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Prognostic Significance of Inflammatory Biomarkers in First-Line Immunotherapy for Metastatic Melanoma: Multicentric Study

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Simple Summary

Patients with advanced melanoma treated with first-line immunotherapy do not all have the same outcome, and simple tools to identify higher-risk patients at treatment start are still needed. In this multicenter retrospective study, we evaluated whether inflammatory biomarkers calculated from routine blood counts before treatment were associated with survival outcomes in 162 patients with unresectable stage III or IV cutaneous melanoma. Patients with less favorable biomarker profiles had shorter progression-free and overall survival. Among the evaluated markers, the pan-immune-inflammation value showed the most consistent independent association with both outcomes after adjustment for clinical factors. These findings suggest that inexpensive and widely available blood-based inflammatory markers may help improve initial risk stratification in advanced melanoma, alongside established clinical factors. However, prospective validation in independent cohorts is required before these markers can be used routinely in clinical practice.

Abstract

Background/Objectives: This study evaluates the prognostic value of baseline inflammatory biomarkers neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), lymphocyte-to-monocyte ratio (LMR), systemic immune-inflammation index (SII) and pan-immune-inflammation value (PIV) in advanced cutaneous melanoma treated with first-line immunotherapy. **Methods:** This multicenter retrospective study included 162 patients with unresectable stage III/IV cutaneous melanoma treated with first-line pembrolizumab, nivolumab, or nivolumab plus ipilimumab. Biomarkers were calculated from complete blood counts obtained within 30 days before treatment start. Cut-offs were defined by ROC analysis. Progression free survival (PFS) and overall survival (OS) were analyzed using Kaplan–Meier and Cox regression. Response was assessed by RECIST v1.1. **Results:** Higher baseline NLR, PLR, MLR, SII and PIV were more common in patients with adverse baseline features, including liver metastases, elevated LDH and poorer ECOG performance status. Patients with biomarker values below the cut-offs had significantly longer PFS and OS. In multivariable models adjusted for clinical covariates, PIV remained independently associated with the duration of PFS and OS; MLR independently predicted PFS, while PLR independently predicted OS. **Conclusions:** Baseline inflammatory biomarkers from routine blood counts provide useful prognostic information in advanced melanoma treated with first-line ICIs. PIV showed the most consistent independent association with survival outcomes and may

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support initial risk stratification alongside LDH, ECOG and metastasis pattern. However, prospective validation in independent cohorts is needed before routine clinical implementation.

Keywords: metastatic melanoma; first-line immunotherapy; immune checkpoint inhibitors; inflammatory biomarkers; progression-free survival; overall survival; prognostic factors

1. Introduction

Melanoma is a significant global health concern, with rising incidence and mortality rates in recent years [1]. The main predictor for survival is tumor stage at diagnosis, ranging from 85% to 95% at 10 years for localized disease while survival rates for advanced melanoma are much lower [2].

Immunotherapy has reshaped the management of metastatic disease. Anti–PD-1 monotherapy with pembrolizumab or nivolumab yields durable benefit, with five-year survival rates of 38.7% and 39%, respectively [3,4]. Combination of nivolumab and ipilimumab provides additional advantage, achieving five-year overall survival of up to 52%[5]. These data underscore the central role of immunotherapy in prolonging survival in advanced melanoma. Although immunotherapy improves outcomes in metastatic melanoma, primary resistance is still common. Up to 58% of patients receiving anti–PD-1 monotherapy and up to 42% receiving anti–PD-1 plus anti–CTLA-4 do not achieve an objective response in the first-line setting [4–6]. In view of persistent primary and acquired resistance, priority should be given to individualizing management and to early identification of patients who will benefit from first-line immunotherapy.

So far, some tumor-related factors have been suggested as predictive markers of efficacy for immunotherapy [7–9]. In routine practice, however, rapid and low-cost biomarkers are needed to support treatment individualization [10,11]. Growing evidence implies that systemic inflammatory response impacts disease progression and course in different cancers, including melanoma [11,12]. Hence, several immune-based scores such as neutrophil count, lymphocyte count, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and monocyte-to-lymphocyte ratio (MLR) were usually employed to assess the systemic inflammation in cancer patients [10,13–15]. Low lymphocyte count, increased neutrophil or platelet count are frequently observed in cancer patients, indicating poor survival outcome [11,14–17]. These counts can be used for calculation of pan-immune-inflammation value (PIV), a new comprehensive biomarker that has been proven to be a strong predictor of survival in patients with metastatic colorectal cancer, non-small cell lung cancer and breast cancer [18–20].

