer,[16] and renal cell carcinoma,[17] have received extensive attention. Our study aimed to clarify the significance of potential indicators in efficacy monitoring and prognostic assessment in stage IV AM and CM patients treated with PD-1 inhibitors, and developed a prognostic model to evaluate individualized survival outcomes of AM and CM patients more intuitively, further guiding clinical practice.

The multicenter study comprised 57 (60.6%) patients with AM and 37 (39.4%) with CM. There were no significant differences between the baseline characteristics of the two groups. Therefore, we integrated acral and cutaneous melanomas for further analysis. We observed the tight correlation between dynamic changes in inflammatory markers and short-term outcomes in AM and CM patients. According to multivariate stepwise Cox regression analysis, we found NLR and PNI to be independent prognostic factors. Moreover, we constructed a prognostic prediction model based on the 3 independent predictors obtained, for better differentiating the patients' clinical prognosis. To the best of our knowledge, our study reports the value of inflammatory markers in efficacy and prognosis of stage IV AM and CM patients receiving PD-1 inhibitors for the first time, which is of great significance.

Concerning baseline characteristics, the relationship between inflammatory markers and clinicopathological features did not correspond precisely. We found that lymph node metastasis was accompanied by high baseline NLR. To date, researchers have found that multiple sentinel lymph node (SLN)-positive and non-SLN metastatic lymph node metastasis portended an adverse prognosis.[28] On the other side, patients with 3 or more distant metastatic sites were relevance to the NLR-H and PNI-L groups. Porcelli Letizia et al reported that patients with 3 or more metastatic sites had worse OS than patients with 1 or 2 metastatic sites.[29] The above results indirectly showed the association between elevated inflammatory markers and a potentially dismal prognosis in MM patients. On the contrary, poorer ECOG PS scores were associated with low PNI levels, similar to previous studies.[30]

There is growing evidence that inflammatory markers reflect the biological characteristics of MM,[18,31] but rare studies have described the dynamics of these markers with efficacy. Our research identified that inflammatory markers were intimately tied to short-term outcomes in stage IV AM and CM patients receiving ICIs. RLR levels were significantly higher in the DCR and SD groups. Interestingly, the declining trend did not occur in the PD group. Instead, even though none of them were statistically significant, the inflammatory markers' levels all trended upward. This may stem from the relatively low number of patients who achieved the initial efficacy of PD in this cohort, potentially weakening the test power. Therefore, we further depicted the variations in inflammatory markers in all PD patients before the deadline for follow-up (Figure 2g-i). In the baseline values, high NLR level and low PNI level were associated with poor PFS, which reaffirmed the statistical significance of NLR and PNI in OS, suggesting that the short-term efficacy of immunotherapy may provide a favorable judgement for long-term prognosis. However, RLR was significantly higher after the failure of PD-1 inhibitors, while RLR did not correlate with OS. This may be caused by many factors influencing the translation of recent tumor control into long-term survival as well as limited sample size. Besides, the immune microenvironment plays a crucial role in the response to melanoma immunotherapy. It is worth noting that tumor microenvironment (TME) metabolically reprograms immune cells by producing metabolites, and such changes may influence clinical efficacy.[32] Infiltrating lymphocytes in TME are generally indicators of the organism's immune state, whereby lymphocytes and their subpopulations (CD8+ T cells and CD3+ T cells) have been associated with the efficacy of some tumors.[33] In conclusion, inflammatory marker levels were significantly lower than the baseline levels when better short-term outcomes were achieved. However, these levels increased again as the tumor advanced. Dynamic changes in inflammatory markers show great potential for forecasting the short-term outcomes of ICIs and disease progression in AM and CM patients.

Considering the prognostic value of inflammatory markers, we found that high NLR levels were independent factors of short OS in stage IV AM and CM patients. NLR reflects the balance between two aspects of the immune process: innate immunity (neutrophil-dominated) and adaptive immunity (lymphocyte-dominated). Tumors influence the immune response through a storm of pro-inflammatory cytokines and chemokines, directly stimulating tumor associated

neutrophils (TANs) to promote metastasis and systemic neutrophilic inflammation.[34] Lymphocytes engage in the host response to neoplastic cells, and a lower NLR is typically accompanied by favorable prognostic factors reflecting a preserved immune homeostasis. This supports the view that the NLR can be considered a reliable and inexpensive element of active tumor-associated inflammation and a potent marker of prognosis in solid tumors. Similar to our findings in AM and CM, high NLR levels have been reported to confer poor prognosis in patients with non-small cell lung cancer[35] and breast cancer[36] patients, with a standard threshold value under debate. Changes in NLR over time are markers of immune system disorders, even without a fixed threshold. In our study, low PNI levels served as an independent predictor of poor OS in stage IV AM and CM patients. As an indicator of nutritional status and systemic immunocompetence, PNI is significantly associated with TILs.[37] Reduced PNI levels have correlations with local immune responses in tumors and poor prognosis in diverse cancers, including hepatocellular carcinoma,[38] cervical cancer,[39] and colorectal cancer.[40] Likewise, NLR and PNI could serve as independent prognostic factors in primary CM[41] patients, similar to our findings. In summary, previous studies have focused on the prognostic significance of markers dominated by pathological features of early (non-stage IV) CM for immunotherapy. Small sample studies have suggested that the level of a single inflammatory marker is associated with survival in MM patients not receiving immunotherapy. However, none of the studies have built prognostic models. Our report is the first to show that baseline high NLR and low PNI are independent predictors of poor OS in stage IV AM and CM patients treated with PD-1 inhibitors, which enriches the value of inflammation markers in MM immunotherapy, especially in AM poorly responsive to immunotherapy.

