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der Universität zu Köln
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Prognostic value of routinely collected inflammatory blood parameters in stage III melanoma patients with microscopic sentinel lymph node metastasis

Inaugural-Dissertation zur Erlangung der Doktorwürde
der Medizinischen Fakultät
der Universität zu Köln

vorgelegt von
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promoviert am 21.Mai 2025

Gedruckt mit Genehmigung der Medizinischen Fakultät der Universität zu Köln
2025

Dekan: Universitätsprofessor Dr. med. G. R. Fink

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Frau Cindy Franklin
Frau Cornelia Mauch
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Die für diese Arbeit benötigten Daten sowie die der Arbeit zugrunde liegende Publikation wurden von mir gemäß den entsprechenden Anweisungen von Cindy Franklin erhoben und verfasst. Die Analyse und Auswertung der Daten erfolgte durch mich mit Unterstützung von Cindy Franklin, Susanne Horn und Guillermo Hidalgo-Gadea unter Verwendung der angegebenen Programme.

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Danksagung

Mein besonderer Dank gilt PD Dr. Cindy Franklin für die Überlassung des Themas, ihre ausgezeichnete Betreuung sowie ihre große Unterstützung während des gesamten Arbeitsprozesses.

Ebenso danke ich ausdrücklich allen Patientinnen und Patienten, die durch ihre Teilnahme einen wesentlichen Beitrag zu dieser Studie geleistet haben.

Ich bin sehr dankbar allen Mitarbeitenden der Klinik für Dermatologie und Venerologie der Uniklinik Köln für ihre Unterstützung bei der Datenerhebung.

Mein tiefer Dank gilt auch meinen Eltern, meiner Schwester, meinen Brüdern, meinem Partner Jonas Zimmermann sowie meinen guten Freunden Hanna Pietschmann, Greta Steinmetz und Tobias Esser für ihre unermüdliche Unterstützung und Ermutigung.

Inhaltsverzeichnis

Abkürzungsverzeichnis

AJCC classification	American Joint Committee on Cancer classification
AUC	area under the curve
CRP	C-reactive protein
CTLs	cytotoxic T cells
DFS	disease-free survival
dNLR	derived neutrophil-to-lymphocyte ratio
HR	hazard ratio
ICI	immune checkpoint inhibitor
ICIs	immune checkpoint inhibitors
LDH	lactate dehydrogenase
LMR	lymphocyte-to-monocyte ratio
MALT lymphoma	mucosa-associated lymphoid tissue lymphoma
MSS	melanoma-specific survival
NLR	neutrophil-to-lymphocyte ratio
OS	overall survival
PFS	progression-free survival
PLR	platelet-to-lymphocyte ratio
RFS	recurrence-free survival
ROC	receiver operating characteristic
SLN	sentinel lymph node
SLNB	sentinel lymph node biopsy
Th cells	T helper cells
TME	tumor microenvironment
TNF-α	tumor necrosis factor-α
TNM classification	Tumor-, Nodes- and Metastases-classification
Tregs	regulatory T cells
VEGF	vascular endothelial growth factor
WBC	white blood count

1. Zusammenfassung

Adjuvante Therapien mit Immun-Checkpoint-Inhibitoren (ICIs) und BRAF/MEK-Inhibitoren verbessern das rezidivfreie Überleben von Patienten mit malignem Melanom im Stadium III signifikant. Allerdings können sie schwerwiegende Nebenwirkungen verursachen. Daher ist es von entscheidender Bedeutung, Patienten mit einem hohen Rezidivrisiko zu identifizieren, die von diesen adjuvanten Therapien tatsächlich profitieren könnten ist. Ziel der vorliegenden Studie war es, das prognostische Potenzial routinemäßig erhobener Blutparameter bei Patienten mit malignem Melanom im Stadium III und mikroskopischen Metastasen im Sentinel-Lymphknoten (SLN) zu evaluieren.

Im Rahmen dieser retrospektiven Studie wurden 138 Patienten mit malignem Melanom im Stadium III, die zwischen 2011 und 2020 am Hautkrebszentrum der Uniklinik Köln mit mikroskopisch detektierbaren SLN-Metastasen diagnostiziert wurden und zuvor keine adjuvante Therapie mit ICIs oder BRAF/MEK-Inhibitoren erhalten hatten, analysiert.
Die Auswertung erfolgte mittels univariater und multivariater Cox-Regressionsanalysen, Kaplan-Meier-Überlebensanalysen sowie Receiver Operating Characteristic (ROC)-Kurven. Im Fokus der Untersuchung standen Blutparameter, die bis zu 15 Tage vor der Sentinel-Lymphknotenbiopsie erhoben wurden, sowie deren Verhältnisse untereinander hinsichtlich ihres Einflusses auf das rezidivfreie Überleben und Gesamtüberleben. Das rezidivfreie Überleben stellte dabei den primären Endpunkt dar.

Die multivariate Analyse ergab, dass ein hohes Neutrophil-Lymphozyten-Verhältnis (NLR), ein niedriges Lymphozyten-Monozyten-Verhältnis (LMR) und ein hohes C-reaktives Protein (CRP) signifikant mit einem kürzeren rezidivfreien Überleben assoziiert waren. Der Effekt blieb auch nach der Dichotomisierung für die LMR (Cut-off 3,5) und den CRP-Wert (Cut-off 3,0) bestehen, jedoch nicht für die NLR (Cut-off 3,5). Bemerkenswerterweise zeigte der CRP-Wert eine noch stärkere Assoziation mit dem rezidivfreien Überleben als die NLR oder die LMR (sowohl als kontinuierlicher Wert als auch dichotomisiert). Die signifikanteste Assoziation mit der größten Aussagekraft im Hinblick auf ein Rezidiv wurde für die Kombination von einer niedrigem LMR (< 3,5) und einem hohem CRP-Wert (< 3,0) beobachtet. Darüber hinaus war eine abgeleitete (derived) NLR (dNLR) ≥ 2,0 in der multivariaten Analyse signifikant mit einem kürzeren Gesamtüberleben assoziiert.

In der Gesamtschau meiner Studienergebnisse lässt sich ableiten, dass die Kombination von CRP und LMR als potenzieller prognostischer Marker für das rezidivfreie Überleben von Melanom-Patienten im Stadium III mit mikroskopischen SLN-Metastasen dienen kann. Um

meine Ergebnisse zu validieren, sind jedoch weitere Studien in größeren, prospektiv gesammelten Patientenkohorten erforderlich.

2. Einleitung

2.1. Epidemiologische und klinische Aspekte des malignen Melanoms

Malignant melanoma is a type of skin cancer that results from the uncontrolled proliferation of melanocytes. It has become a significant public health concern worldwide as its incidence is increasing. Despite new effective therapeutic options, it remains the leading cause of death by skin cancer [1]. Its incidence has also increased in Germany. However, it should be noted that this increase in melanoma cases is most likely also due to earlier detection of melanoma after the introduction of a regular skin cancer screening in 2008. Therefore, since 2008, the incidence of early-stage melanoma has increased significantly, but the rate of advanced melanoma has not increased substantially [2].