The aim of this study was to assess the prognostic value of baseline inflammatory biomarkers (NLR, PLR, MLR, lymphocyte-to-monocyte ratio (LMR), systemic immune-inflammation index (SII) and PIV for response to first-line immunotherapy in metastatic melanoma, and to strengthen the evidence base for their clinical utility in this setting.

2. Materials and Methods

Patients

The study enrolled 162 patients with advanced cutaneous melanoma treated at three centers in the Western Balkans (South Eastern Europe)—two in Serbia (the Clinic of Dermatovenereology, Military Medical Academy, Belgrade [n=68] and the Clinic of Oncology, University Clinical Center Niš [n=88]) and one in Bosnia and Herzegovina (the Clinic of Oncology, Clinical Center Sarajevo [n=6]). Inclusion criteria for participation in the study were: 18 years of age or older, unresectable stage III/IV cutaneous melanoma, first-line mono-immunotherapy (pembrolizumab or nivolumab) or combined immunotherapy (nivolumab and ipilimumab) and availability of a complete blood count with differential obtained within 30 days before initiation of the first treatment cycle. Exclusion criteria were: age under 18 years, patients with insufficient follow-up (<12 months) without documented progression or death.

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Treatment

In the pembrolizumab regimen, 200 mg of pembrolizumab was administered intravenously every 3 weeks or 400 mg every 6 weeks. In the nivolumab regimen, 480 mg of nivolumab was administered intravenously every 4 weeks. In the ipilimumab plus nivolumab regimen, 3 mg/kg of ipilimumab was administered intravenously following 1 mg/kg of nivolumab, or 1 mg/kg of ipilimumab following 3 mg/kg of nivolumab, every 3 weeks for up to 4 cycles, followed by continued administration of 480 mg of nivolumab every 4 weeks. Dose and schedule modifications were permitted based on the patient's condition.

Response to therapy was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST v1.1) guidelines [21].

Data Collection and Ethics

Patients' data were extracted from the National Melanoma Registries, which are part of the Central South Eastern European Melanoma Expert Group and integrated into the European Melanoma Registry (EuMelaReg).

The collection of patient data and its entry into the National Melanoma Registries were approved by the Ethics Committees of the respective institutions from which the data were obtained (Ethics Committee of the Military Medical Academy, Serbia, Number 55/2019 from 04 July 2019; Ethics Committee of the Clinical Center Niš, Serbia, Number 13872/502 from 06 June 2020; Ethics Committee of the University Clinical Center Sarajevo, Bosnia and Herzegovina, Number 03-02-282 from 24 December 2019).

All participants had previously signed informed consent forms allowing their clinicopathological data to be entered into an anonymized melanoma registry and used for research purposes.

Statistical Analysis

The baseline clinicopathological variables were summarized using descriptive statistics.

Calculation of biomarker ratios included absolute cell counts at baseline (within 30 days prior to the initiation of immunotherapy). PIV was calculated as (neutrophil count × platelet count × monocyte count)/lymphocyte count, while SII was calculated as (neutrophil count × platelet count)/lymphocyte count. All baseline absolute cell counts were in 10^9/L.

The cut-off values were calculated using ROC-Curves predicting a survival of ">1 year", choosing the value with the highest distance from the diagonal.

Progression-free survival (PFS) was calculated from treatment initiation to radiologically confirmed disease progression according to RECIST criteria or death from any cause. Participants who started a new therapy before progression/death were censored at the start of the next therapy. Participants without progression/death and no further therapies were censored at the last date they were known to be alive. Overall survival (OS) was calculated from start of treatment until death. Participants not documented as dead were censored at the last date they were known to be alive. Survival curves were plotted by Kaplan-Meier method.

P values were calculated using Fisher's exact test for categorical variables, Student's t-test for continuous variables and Log-Rank-Test for Kaplan-Meier-estimates.

Missing data are presented, but not imputed. P values less than 0.05 were considered as statistically significant.

3. Results

3.1. Clinical Characteristics and Therapy

This study included, 61 females (37.65%) and 101 men (62.35%) with advanced cutaneous melanoma. The mean age at initiation of immunotherapy was 65 years (range 28–96, median 67). The

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most frequent histological subtype was nodular melanoma (54.94%). Median Breslow thickness was 4.45 mm (range 0.4–38 mm) and ulceration was present in 108 patients (66.67%), indicating that more than half of the cohort initially had histopathologically high-risk cutaneous melanoma. BRAF mutation was detected in 63 patients (38.89%). At initial diagnosis, most patients were stage II (55.56%), whereas 7.4% were already stage IV. The mean interval from initial diagnosis to relapse to stage III or IV was 28.1 months (range 0-165.16; median 17.01). Table 1 presents clinicopathologic characteristics at baseline.

Table 1. Baseline clinicopathologic characteristics of the cohort.