Subsequently, we explored the prognostic value of general baseline characteristics other than inflammatory markers for AM and CM. Survival analysis showed prolonged OS in patients with low ECOG PS scores at baseline compared to those with high ECOG PS scores. It is well known that individuals suffering from adverse ECOG PS generally cannot endure routine drug dosages and harbor a dismal prognosis.[42] However, according to the multivariate stepwise Cox regression analysis, the ECOG PS level was not a statistically significant predictor of OS. This may be owing to the fact that many factors, including the limited sample volume, influence the translation of short-term outcomes to survival benefits. Similar to the traditional TNM classification as an independent prognostic factor for advanced MM,[43] our study also confirmed that a later M stage was a poor prognostic factor. However, lymph node metastasis status was not an independent prognostic factor in our study, which probably relates to the fact that widely used immunological drugs dramatically improve the prognosis of melanoma patients,[44] further compensating for the preexisting survival differences between the presence or absence of lymph node metastasis. However, we did not include the key features of Clark level, Breslow thickness, ulceration, and BRAF mutation because of the lack of pathological data.[10,45]

A comprehensive assessment of the OS rate and an objective evaluation of the risk-benefit ratio of medical interventions are valuable for clinicians to facilitate optimal medical decision-making for AM and CM patients. In our study, we developed the first nomogram combining clinicopathological features and routine blood tests to foresee survival outcomes in stage IV AM and CM patients receiving anti-PD-1 therapy which needed only 3 indicators (M stage, RLR, and PNI). All parameters required for the model were easily available at baseline, which enriched the predictive value of efficacy and prognosis for inflammatory markers in immunotherapy, thus providing important survival information for patient classification through nomogram. Furthermore, compared with previous prognostic models for AM, the predominant subtype in the Asian populations,[46] our longer follow-up for different MM subtypes provided more reliable prognostic predictions with higher forecast performance. And the test power of the model was superior. In addition, the model was validated based on t-ROC curves and showed excellent discrimination, accounting for temporal dynamics and avoiding the limitations of a single time-point assessment.[47] This may assist in identifying patients who would benefit from immunotherapy, potentially rationalizing therapeutic strategies.

There are several limitations to note. Firstly, this study is a retrospective real-world study, which may introduce selection bias due to patient selection and missing data. Secondly, we included cases from two centers with a limited sample size, affecting the representativeness of our findings and limiting their broad applicability. Additionally, our study lacks key molecular biological characteristics and other potential biomarkers associated with immunotherapy response, such as Clark level, Breslow thickness, BRAF mutation, tumor-associated antigens, T cell subsets, and cytokines that are typically important for melanoma prognosis, which may affect the completeness of the model. Furthermore, most

inflammatory markers lack recognized cut-off values. We determined the cut-off values of these markers by t-ROC. Therefore, further prospective studies with large samples are needed to determine the common cut-off values. Lastly, this model lacks data support for external validation, which limits generalization ability. Therefore, multicenter prospective studies with larger samples are necessary to confirm the conclusions.

Conclusions

Overall, lymph node metastasis was strongly correlated with high levels of NLR. For stage IV AM and CM patients who received PD-1 inhibitors, 3 or above metastatic sites occurred more commonly in patients with high NLR levels and low PNI levels. Elevated NLR and RLR levels and decreased PNI levels at baseline indicate disease progression. M1c/M1d stage, high NLR, and low PNI were poor independent prognostic factors that affected OS. Therefore, we developed and validated a prognostic prediction model based on the above results, including clinicopathological features and inflammatory markers, which holds great promise for clinical applications in stage IV AM and CM patients treated with anti-PD-1 therapy.

Abbreviations

MM, malignant melanoma; AM, acral melanoma; CM, cutaneous melanoma; ICIs, immune checkpoint inhibitors; OS, overall survival; NLR, neutrophil-to-lymphocyte ratio; PNI, prognostic nutritional index; RLR, red blood cell distribution width-lymphocyte ratio; KM, Kaplan-Meier; t-ROC, time-dependent receiver operating characteristic curves; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; CNS, central nervous system; AUC, area under the curve; mOS, median overall survival; anti-PD-1, anti-programmed death protein-1; TME, tumor microenvironment; PFS, progression-free survival.

Data Sharing Statement

Datasets related to this article are available from the corresponding author on reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Quick-PJ2024-06-43). All authors have provided their consent for publication.

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Disclosure

The authors report no conflicts of interest in this work.

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