Clinically and histologically, there are different subtypes of melanoma with the main types being: superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acrolentiginous melanoma, amelanotic melanoma, and desmoplastic melanoma [3]. Malignant melanoma is most commonly found on the trunk (33%), followed by the upper (24%) and lower extremities (22%). There are also gender differences in the location of melanoma. In men, 42% of melanomas occur on the trunk and only 12% on the lower extremities. The reverse is true for women—their lower extremities are more commonly affected (32%) than their trunk (24%) [2]. According to the latest American Joint Committee on Cancer (AJCC) classification (8^{th} edition), there are four stages of melanoma: melanoma with low to moderate risk of metastasis (stage I), high risk melanoma without detectable metastases at initial diagnosis (stage II), melanoma with locoregional metastases at initial diagnosis (stage III) and melanoma with distant metastases at initial diagnosis (stage IV). The 5-year melanoma-specific survival rate in a large American analysis was 97%–99% in stage I and 82%–94% in stage II. For stage III melanoma, the 5-year survival rates were 93% (stage IIIA), 83% (stage IIIB), 69% (stage IIIC), and 32% (stage IIID). Survival rates for stage IV melanoma were not assessed in that study [4].

In recent years, several routinely available blood-based parameters, such as the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and C-reactive protein (CRP) have been associated with prognosis in various cancers. However, their relevance in the context of stage III malignant melanoma with microscopic SLN metastases remains unclear. This study aims to address this gap.

2.2. Wissenschaftlicher Hintergrund

2.2.1. Diagnostik und Therapie des malignen Melanoms

At the time of initial diagnosis, a complete surgical excision of the melanoma is performed, along with a histopathological evaluation to determine both the Breslow thickness and the presence of primary ulceration. These characteristics of the primary tumor are used to determine the T-classification value according to the Tumor-, Nodes- and Metastases- (TNM) classification system. For melanomas with a Breslow thickness of 1 mm or greater, a sentinel lymph node biopsy (SLNB) is advised in addition to wide local excision of the primary tumor. To locate the sentinel lymph node (SLN), blue dye, a radioactive substance, or a combination of both is injected next to the previous melanoma. A probe is then used to detect the highest concentration of the substance in nearby lymph nodes. The lymph node with the strongest signal is identified as the SLN, the first node to which the tumor drains. After its removal, the SLN is examined for the presence of metastatic cells [1]. The detection of regional lymph node metastases, and thus the N-classification, plays a critical role in determining disease progression in newly diagnosed melanoma patients [4,5]. The N-status ranges from N0 to N3c, depending on the number of lymph nodes involved, whether the metastases are clinically occult (microscopic) or clinically detectable (macroscopic), and the potential presence of microsatellite, satellite, or in-transit metastases [6]. After complete (R0) resection of the primary tumor and lymph node metastases, adjuvant therapy is recommended in most patients. Randomized clinical trials have shown improved recurrence-free survival (RFS) in stage III melanoma patients who receive adjuvant systemic therapies, such as immune checkpoint inhibitors (ICIs) or targeted therapies like BRAF/MEK inhibitors. However, many patients do not benefit from adjuvant treatment, either due to primary or acquired resistance or because they may not have developed metastases in the first place without the treatment [7,8]. As a result, these patients are unnecessarily exposed to potential therapy-related side effects, including inflammation of various organ systems [9,10]. Therefore, additional parameters are needed to identify patients at risk for recurrence and progression of stage III melanoma. The pursuit of personalized and effective therapeutic strategies requires an increased focus on the identification of valuable parameters that facilitate the early detection of individuals who are likely to experience relapse and disease progression and who would benefit most from systemic therapies.

2.2.2. Entzündungsmarker bei Melanompatienten

The role of systemic inflammation in cancer development and progression is increasingly recognized [11], prompting investigations on the potential utility of specific inflammatory blood values for predicting survival outcomes. Several cell types, such as neutrophils, monocytes and platelets play a role in releasing cytokines that promote tumor growth, including tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF) [12].

Especially the neutrophil-to-lymphocyte ratio (NLR) has gained recognition in the literature as a strong prognostic marker, showing relevance for predicting outcomes such as overall survival (OS), recurrence-free survival (RFS), and progression-free survival (PFS) across a variety of solid tumors [13,14]. In the context of melanoma, the potential of the NLR as a prognostic indicator at various tumor stages has been studied by different groups [4,15-18]. Furthermore, additional blood cell ratios, including the lymphocyte-to-monocyte ratio (LMR) [17,19], platelet-to-lymphocyte ratio (PLR) [20,21], and derived neutrophil-to-lymphocyte ratio (dNLR) [22,23], along with serological markers of inflammation such as C-reactive protein (CRP), have been explored for their potential to predict disease progression and survival outcomes. These studies have mainly focused on advanced melanoma patients [24,25].
Although many studies have demonstrated links between these blood parameters and factors like tumor burden, recurrence, or survival, the findings are not always consistent. This is particularly true in early-stage melanoma, where there is still no agreement on which blood markers offer additional insights beyond well-established prognostic indicators such as tumor stage and lymph node involvement [15,16,18,23,26]. As a consequence, it remains uncertain which inflammatory blood markers hold the greatest prognostic significance, particularly for patients with stage III melanoma. Resolving this uncertainty is of importance to refine prognostic assessments and to be able to tailor therapeutic strategies to the specific needs of each individual patient.

2.3. Fragestellungen und Ziel der Arbeit

The present study aimed to examine different blood variables on their ability to predict recurrence-free survival (RFS) and overall survival (OS) specifically in stage III melanoma patients with microscopically detectable sentinel lymph node (SLN) metastasis. For this purpose, I collected and analyzed the data from 138 consecutive patients with stage III melanoma and microscopically detectable SLN metastasis, who underwent SLNB at the Department of Dermatology and Venerology, University Hospital Cologne, between January 2011 and December 2020.

In this study, I wanted to address the following hypothesis:
The level of specific immunological blood values is associated with shorter recurrence-free (RFS) and/or overall survival (OS) in patients with malignant melanoma with microscopic sentinel lymph node metastasis (stage III melanoma).

3. Material, Methoden und Ergebnisse

This part is covered by the publication "C-Reactive Protein and Lymphocyte-to-Monocyte Ratio Predict Recurrence in Stage III Melanoma Patients with Microscopic Sentinel Lymph Node Metastasis" [27].

3.1. Publikation

cancers

MDPI

Article

C-Reactive Protein and Lymphocyte-to-Monocyte Ratio Predict Recurrence in Stage III Melanoma Patients with Microscopic Sentinel Lymph Node Metastasis

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Simple Summary: Indicators of a potential recurrence of melanoma in patients after the detection of lymph node metastasis are needed in order to not treat patients unnecessarily with a systemic therapy. Blood parameters such as the number of white blood cells and ratios of different white blood cell subtypes are collected in the clinical routine and could be useful indicators of a possible disease relapse. The aim of our present study was to identify blood parameters which predict the recurrence of melanoma in melanoma patients with microscopic sentinel lymph node metastasis. We identified the lymphocyte-to-monocyte ratio (LMR) and C-reactive protein (CRP) to be the strongest predictors for melanoma recurrence.