Characteristic	N (%)
DEMOGRAPHICS	
Gender	
Male	101 (62.35)
Female	61 (37.65)
Age at the start of immunotherapy	
≤60 years	53 (32.72)
>60 years	109 (67.28)
HISTOPATHOLOGY	
Melanoma subtype	
Superficial spreading melanoma	39 (24.07)
Nodular melanoma	89 (54.94)
Lentigo maligna melanoma	2 (1.23)
Acral lentiginous melanoma	10 (6.17)
Others	22 (13.58)
Breslow thickness	
≤4 mm	63 (38.89)
>4 mm	81 (50)
Unknown/missing	18 (11.11)
Clark level	
II	5 (3.09)
III	25 (15.43)
IV	81 (50)
V	24 (14.81)
Not specified	18 (11.11)
Unknown/missing	9 (5.56)

Characteristic	N (%)
HISTOPATHOLOGY (cont.)	
Ulceration	
Present	108 (66.67)
Absent	31 (19.14)
Unknown/missing	23 (14.20)
Mitotic rate	
≤1	6 (3.70)
>1-2	10 (6.17)
>2-4	23 (14.2)
>4	64 (39.51)
Unknown/missing	59 (36.42)
Tumor infiltrating lymphocytes	
Present	74 (45.68)
Absent	14 (8.64)
Unknown/missing	74 (45.68)
Microsatellites	
Absent	156 (96.30)
Present	6 (3.7)
Lymphovascular invasion	
Present	29 (17.9)
Absent	66 (40.44)
Unknown/missing	67 (41.36)

Characteristic	N (%)
HISTOPATHOLOGY (cont.)	
Perineural invasion	
Present	8 (4.94)
Absent	74 (45.68)
Unknown/missing	80 (49.38)
BRAF mutation status	
Positive	63 (38.89)
Wild type	97 (59.88)
Unknown	2 (1.93)
CLINICAL CHARACTERISTICS	
Initial clinical stage	
I	11 (6.79)
II	90 (55.56)
III	46 (28.4)
IV	12 (7.41)
Unknown	3 (1.85)
Clinical stage at the start of immunotherapy	
III	28 (17.28)
IV	134 (82.72)

Characteristic	N (%)
CLINICAL CHARACTERISTICS (cont.)	
Metastatic sites	
Skin/subcutaneous	51 (31.48)
Lymph node	90 (55.56)
Lungs	82 (50.62)
Liver	44 (27.16)
Bones	17 (10.49)
Brain/CNS	17 (10.49)
Adrenal gland	18 (11.11)
Other	35 (21.6)
LDH level at baseline	
Elevated	31 (19.14)
>2× elevated	26 (16.05)
Normal	100 (61.73)
Unknown/missing	5 (3.09)
S-100 level at baseline	
Elevated	16 (9.88)
Normal	20 (12.35)
Unknown/missing	126 (77.78)
ECOG performance status1	
0	104 (64.2)
1	48 (29.63)
2	2 (1.23)
3	1 (0.62)
Unknown/missing	7 (4.32)

1 ECOG, Eastern Cooperative Oncology Group.

As first-line treatment, 59.88% received pembrolizumab, 36.42% received nivolumab and 3.70% received combination nivolumab/ipilimumab in standard regime. At the start of immunotherapy, most patients had involvement of two or fewer metastatic organ sites (69.75%). The most common sites of metastasis were lymph nodes (55.56%), lungs (50.62%), skin/subcutaneous (31.48%) and liver (27.16%). Central nervous system metastases were present in 17 patients (10.49%). Baseline LDH values were elevated in 57 patients (35.19%). Most patients had an ECOG performance status of 0 or 1 (93.83%). Best overall response to first-line therapy comprised a complete response (CR) in 15.43% of patients; the disease control rate (DCR) was 66.05% and the objective response rate (ORR) 37.04%. Immune-related adverse events were documented in 58 patients.

3.2. ROC analyses and cut-off values of inflammatory biomarkers

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The ROC curves were calculated against OS>1 year vs. OS≤1 year. Baseline cut-offs for inflammatory biomarkers were: NLR 3.748 [area under the curve (AUC) 0.66; p=0.012], PLR 180.741 (AUC 0.614; p=0.003), MLR 0.298 (AUC 0.658; p=0.005), LMR 3.351 (AUC 0.652; p=0.083), SII 763.958 (AUC 0.623; p=0.001) and PIV 277.269 (AUC 0.62; p=0.003).