Abstract: Although adjuvant therapies with immune checkpoint inhibitors (ICI) and BRAF/MEK inhibitors improve recurrence-free survival (RFS) in stage III melanoma patients significantly, prognostic factors are needed to identify patients with a high risk of disease recurrence. Therefore, the aim of our study was to investigate the prognostic potential of routinely collected blood parameters for stage III melanoma patients with microscopic sentinel lymph node (SLN) metastasis. Altogether, we retrospectively analyzed 138 stage III melanoma patients who were diagnosed with microscopic SLN metastasis at the skin cancer center of the University Hospital Cologne between 2011 and 2020 and who did not receive prior adjuvant therapy with ICI or BRAF/MEK-inhibitors. Univariate and multivariate Cox regression analyses, Kaplan–Meier survival analyses and receiver operating characteristic (ROC) curves were performed to assess the impact of preoperatively collected blood parameters and blood ratios on recurrence-free survival (RFS; primary endpoint) and overall survival (OS). A high neutrophil-to-lymphocyte ratio (NLR), low lymphocyte-to-monocyte ratio (LMR) and high C-reactive protein (CRP) value were significantly associated with shorter RFS in multivariate analysis. For LMR (cut-off 3.5) and for CRP (cut-off 3.0) this effect remained after dichotomization. CRP showed a stronger association with RFS than NLR or LMR, with the highest association being detected for the combination of low LMR and high CRP. Additionally, derived NLR $\geq$ 2.0 was significantly associated with shorter OS in multivariate analysis. In summary, our data suggest that CRP in combination with LMR should be considered as a marker for melanoma recurrence in stage III melanoma patients with microscopic SLN metastasis.

Keywords: C-reactive protein (CRP); lymphocyte-to-monocyte ratio (LMR); neutrophil-to-lymphocyte ratio (NLR); derived NLR; platelet-to-lymphocyte ratio (PLR); melanoma; recurrence-free survival; sentinel lymph node metastasis; overall survival

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Citation: Schildbach, V.A.S.; Horn, S.; Hidalgo-Gadea, G.; Johannis, W.; Mauch, C.; Franklin, C. C-Reactive Protein and Lymphocyte-to-Monocyte Ratio Predict Recurrence in Stage III Melanoma Patients with Microscopic Sentinel Lymph Node Metastasis. *Cancers* **2023**, *15*, 702. https://doi.org/10.3390/cancers15030702

Academic Editor: Ellen Kapiteijn

Received: 12 December 2022
Revised: 16 January 2023
Accepted: 18 January 2023
Published: 23 January 2023

1. Introduction

Melanoma accounts for 4.5% of all cancer diagnoses in Germany, with a rising incidence worldwide [1]. Despite a number of new therapeutic options, it remains the leading cause of death by skin cancer. At first diagnosis, melanoma is excised completely with histopathological determination of Breslow thickness and ulceration of the primary. For melanoma with a Breslow thickness $\geq$1 mm, a sentinel lymph node biopsy (SLNB) is recommended in addition to wide local excision. To identify the sentinel lymph node (SLN), the surgeon injects a radioactive substance, blue dye or both, at the site of the primary melanoma. After resection of the SLN, it is histopathologically assessed for the presence of lymph node metastases [2].

The presence of regional lymph node metastases is an important prognostic factor for disease progression in newly diagnosed melanoma [3,4]. For patients with loco-regionally metastasized melanoma (stage III melanoma), 5-year melanoma-specific survival ranges from 93% (stage IIIA) to 83% (stage IIIB), 69% (stage IIIC) and 32% (stage IIID) compared to 98% in stage I melanoma patients [5]. Metastases, which are only detectable by histopathological analysis, but not clinically, are defined as microscopic SLN node metastases.

Prospectively randomized trials showed improved recurrence-free survival (RFS) in stage III melanoma patients with adjuvant systemic therapy with immune checkpoint inhibitors (ICI) or BRAF/MEK targeted therapy. Still, a large number of patients do not benefit from adjuvant therapy, either because of primary or secondary resistance to adjuvant treatment or because they would not have developed metastases even without adjuvant treatment [6,7]. These patients unnecessarily endure the risks of therapy-related adverse events such as the inflammation of organ systems. Therefore, parameters which allow the identification of patients at risk for recurrence and progression are needed.

Systemic inflammation plays an important role in cancer development and progression [8]. This opens up the possibility of using specific inflammatory blood values to predict survival outcome. Amongst other cell types, neutrophils, monocytes and platelets secrete pro-tumorigenic cytokines such as tumor necrosis factor-α and vascular endothelial growth factor [9]. As the neutrophil-to-lymphocyte ratio (NLR) is a prognostic factor for overall (OS), recurrence-free (RFS) and progression-free survival (PFS) in different solid tumors [10,11], several studies investigated its role as a prognostic factor for melanoma in different tumor stages [12–16]. Additionally, further blood cell ratios such as the lymphocyte-to-monocyte ratio (LMR) [14,17], the platelet-to-lymphocyte ratio (PLR) [18,19] and the derived neutrophil-to-lymphocyte ratio (dNLR) [20,21], as well as serological inflammatory parameters (e.g., the acute phase reactant C-reactive protein; CRP) have been assessed for their ability to predict progression and survival in melanoma patients [22,23]. While many studies found associations between these blood parameters with disease recurrence, tumor load or survival, there are also contradictory results. Especially in localized melanoma stages, there is no consensus on which blood cell variables may add information to the established prognostic parameters (such as tumor stage) [12,15,16,21,24]. At present, it is therefore unclear which inflammatory blood parameters have the highest prognostic value, specifically in stage III melanoma patients with microscopic SLN metastasis.

The aim of our present study was to identify specific blood values (leukocyte count, neutrophil count, CRP) and blood ratios (NLR, dNLR, LMR) that correlate with RFS and OS in melanoma patients with microscopic SLN metastasis and to determine optimal cut-off values to facilitate their use in clinical routine. Moreover, we wanted to assess which of these markers have the highest prognostic potential and could help to identify patients at risk of melanoma recurrence who should be monitored more closely and receive adjuvant therapy.

2. Materials and Methods

2.1. Study Design

Patients with cutaneous melanoma who received a SLNB with histologically, but not clinically, detectable (microscopic) lymph node metastasis between January 2011 and

2. Materials and Methods

2.1. Study Design

Patients with cutaneous melanoma who received a SLNB with histolc clinically, detectable (microscopic) lymph node metastasis between Januar cember 2020 at the skin cancer center of the Department of Dermatology a Hospital Cologne were identified from the electronic database. Of 183 pat we excluded those who received adjuvant treatment with PD-1- or BRAF/M Patients who received adjuvant interferon-α (32.6%), however, were incl we identified 138 melanoma patients who received a SLNB at our departm receive adjuvant therapy with PD-1- or BRAF/MEK-inhibitors. These w our final analysis (see Figure 1).

December 2020 at the skin cancer center of the Department of Dermatology at the University Hospital Cologne were identified from the electronic database. Of 183 patients identified, we excluded those who received adjuvant treatment with PD-1- or BRAF/MEK-inhibitors. Patients who received adjuvant interferon-α (32.6%), however, were included. Thus, we identified 138 melanoma patients who received a SLNB at our department and did not receive adjuvant therapy with PD-1- or BRAF/MEK-inhibitors. These were included in our final analysis (see Figure 1).

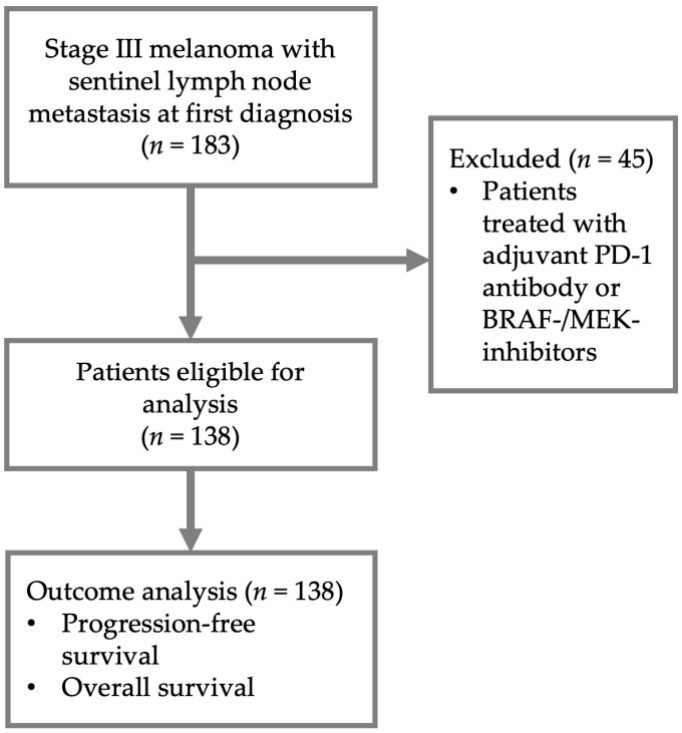

Figure 1. Study flow. 183 patients with stage III melanoma with lymphoscintigraphic sent metastasis were identified at the Department of Dermatology at the University Hospital Cologne. Of these, 138 patients were eligible for analysis.

Figure 1. Study flow. 183 patients with stage III melanoma with lymphoscintigraphic sentinel metastasis were identified at the Department of Dermatology at the University Hospital Cologne. Of these, 138 patients were eligible for analysis.

Patient and tumor characteristics (age, immunosuppressive co-medication, tumor stage, presence of an ulceration of the primary tumor, number of affected SLN and maximal size of SLN metastasis, capsule invasion by the metastasis), blood test results of blood samples drawn within 15 days before SLNB and the overall course of the disease (adjuvant treatment, recurrence, progression, survival) were collected from clinical records of the Department of Dermatology and Venereology at the University Hospital Cologne. The following blood ratios were calculated from the blood test results already provided: NLR, dNLR, LMR and PLR. These ratios were calculated from absolute blood values as follows: NLR = absolute neutrophil count/absolute lymphocyte count, dNLR = absolute neutrophil count/(absolute leukocyte count − absolute neutrophil count), LMR = absolute lymphocyte count/absolute monocyte count and PLR = absolute platelet count/absolute lymphocyte count. Laboratory analyses took place at the Institute for Clinical Chemistry at the University Hospital Cologne. The complete blood counts were performed on an automated

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and performing leukocyte differentiation. CRP values were measured on a Cobas c702 analyzer (Roche Diagnostics) issuing a latex-enhanced immunoturbidimetric assay. The analytical measurement range was 0.3–350.0 mg/L. The primary study endpoint was RFS and secondary endpoint was OS. The data cut point was 1 January 2022 and events after this date were censored in later survival analyses (right-censoring). This research was approved by the Ethics Committee of the University of Cologne (approval no. 20-1584).

2.2. Statistical Analysis

We performed univariate and multivariate Cox proportional hazards regression analyses, Kaplan–Meier survival analyses and ROC-curves to assess the impact of routinely collected blood parameters and their respective ratios on RFS and OS.

In an initial explorative analysis, we checked for intercorrelations among our clinical variables before constructing the multivariate Cox regression model. For the considered blood parameters, we conducted a manual parameter selection following a set of clinical and statistical exclusion criteria to ensure parsimony of the multivariate model and to reduce multicollinearity. These exclusion criteria were as follows: (1) blood values were excluded if they were not significant predictors of RFS or OS at an alpha level of 0.05 in univariate or multivariate Cox regression analysis; (2) blood values with missing data in >25% of the blood sample, i.e., less than 100 reported cases, were excluded to avoid a reduction in the sample size; (3) whenever absolute blood values were provided, we excluded relative blood values, as absolute counts are suggested to provide better diagnostic support [25]; (4) for blood parameters with high intercorrelations (pairwise Pearson $r \geq 0.7$), the parameter with the weaker effect on RFS or OS was dropped, respectively. The following parameters were then included in the multivariate Cox regression analysis: age, tumor stage, presence of capsule invasion, adjuvant therapy with interferon-α, diameter of the largest SLN metastasis, as well as, respectively, one of the blood parameters selected above (NLR, dNLR, LMR, CRP; absolute leukocyte count, absolute neutrophil count).

To facilitate the clinical use of the recommended diagnostic blood parameters, we determined cut-off values by contrasting sensitivity and specificity from ROC curves for RFS and OS, respectively. For cases in which cut-off points were not immediately apparent, we evaluated the effects of different values using Kaplan–Meier estimates, as well as their effects in the univariate and multivariate Cox proportional hazards analysis.

Median follow-up time was calculated as time from first diagnosis until last patient contact or in case of death, until cut-off date. RFS was defined as time from first diagnosis until disease recurrence (locoregional or distant metastasis) or last patient contact (censored RFS) and OS as time from first diagnosis until death or last patient contact (censored OS). Differences in Kaplan–Meier estimates were assessed by two-sided log-rank test. p-values < 0.05 were considered statistically significant. Patients with missing data were excluded from the respective analyses. All statistical analyses were performed with IBM SPSS Statistics 27.

3. Results

3.1. Patient and Disease Characteristics

Of the 138 patients eligible for analysis, 83 patients (60%) were older than 65 years. The median age at the first diagnosis was 59 years. Seventy-one patients (51%) were male. Median follow-up time was 53.4 months (interquartile range: 28.5–70.3) after first diagnosis. According to AJCC 8th edition, 36 patients (26%) had stage IIIA melanoma at first diagnosis, 32 (23%) stage IIIB and 70 patients (51%) stage IIIC. No cases with stage IIID were recorded. Forty-five patients (33%) received adjuvant interferon-α therapy. Median Breslow thickness was 3.05 mm and ranged from 0.82 to 16.00 mm. Sixty-four primary melanomas (46%) were ulcerated and ninety-five (69%) had a mitosis rate > 1/mm^2. The number of SLN metastases ranged from one lymph node metastasis (108 patients, 78%) to two (22 patients, 16%) and up to three lymph node metastases (8 patients, 6%), with the median size of the biggest metastasis being 1.25 mm and 11% showing capsule invasion.

Median RFS time was 1.9 years, with a minimum time of 51 days and a maximum of 9.6 years. Seventy-six patients (55%) showed tumor progression within the study period. Of these, sixty-one (80%) developed distant metastases and thereby showed a stage shift from stage III to IV. For OS, the median survival time was 4.5 years with a minimum of 51 days and a maximum recorded time of 9.9 years. Forty-three patients (31%) died during the observed study period. For an overview of patient and survival characteristics see Table 1; for further disease characteristics, see Table S1.

Table 1. Baseline characteristics and patient outcome.