3.3. Inflammatory Biomarkers and Clinical Characteristics

Patients with baseline NLR, PLR and MLR values above cut-off more frequently experienced liver metastases (45.65% vs. 19.83%, p=0.002 for NLR; 43.75% vs. 20.35%, p=0.004 for PLR; 36.51% vs. 20.88%, p=0.043 for MLR), and elevated serum LDH (54.35% vs. 27.59%, p=0.001 for NLR; 58.34% vs. 25.66%, p<0.0001 for PLR; 46.03% vs. 25.27%, p=0.001 for MLR). These patients were also of older age at the beginning of immunotherapy (median 70 vs. 66 years, p=0.043 for NLR; 70 vs. 65 years, p=0.015 for PLR; 71 vs. 63 years, p=0.002 for MLR), and had unfavorable ECOG score (54.35% vs. 22.41%, p=0.001 for NLR; 50% vs. 23.89%, p=0.004 for PLR; 41.27% vs. 24.18%, p=0.004 for MLR) and lower rate of overall response (ORR) to therapy (19.57% vs. 43.97%, p=0.004 for NLR; 12.5% vs. 46.9, p<0.0001 for PLR; 25.4% vs. 42.86%, p=0.028 for MLR), respectively. Microsatellites were more frequently present in patients with NLR and PLR values above cut-off than in patients with lower NLR and PLR (8.7 vs. 1.72, p=0.055 and 8.33 vs. 1.77, p=0.065). PLR above threshold was also associated with the presence of more than two metastatic sites (41.67% vs. 24.78%, p=0.039), while MLR values above threshold were also associated with elevated serum S-100 (20.63% vs. 3.3%, p=0.002).

When patients were grouped by at least one high ratio (NLR, PLR or MLR), significant association was observed for older age at the start of immunotherapy (median 70 vs. 63 years, p=0.005), presence of liver metastasis (34.94% vs. 19.99%, p=0.033), elevated serum LDH (46.99% vs. 22.79%, p=0.0001) and S-100 (15.66% vs. 3.8%, p=0.039), unfavorable EGOG score (43.37% vs. 18.99%, p=0.003) and ORR (73.49% vs. 51.9%, p=0.006).

Analysis of LMR showed that patients with values above cut-off were younger at the start of immunotherapy (median 64 vs. 70.5 years, p=0.004), less frequently had metastases in liver (21.35% vs. 35.94%, p=0.066), elevated serum LDH (25.84% vs. 45.32%, p=0.002) and S-100 (3.37% vs. 20.31%, p=0.003). Also, these patients less frequently had poor ECOG score (24.72% vs. 40.63%, p=0.051) and more frequently had better ORR (42.7% vs. 25%, p=0.027).

Patients with baseline SII above cut-off more frequently had liver metastases (40.98% vs. 19%, p=0.0034), more than two metastatic sites (39.34% vs. 24%, p=0.053), elevated serum LDH (52.46% vs. 25%, p=0.0002) and S-100 protein (16.39% vs. 5%, p=0.051). These patients were also of older age at the start of immunotherapy (median 69 vs. 65 years, p=0.031), and more frequently had unfavorable ECOG score (45.9% vs. 23%, p=0.002) and poor ORR to therapy (77.05% vs. 55%, p=0.007), respectively.

PIV values above cut-off were associated with elevated LDH (44.45% vs. 24.69%, p<0.0001) and S-100 (16.67% vs. 3.7, p=0.01), unfavorable ECOG score (41.67% vs. 22.22%, p=0.022) and poor ORR (73.61% vs. 56.79%, p=0.042).

3.4. Inflammatory Biomarkers and Survival Analyses

Patients with baseline values below these thresholds had markedly longer median PFS: 11.48 vs. 3.06 months for NLR (p<0.0001); 11.48 vs. 3.95 months for PLR (p=0.0006); 11.18 vs. 4.93 months for MLR (p<0.0001); 13.91 vs. 3.85 months for PIV (p<0.0001). The same pattern was observed for OS, with significantly longer survival in the below cut-off groups: 29.18 vs. 5.03 months for NLR (p<0.0001); 29.87 vs. 6.78 months for PLR (p<0.0001); 49.90 vs. 8.42 months for MLR (p<0.0001); 33.09 vs. 8.42 months for PIV (p<0.0001).

Using a composite indicator, patients were classified as at least one high biomarker if at least one of NLR, PLR, or MLR exceeded its pre-specified cut-off (with the other two below). Compared with the all-low group, at least one high cohort had markedly shorter survival—median PFS 4.21 vs. 16.02 months and OS 8.42 vs. 52.66 months, both p<0.0001.