Patient Characteristics and Outcome	All Patients
	***n* = 138 (100%)**
Age	
≤65 years	83 (60.1)
>65 years	55 (39.9)
Median in years (IQR) *	59 (45.0–72.0)
Gender	
Male	71 (51,4)
Female	67 (48.6)
Adjuvant interferon-α	
No	92 (66.7)
Yes	45 (32.6)
Unknown	1 (0.7)
AJCC-stage	
IIIA	36 (26.1)
IIIB	32 (23.2)
IIIC	70 (50.7)
IIID	/
Recurrence (locoregional and/or distant)	
No	62 (44.9)
Yes	76 (55.1)
-only locoregional	15 (19.7)
-locoregional and distant	61 (80.2)
Death	
No	95 (68.8)
Yes	43 (31.2)
Recurrence-free survival	
Median in months (IQR)	23.3 (8.0–56.9)
Overall survival **	
Median in months (IQR)	53.4 (28.5–70.3)

* IQR = Interquartile range (Q1–Q3). ** Censored values: 95 patients lived longer than 10 years.

3.2. Exploratory Analysis

Univariate Cox proportional hazards analyses revealed multiple parameters that were significantly associated with shorter RFS: age > 65 years (HR = 1.985, 95%CI = 1.261–3.125, $p = 0.003$), largest diameter of the SLN metastasis > 1 mm (HR = 1.849, 95%CI = 1.139–3.004, $p = 0.013$), the presence of an ulceration in the primary tumor (HR = 2.390, 95%CI = 1.505–3.794, $p < 0.001$) and AJCC-stage IIIC versus stage IIIA (HR = 4.303, 95%CI = 2.114–8.759, $p < 0.001$). There was no significant difference for AJCC-stage IIIB compared to stage IIIA. Patients who received a CLND had a higher risk of recurrence in the univariate analysis (HR = 1.710, 95%CI = 1.072–2.729, $p = 0.024$). Figure 2 (RFS) and Figure S1 (OS) show Kaplan–Meier survival curves of some important variables.

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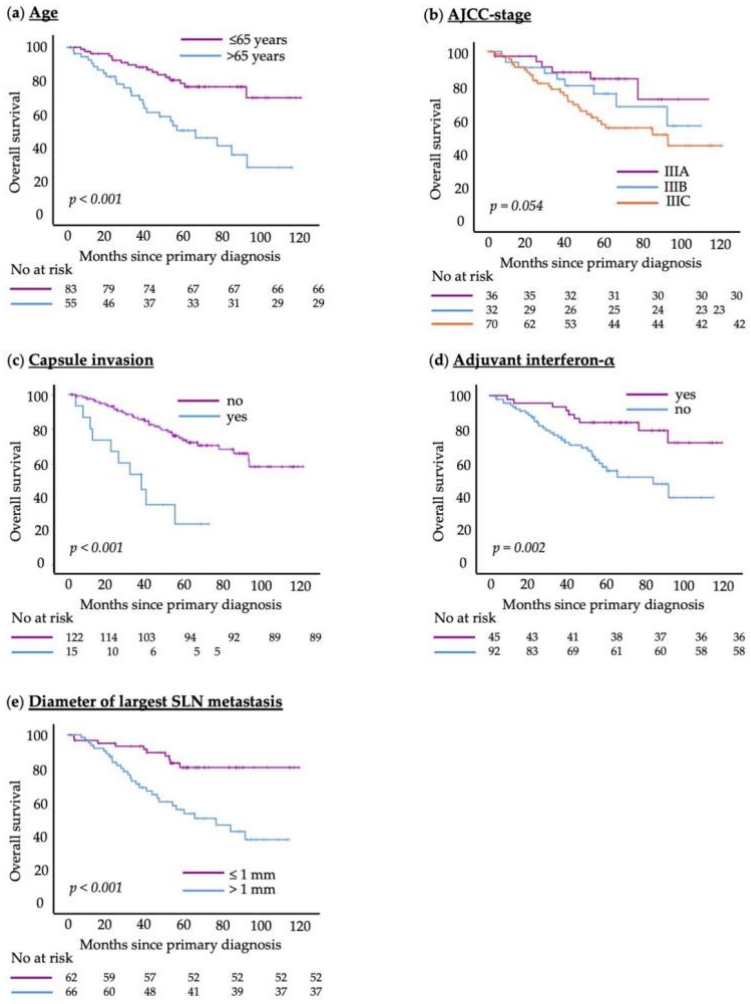

Figure 2. Kaplan–Meier survival curves showing recurrence-free survival for covariates of the multivariate Cox regression model: (**a**) patient age; (**b**) AJCC stage; (**c**) capsule invasion of SLN metastasis; (**d**) adjuvant interferon-α therapy; (**e**) diameter of largest SLN metastasis. The log-rank test was used to compare between groups; $p < 0.05$ was considered significant.

Similarly, in the univariate analysis, the following parameters were associated with shorter OS: age > 65 years (HR = 3.060, 95%CI = 1.657–5.650, $p < 0.001$), higher AJCC-stage (HR = 2.685, 95%CI = 1.111–6.490, $p = 0.028$, specifically, stage IIIC versus stage IIIA), largest diameter of the SLN metastasis (HR = 3.060, 95%CI = 1.657–5.650, $p < 0.001$), higher and the presence of ulceration of the primary tumor (HR = 2.044, 95%CI = 1.108–3.770, $p = 0.022$). Capsule invasion of the SLN by the metastasis (HR = 4.394, 95%CI = 2.120–9.109, $p < 0.001$) was also associated with significantly decreased OS. Patients who received adjuvant interferon-α therapy (HR = 0.329, 95%CI = 0.156–0.692, $p = 0.003$) had a significantly lowered risk of death and therefore a better OS in the univariate analysis.

Gender, type of melanoma, number of affected lymph nodes in SLNB,

Gender, type of melanoma, number of affected lymph nodes in SLNB, mitosis rate (>1/mm^2 versus $\leq$1/mm^2) and presence of locoregional cutaneous metastasis showed no significant association with any of the defined endpoints in the univariate analysis (Table S3).

3.3. Pre-Selection of Relevant Blood Parameters

After a manual parameter selection following the criteria outlined above (direct effect on endpoints, patient number, feature simplicity and multicollinearity), three blood parameters out of sixteen were selected as relevant predictors of recurrence (see Figure 3 and Table S2a for details). NLR, LMR and CRP were significantly associated with RFS in the univariate Cox regression analysis (NLR: HR = 1.236, 95%CI = 1.064–1.437, $p = 0.006$; LMR: HR = 0.689, 95%CI = 0.564–0.841, $p < 0.001$; CRP: HR = 1.065, 95%CI = 1.026–1.105, $p < 0.001$, respectively). These parameters were available for $\geq$100 patients, were calculated based on absolute blood values, or were absolute values themselves and were clinically and statistically more relevant than other intercorrelated values.

The same exclusion criteria revealed three different blood parameters to be particularly relevant for the prediction of OS: dNLR, absolute leukocyte count and absolute neutrophil count. Of note, the selected parameters were not significantly associated with OS in the univariate analysis, but had a significant independent effect when they were controlled for age, AJCC-stage, capsule invasion, adjuvant interferon-α therapy and diameter of the largest metastasis in the multivariate analysis (dNLR: HR = 1.410, 95%CI = 1.024–1.942, $p = 0.035$; absolute leukocyte count: HR = 1.334, 95%CI = 1.022–1.742, $p = 0.034$; absolute neutrophil count: HR = 1.404, 95%CI = 1.086–1.815, $p = 0.010$, see Figure 3 and Table S2b).

3.4. Cut-off Points for Blood Values

For the set of selected blood parameters listed above (NLR, LMR and CRP for RFS; dNLR, absolute leukocyte count and absolute neutrophil count for OS), we determined cut-off values by contrasting sensitivity and specificity from ROC curves, evaluated the effects of different alternative values using Kaplan–Meier estimates and analyzed their effects in the univariate and multivariate Cox proportional hazards analysis. This enabled dichotomization (separation of the respective parameters into two groups: above and below the cut-off point). These cut-off points of the selected blood parameters were used in later analyses and considerably simplify the clinical applications of these parameters.

Figure 4 shows the ROC curves of these parameters for RFS and OS, respectively. For NLR, the optimal cut-off point was rounded to 3.5 (sensitivity 0.314; 1-specificity 0.145). The LMR showed the highest significance for RFS prediction at a cut-off point of 3.5 (sensitivity 0.414; 1-specificity 0.691). A clear cut-off for CRP could not be identified in the ROC analysis (range 3.0–5.0). It was set to 3.0 (sensitivity 0.400; 1-specificity 0.091), as thereby the two groups were most balanced in size. For OS, the optimal cut-off point for the dNLR was 2.0 (sensitivity 0.513; 1-specificity 0.256). For the absolute leukocyte count and absolute neutrophil count no cut-off values could be identified, as the area under the curve (AUC) was approximately 0.5 for both parameters, which indicates a low predictive value. Log rank analysis confirmed the significance of the determined cut-off values for RFS with $p = 0.026$ for NLR, $p < 0.001$ for LMR and $p < 0.001$ for CRP. For OS, only the dichotomized dNLR showed statistically significant results ($p = 0.01$; Figure 5).

3.5. Hierarchical Multivariate Cox Regression Analysis

With the parameters pre-selected and investigated above, we proceeded to build a multivariate Cox regression analysis model with hierarchically organized control variables. First, we controlled for the patients' age. Gender showed no relevance in our previous exploratory analysis (see Table S3). Next, from already described prognostic factors in literature and medical guidelines, we chose the AJCC-stage, which was significantly associated with RFS and OS (see Table S3) and indirectly includes many strong prognostic variables (such as the presence of ulceration of the primary, Breslow thickness, number of

affected lymph nodes, presence of cutaneous metastases). In addition, capsule invasion of the lymph node by the metastasis and whether patients received adjuvant therapy with interferon-α were included as variables. Subsequently, our pre-selected blood values were included individually into the model to determine their respective prognostic value, with respect to the two endpoints. Tables 2 and 3 show the results of the multivariate Cox regression analyses for RFS and OS, while Table 4 shows an overview of the univariate and multivariate Cox regression analyses with continuous and dichotomized blood values described in the previous section.

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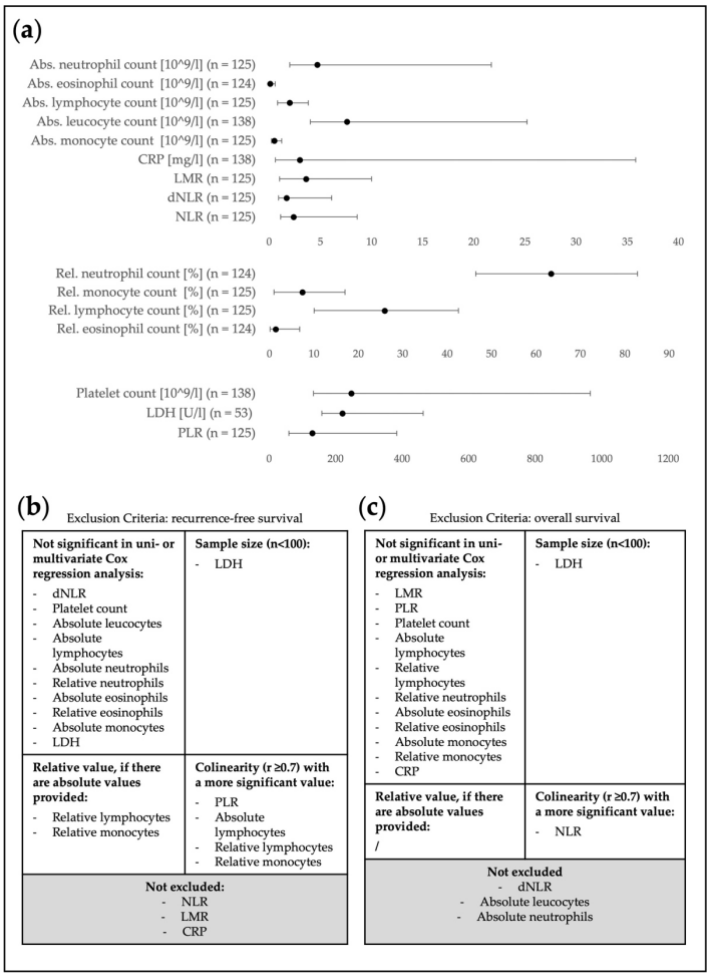

Figure 3. Overview of all blood values with applied exclusion criteria. Panel (**a**) shows a forest plot distribution of median values with 95% CI on three different scales. Panel (**b**) shows a list of excluded blood parameters related to recurrence-free survival and panel (**c**) shows excluded parameters for overall survival. CRP: C-reactive protein, LMR: lymphocyte-to-monocyte ratio, NLR: neutrophil-to-lymphocyte ratio, dNLR: derived NLR, LDH: lactate dehydrogenase, PLR: platelet-to-lymphocyte ratio.

3.4. Cut-off Points for Blood Values

For the set of selected blood parameters listed above (NLR, LMR and CRP for RFS; dNLR, absolute leukocyte count and absolute neutrophil count for OS), we determined cut-off values by contrasting sensitivity and specificity from ROC curves, evaluated the effects of different alternative values using Kaplan–Meier estimates and analyzed their effects in the univariate and multivariate Cox proportional hazards analysis. This enabled

thereby the two groups were most balanced in size. For OS, the optimal cut-off point for the dNLR was 2.0 (sensitivity 0.513; 1-specificity 0.256). For the absolute leukocyte count and absolute neutrophil count no cut-off values could be identified, as the area under the curve (AUC) was approximately 0.5 for both parameters, which indicates a low predictive value. Log rank analysis confirmed the significance of the determined cut-off values for RFS with $p = 0.026$ for NLR, $p < 0.001$ for LMR and $p < 0.001$ for CRP. For OS, only the dichotomized dNLR showed statistically significant results ($p = 0.01$; Figure 5).