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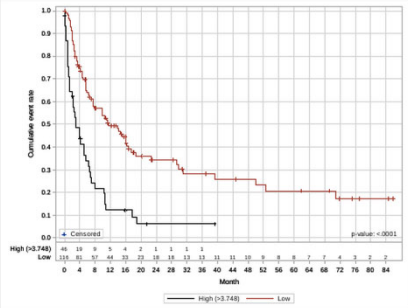
SII values below cut-off were associated with superior median PFS (13.91 vs. 4.21 months, p=0.0083) and OS (29.18 vs. 7.4 months, p=0.0007).

Opposite to NLR, MLR, PLR and SII patients with LMR values below threshold, had poor median PFS (5 vs. 11.18 months, p<0.0001) and OS (8.42 vs. 49.9 months, p<0.0001).

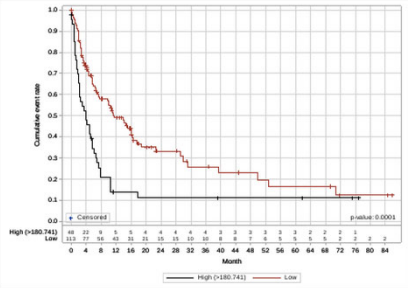
Survival curves are presented by Figures 1 and 2.

Figure 1. Kaplan-Meier curves for progression-free survival (PFS) according to baseline inflammatory biomarkers (ROC-derived cut-offs): (a) NLR (3.748), (b) PLR (180.741), (c) MLR (0.298), (d) LMR (3.351), (e) SII (763.958), (f) PIV (277.269), and (g) all available biomarkers low vs at least one high.

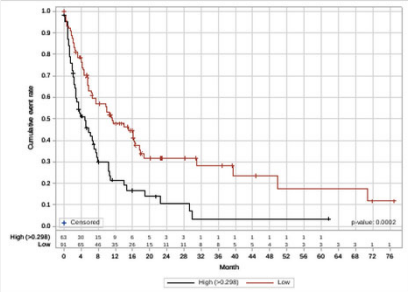
(a) PFS for NLR (cut-off value 3.748)



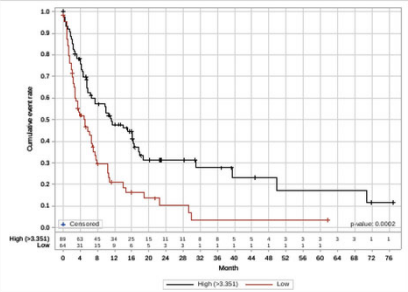
(b) PFS for PLR (cut-off value 180.741)

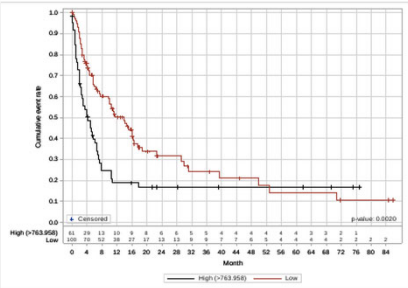


(c) PFS for MLR (cut-off value 0.298)

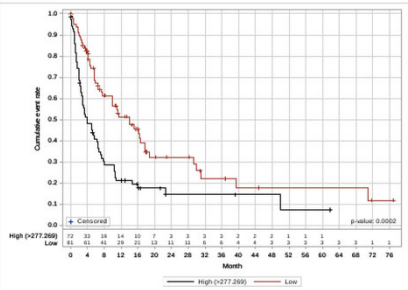


(d) PFS for LMR (cut-off value 3.351)

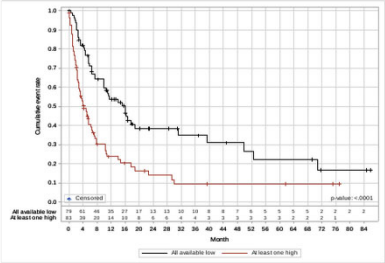




(f) PFS for PIV (cut-off value 277.269)



(g) PFS for all available low versus at least one high inflammatory biomarker



PFS, progression-free survival; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; SII, systemic immune-inflammation index; PIV, pan-immune-inflammation value.

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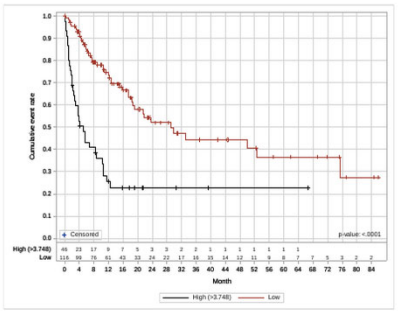
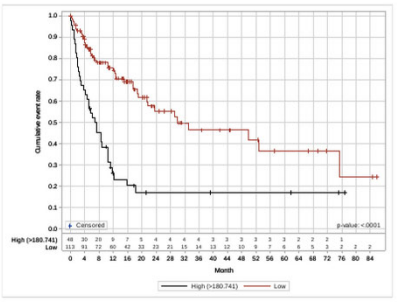
Figure 2. Kaplan–Meier curves for overall survival (OS) according to baseline inflammatory biomarkers (ROC-derived cut-offs): (a) NLR (3.748), (b) PLR (180.741), (c) MLR (0.298), (d) LMR (3.351), (e) SII (763.958), (f) PIV (277.269), and (g) all available biomarkers low vs at least one high.