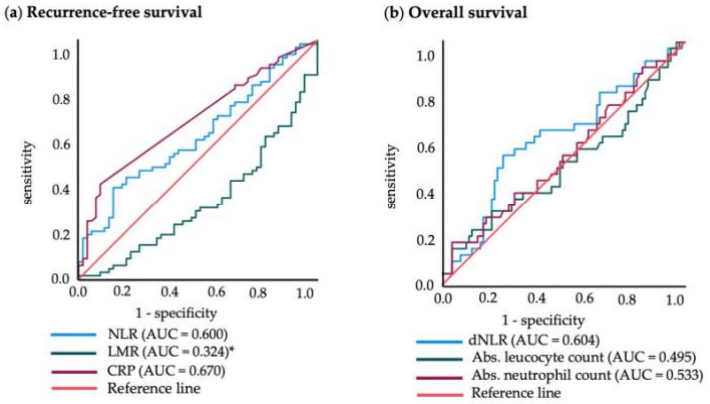

Figure 4. ROC curves for recurrence-free survival (RFS) and overall survival (OS) for dichotomization of blood values (**a**) sensitivity and specificity with neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), C-reactive protein (CRP) for RFS in subanalysis (**b**) sensitivity and specificity with derived NLR (dNLR), absolute leukocyte and neutrophil count for OS (* Note: since the LMR negatively correlates to RFS, the ROC curve is below the reference line).

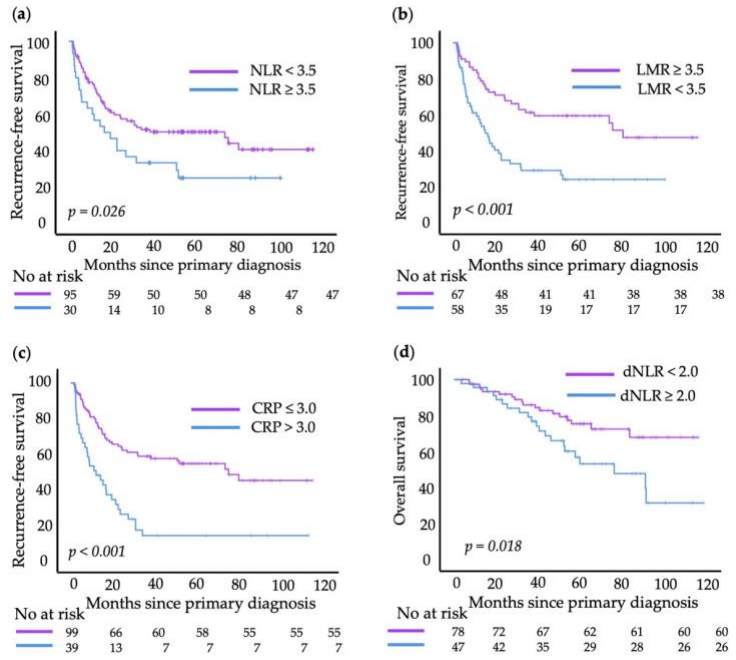

Figure 5. Kaplan–Meier survival curves showing recurrence-free survival (RFS) and overall survival (OS) for dichotomized blood values: (**a**) neutrophil-to-lymphocyte ratio (NLR); (**b**) lymphocyte-to-monocyte ratio (LMR); (**c**) C-reactive protein (CRP); (**d**) derived NLR (dNLR). The log-rank test was used to compare between groups; $p < 0.05$ was considered significant.

3.5. Hierarchical Multivariate Cox Regression Analysis

With the parameters pre-selected and investigated above, we proceeded to build a multivariate Cox regression analysis model with hierarchically organized control variables. First, we controlled for the patients' age. Gender showed no relevance in our previous exploratory analysis (see Table S3). Next, from already described prognostic factors

Table 2. Multivariate Cox regression analysis for recurrence-free survival (RFS) either with C-reactive protein (CRP), lymphocyte-to-monocyte ratio (LMR) or neutrophil-to-lymphocyte ratio (NLR).

	Model 1: RFS		**Model 2: RFS**		**Model 3: RFS**	
Variable (reference bold)	**HR (95%CI)**	***p*-Value**	**HR (95%CI)**	***p*-Value**	**HR (95%CI)**	***p*-Value**
Age (continuous)	1.330 (0.974–1.817)	0.073	1.286 (0.943–1.752)	0.111	**1.402 (1.054–1.865)**	**0.020**
AJCC-stage (A: n = 35) (B: n = 28) (C: n = 63)						
IIIB vs. **IIIA**	2.143 (0.885–5.188)	0.091	2.151 (0.890–5.197)	0.089	2.077 (0.888–4.857)	0.092
IIIC vs. **IIIA**	**3.461 (1.599–7.489)**	**0.005**	**3.410 (1.575–7.384)**	**0.002**	**3.707 (1.784–7.703)**	**<0.001**
IIID vs. **IIIA**	/	/	/	/	/	/
Capsule invasion (n = 14; n = 112) (no vs. **yes**)	0.798 (0.398–1.356)	0.568	0.808 (0.371–1.762)	0.593	1.027 (0.485–2.176)	0.944
Adjuvant interferon-α (n = 84; n = 42) (yes vs. **no**)	0.735 (0.398–1.356)	0.324	0.763 (0.412–1.412)	0.389	0.779 (0.434–1.399)	0.403
Size of largest SLN metastasis (continuous)	1.053 (0.898–1.234)	0.524	1.028 (0.876–1.207)	0.734	1.129 (0.974–1.307)	0.107
NLR (n = 27; n = 87) (≥3.5 vs. **<3.5**)	1.512 (0.845–2.705)	0.164				
LMR (n = 51; n = 63) (<3.5 vs. **≥3.5**)			**2.198 (1.301–3.715)**	**0.003**		
CRP (n = 92; n = 34) (>3.0 vs. **≤3.0**)					**3.355 (2.017–5.582)**	**<0.001**

Note: values in bold indicate significant results.

Table 3. Multivariate Cox regression analysis for overall survival with derived NLR (dNLR).

	Model 4: OS	
Variable (Reference bold)	**HR (95%CI)**	***p*-Value**
Age (continuous)	1.438 (0.935–2.209)	0.098
AJCC-stage (n = 35, n = 28, n = 63, n = 0)		
IIIB vs. **IIIA**	2.311 (0.699–7.647)	0.170
IIIC vs. **IIIA**	2.199 (0.730–6.624)	0.161
IIID vs. **IIIA**	/	/
Capsule invasion (n = 14; n = 112) (no vs. **yes**)	0.482 (0.196–1.1885)	0.113
Adjuvant interferon-α (n = 84; n = 42) (yes vs. **no**)	0.404 (0.161–1.014)	0.053
Size of biggest SLN metastasis (continuous)	**1.272 (1.071–1.511)**	**0.006**
dNLR (n = 70; n = 44) (≥2.0 vs. **<2.0**)	**2.428 (1.186–4.968)**	**0.015**

Note: values in bold indicate significant results.

Table 4. Significance of neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and C-reactive protein (CRP) for recurrence-free survival (RFS) and derived NLR (dNLR) for overall survival (OS) as continuous and dichotomized variables in univariate and multivariate Cox regression analysis.