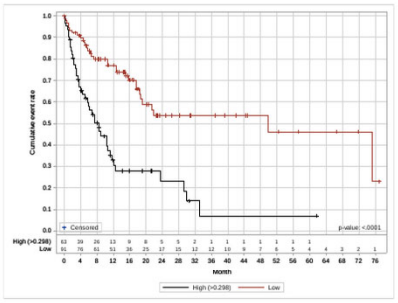
(a) OS for NLR (cut-off value 3.748)

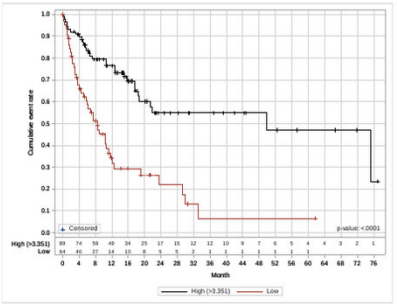


(b) OS for PLR (cut-off value 180.741)

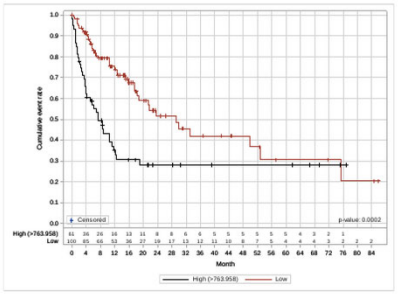


(c) OS for MLR (cut-off value 0.298)

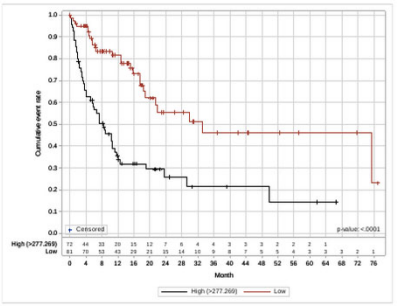




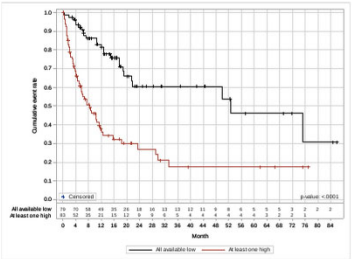
(e) OS for SII (cut-off value 763.958)



(f) OS for PIV (cut-off value 277.269)



(g) OS for all available low versus at least one high inflammatory biomarker



OS, overall survival; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; SII, systemic immune-inflammation index; PIV, pan-immune-inflammation value.

3.5. Cox Regression Analysis

Univariate Cox regression for PFS and OS, respectively, included the following variables: NLR, PLR, MLR, LMR, SII, PIV, gender, age, histologic subtype, Breslow thickness, the presence of ulcerations, lymphovascular and/or perineural invasion, tumor infiltrating lymphocytes, microsatellites before the first line of therapy, *BRAF* status, number of metastatic sites, the presence of CNS and/or liver and/or lung metastases, clinical stage, baseline levels of LDH and S100, ECOG score and prior adjuvant treatment.

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In univariate Cox analysis, higher baseline NLR (p=0.006), PLR (p=0.001), MLR (p<0.0001), PIV (p<0.0001) and SII (p<0.0001), as well as the presence of acral lentiginous melanoma (p=0.003), LDH >2× Upper Limit of Normal (ULN) (p=0.0002) and ECOG score >0 (p=0.002) were associated with shorter PFS. Opposite, higher baseline LMR (p=0.015) and the absence of perineural invasion (p=0.011) indicate better PFS.

For OS, all five indices were likewise significant: NLR (p=0.002), PLR (p<0.0001), MLR (p<0.0001), PIV (p<0.0001) and SII (p<0.0001). Inferior OS was also associated with age over 60 years (p=0.016), the presence of nodular (p=0.028) or acral lentiginous melanoma (p=0.0001), *wild type* BRAF (p=0.019), baseline LDH elevated (p=0.004) or LDH >2× ULN (p<0.0001) and ECOG score >0 (p<0.0001). Similarly to PFS, LMR above cut-off values (p=0.002) and the absence of perineural invasion (p=0.009) indicate better OS. The most impactful variables of the univariable Cox proportional-hazards analyses for PFS and OS are summarized in Tables 2 and 3.

Table 2. Univariable Cox regression analysis for PFS (key variables).

Variable	HR (95% CI)	p-value
NLRa	1.055 (1.015-1.096)	0.0064
PLRa	1.003 (1.001-1.005)	0.0011
LMRa	0.885 (0.803-0.976)	0.0146
MLRa	4.234 (2.188-8.193)	<.0001
PIVa	1.001 (1.000-1.001)	<.0001
SIIa	1.001 (1.000-1.001)	<.0001
Age	1.015 (1.001-1.030)	0.0402
Subtype Acral lentiginous melanoma	3.307 (1.505-7.265)	0.0029
Perineural invasion absent	0.350 (0.156-0.786)	0.0109
LDH Elevated	1.499 (0.940-2.390)	0.0893
LDH >2x Elevated	2.537 (1.551-4.151)	0.0002
ECOG >0	1.879 (1.273-2.774)	0.0015

a continuous variables (per unit increase).

Table 3. Univariable Cox regression analysis for OS (key variables).

Variable	HR (95% CI)	p-value
NLRa	1.071 (1.026-1.118)	0.002
PLRa	1.004 (1.003-1.006)	<.0001
LMRa	0.820 (0.722-0.931)	0.002
MLRa	4.318 (2.152-8.664)	<.0001
PIVa	1.001 (1.001-1.001)	<.0001
SIIa	1.001 (1.001-1.001)	<.0001
Agea	1.029 (1.011-1.048)	0.002
Age >60	1.872 (1.122-3.122)	0.016
Subtype Nodular melanoma	2.024 (1.078-3.803)	0.028
Subtype Acral lentiginous melanoma	5.906 (2.384-14.627)	0.0001
Perineural invasion absent	0.307 (0.127-0.741)	0.009
BRAF Wild-type	1.797 (1.102-2.930)	0.019
LDH Elevated	2.240 (1.291-3.888)	0.004
LDH >2x Elevated	4.001 (2.315-6.913)	<.0001
CNS metastasis absent	0.542 (0.286-1.026)	0.06
Liver metastasis absent	0.639 (0.399-1.024)	0.0624
ECOG >0	2.641 (1.673-4.170)	<.0001

a continuous variables (per unit increase).

In multivariable Cox models fitted separately for each biomarker and adjusted for clinical covariates, MLR (p=0.006) and PIV (p=0.001) retained independent associations with shorter PFS. For OS, PLR (p=0.032) and PIV (p=0.032) remained independently associated with mortality, whereas NLR and MLR were not significant after adjustment. In these adjusted models, additional covariates showed consistent associations: ECOG performance status >0 predicted shorter PFS in the NLR (p=0.013), PLR (p=0.02) and MLR (p=0.016) models. Baseline LDH >2× ULN predicted worse PFS in

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the NLR (p=0.002) and PLR (p=0.011) models, and worse OS in the NLR (p<0.0001), PLR (p=0.001) and MLR (p=0.004) models. For OS, older age was independently associated with higher mortality in the PLR (p=0.009), MLR (p=0.01) and PIV (p=0.002) models. The acral lentiginous subtype conferred higher risk for PFS in the NLR (p=0.043), MLR (p=0.02) and PIV (p=0.01) models, and for OS in the PLR (p=0.023), MLR (p=0.01) and PIV (p=0.013) models. Finally, absence of CNS metastases was associated with better OS in the PLR (p=0.0392), MLR (p=0.024) and PIV (p=0.012) models.

Results of biomarker-specific multivariable Cox regression models for PFS and OS are provided in **Supplementary** Tables S1 and S2.

4. Discussion

In this multicenter, real-world cohort of patients with unresectable stage III/IV cutaneous melanoma receiving first-line immune checkpoint inhibitors (ICIs), baseline inflammatory biomarkers derived from routine complete blood counts showed clinically meaningful and partly independent prognostic associations. On univariable analyses, higher NLR, PLR, monocyte–lymphocyte ratio and PIV were each linked to shorter PFS and OS. After adjustment for clinical covariates in biomarker-specific multivariable Cox models, monocyte–lymphocyte ratio and PIV retained independent value for PFS, while PLR and PIV remained independently associated with OS. These findings support the concept that composite complete blood count–derived markers reflecting the balance between innate (neutrophils, monocytes, platelets) and adaptive (lymphocytes) immunity have been investigated as baseline tools for risk stratification in ICI-treated melanoma, although results across cohorts are not fully consistent [13,22–24].

Our results are consistent with a meta-analysis of melanoma cohorts treated with ICIs (n=3,235), in which elevated baseline NLR and PLR were associated with poorer OS and PFS, supporting the prognostic utility of systemic inflammatory biomarkers at treatment initiation [13].

For PIV, published data in melanoma are not fully concordant. In a retrospective, single-center cohort of metastatic melanoma receiving first-line systemic therapy (including both immunotherapy and targeted therapy), higher baseline PIV was independently associated with shorter PFS and OS, whereas a smaller single-center study restricted to ICI-treated advanced melanoma did not confirm significant associations with response or survival outcomes. A recent meta-analysis across ICI-treated cancers further supported the adverse prognostic impact of elevated PIV on both PFS and OS [22–24].

The observed links between higher inflammatory biomarkers and worse baseline features (higher LDH, poorer ECOG performance status, and visceral disease) are biologically and clinically plausible. LDH is widely used as a marker of tumor burden, but it also reflects a more glycolytic tumor environment with higher lactate production, which can impair effector T-cell function and contribute to weaker antitumor immunity [25,26]. Regarding liver involvement, we interpret it as a feature of high-risk visceral spread rather than a single isolated factor; importantly, a recent multicenter nomogram in unresectable stage IV melanoma treated with first-line anti-PD-1–based therapy included the presence of liver or brain metastases together with LDH, NLR, melanoma subtype and other variables as key determinants of early progression risk [27]. This approach is consistent with current ESMO guidance, which stresses baseline clinical assessment—including LDH, performance status, and the pattern of visceral disease—when choosing and sequencing systemic treatment in advanced melanoma [28].

From a biological perspective, CBC-derived inflammatory markers have a clear immune rationale. Neutrophils can support tumor progression and, when highly activated, may form neutrophil extracellular traps (NETs) that facilitate immune evasion and have been linked to resistance mechanisms relevant to anticancer therapies, including immunotherapy [29]. Platelets are also active players in cancer biology: they can enhance tumor growth signals, contribute to angiogenesis/vascular remodeling, and promote metastatic spread and early metastatic niche formation [30–33]. In contrast, the circulating lymphocyte compartment can be viewed as a practical surrogate of systemic immune competence, and lower lymphocyte levels have been associated with worse outcomes in immunotherapy-treated melanoma cohorts [34]. At the tumor level, tumor-

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infiltrating lymphocytes (TILs) are consistently associated with improved survival and are increasingly discussed in the context of treatment selection and response in the ICI era[35]. Taken together, it is therefore expected that higher indices weighting innate components (neutrophils/monocytes/platelets) relative to lymphocytes—such as NLR, PLR, MLR (as defined in our study) and especially PIV—correspond to a more immunosuppressive milieu and worse clinical outcomes [29–35].

Clinically, these markers are acceptable because they are inexpensive, readily available, and reproducible. In our cohort, PIV emerged as the most consistently independent predictor across endpoints, suggesting potential utility as a primary stratifier, while MLR (for PFS) and PLR (for OS) may provide additional prognostic information.

Prospectively, further research should address this study limitations by: validating the observed effects of PIV, NLR, PLR, MLR and LMR in independent cohorts using pre-specified cut-offs; evaluating on-treatment dynamics of these biomarkers (e.g., during the first two cycles) and their predictive value; integrating inflammatory biomarkers with LDH, ECOG performance status, sites of disease (particularly liver/CNS), and molecular features into multivariable nomograms for prediction of treatment response; testing whether high-risk inflammatory profiles identify patients more likely to benefit from intensified combination regimens versus monotherapy; and combining inflammatory biomarkers with circulating tumor DNA and cell-free DNA in multivariable nomograms to improve predictive performance.

5. Conclusions

Baseline inflammatory biomarkers derived from routine blood counts provide independent and accessible prognostic information at initiation of first-line ICI for advanced melanoma. When interpreted alongside LDH, ECOG status and metastatic pattern, these biomarkers can refine initial risk stratification and treatment individualization. Prospective validation with pre-specified thresholds and external cohorts is needed before clinical implementation.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Conflicts of Interest: A.P. received payment/honoraria for lectures/educational events from MSD, Roche, Novartis and BMS, and won ICF/ESMO fellowship grant. M.B. received payment/honoraria for lectures/educational events from MSD, Roche, Novartis and BMS. I.S. was supported to attend meetings by MSD. J.P. received payment/honoraria for presentations/educational events from Novartis and support for attending meetings by Amicus, Medison. P.S. is employed at Alcedis GmbH, a CRO with contracts to several pharmaceutical companies and EUMelaReg gGmbH. T.M. received payment/honoraria for lectures/educational events from Servier, Merck, BMS, AstraZeneca, Medis and Medison. L.K. is a member of the advisory boards of MSD, Novartis and Medison, received payment/honoraria for lectures/educational events from MSD, BMS, Immunocore, Novartis and Abbvie, and support for attending meetings and/or travel from Abbvie and MSD. All of the other authors have no declarations of interest.

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