Blood Values	Univariate Cox Regression Analysis *n*	HR (95%CI) *p*-Value	Multivariate Cox Regression Analysis * *n*	HR (95%CI) *p*-Value
Outcome: RFS				
Model 1: NLR	continuous (n = 125)	**1.236 (1.064–1.437) 0.006**	continuous (n = 114)	**1.340 (1.050–1.711) 0.019**
	cut-off 3.5 (n = 30; n = 95) (≥3.5 vs. <3.5)	**1.761 (1.063–2.919) 0.028**	cut-off 3.5 (n = 27; n = 87) (≥3.5 vs. **<3.5**)	1.512 (0.845–2.705) 0.164
Model 2: LMR	continuous (n = 125)	**0.689 (0.564–0.841) <0.001**	continuous (n = 114)	**0.608 (0.422–0.877) 0.008**
	cut-off 3.5 (n = 58; n = 67) (<3.5 vs. ≥3.5)	**2.433 (1.505–3.934) <0.001**	cut-off 3.5 (n = 51; n = 63) (<3.5 vs. **≥3.5**)	**2.198 (1.301–3.715) 0.003**
Model 3: CRP	continuous (n = 138)	**1.065 (1.026–1.105) <0.001**	continuous (n = 126)	**1.457 (1.214–1.747) <0.001**
	cut-off 3.0 (n = 39 vs. 99) (>3.0 vs. ≤3.0)	**2.841 (1.791–4.508) <0.001**	cut-off 3.0 (n = 34 vs. 92) (>3.0 vs. **≤3.0**)	**3.355 (2.017–5.582) <0.001**
Outcome: OS				
Model 4: dNLR	continuous (n = 124)	1.287 (0.945–1.753) 0.109	continuous (n = 114)	**1.410 (1.024–1.942) 0.035**
	cut-off 2.0 (n = 47; n = 78) (≥2.0 vs. <2.0)	**2.102 (1.119–3.948) 0.021**	cut-off 2.0 (n = 44; n = 70) (≥2.0 vs. **<2.0**)	**2.428 (1.186–4.968) 0.015**

* Following parameters were additionally included in the multivariate Cox regression analysis: age, AJCC-stage, capsule invasion, adjuvant interferon-α, size of largest SLN metastasis. Note: values in bold indicate significant results.

As shown in Table 2, a low LMR-value (<3.5) and a high CRP-value (>3.0) were independently associated with an increased risk of progression in the multivariate analysis (LMR: HR = 2.198, 95%CI = 1.301–3.715, p = 0.003; CRP: HR = 3.355, 95%CI = 2.017–5.582, $p < 0.001$). Note that the NLR was independently associated with RFS as a continuous factor (HR = 1.340, 95%CI = 1.050–1.711, p = 0.019), but not as dichotomized parameter with a cut-off at 3.5 (HR = 1.512, 95%CI = 0.845–2.705, p = 0.164; see Table 4). Comparing the effect sizes of CRP and LMR above, CRP had a higher effect on RFS than LMR (CRP > 3.0 associated with a 3.4-fold increased risk of progression compared to LMR ≥ 3.5 with a 2.2-fold increased risk of progression). Furthermore, in the multivariate Cox regression analysis for RFS with CRP as the dichotomized value (see Table 2), AJCC-stage (IIIC versus IIIA: HR = 3.707, 95%CI = 1.784–7.703, $p < 0.001$) and age at first diagnosis (HR = 1.402, 95%CI = 1.054–1.865, p = 0.020) were also independently associated with RFS. Capsule invasion, the maximum diameter of the biggest SLN metastasis and adjuvant treatment with interferon-α were not independently associated with RFS in our model.

In the multivariate Cox regression model for OS (Table 3) with dNLR as the dichotomized value with a cut-off at 2.0, a higher dNLR was significantly associated with a shorter OS (HR = 2.428, 95%CI = 1.186–4.968, p = 0.015). In this model, the maximum diameter of the biggest SLN metastasis was also an independent significant prognostic factor (HR = 1.272, 95%CI = 1.071–1.511, p = 0.006). Age, AJCC-stage, capsule invasion and adjuvant therapy with interferon-α lost the previously found univariate effect on OS after controlling for dNLR (see Figure S1). The NLR was also significantly associated with OS in

the multivariate Cox regression analysis. As this effect was smaller than the one of dNLR, we further analyzed dNLR.

3.6. Hierarchical Multivariate Cox Regression Analysis

Next, the power of the final multivariate Cox regression models was tested in a ROC curve analysis. A model for RFS with the defined covariates, in addition to one of our analyzed blood values, showed the following total area under the curve (AUC): NLR 0.748; LMR 0.772 and CRP 0.801. In comparison, the models without the additional blood parameters showed a baseline AUC of 0.744. For OS, the area under the curve for the model with dNLR was higher (AUC = 0.767) than the baseline model without dNLR (AUC = 0.733).

3.7. Combination of Dichotomized Blood Values

We created new variables denoting samples with NLR $\geq$ 3.5 plus CRP > 3.0, LMR < 3.5 plus CRP > 3.0, NLR $\geq$ 3.5 plus LMR < 3.5 and NLR $\geq$ 3.5 plus LMR < 3.5 plus CRP > 3.0, to assess whether these combinations were associated with a bigger prognostic effect than the single blood values. The combination of high NLR and high CRP shows a HR = 4.838 (95%CI = 2.009–11.652, $p < 0.001$), compared to no elevation of these blood values. The further addition of low LMR to high NLR and high CRP led to a significantly better prognostic effect on RFS (HR = 7.690, 95%CI = 2.789–21.202, $p < 0.001$), although the highest prognostic value for RFS was seen in patients with a combination of low LMR and high CRP compared to patients with LMR $\geq$ 3.5 and CRP < 3.0 (HR = 7.700, 95%CI = 3.436–17.255, $p < 0.001$). The prognostic effect of these combined blood values was stronger than the effects of the single blood cell ratios (LMR < 3.5: HR = 2.198 95%CI = 1.301–3.715, $p = 0.003$; CRP > 3.0: HR = 3.355, 95%CI = 2.017–5.582, $p < 0.001$; see Table 2 and Table S4 for a comparison of effect sizes).

4. Discussion

In our present study, we analyzed the effectiveness of different blood variables to predict RFS and OS in stage III melanoma patients with microscopically detectable SLN metastasis. To the best of our knowledge, this is the first study to comparatively assess the prognostic value of ratios of different blood cells and CRP, specifically in stage III melanoma patients with microscopic SLN metastasis. This specific setting was chosen to identify patients with a higher risk of disease recurrence from the large number of patients who are regularly diagnosed with SLN metastasis, and are potential candidates for adjuvant therapy with immune checkpoint inhibitors or BRAF/MEK-inhibitors. We therefore intentionally excluded patients who received these adjuvant therapies. Our findings demonstrate that NLR, LMR and CRP provide useful prognostic information about RFS, in addition to the predictiveness of other well-established prognostic factors. Nevertheless, the factor with the strongest association with RFS was high CRP, with the combination of high CRP and low LMR having the strongest predictive power for melanoma recurrence in our patient cohort. Regarding OS, a high dNLR and NLR were associated with shorter OS.

The NLR contains information about the number of lymphocytes, which are known for their strong anti-tumoral effects [26], and about the number of neutrophils, which are often reported to be pro-tumorigenic [15]. While neutrophils in the later tumor stages are mainly pro-tumorigenic, they can also exert anti-tumoral effects. These are most often reported in early tumor stages [15,27,28]. An increased NLR has been regarded to mirror sustained angiogenesis and proliferation of tumor cells [29].

Although there are numerous studies on the prognostic value of NLR in advanced melanoma and on the predictive potential of NLR in the treatment with ICI or BRAF/MEK-inhibitors, few studies analyze the prognostic potential of NLR and other blood ratios in localized melanoma stages, especially in patients who did not receive adjuvant ICI or targeted therapy. For example, a retrospective study by Ma et al. investigated patients with stage III melanoma and described an NLR $\geq$ 2.5 to be a strong predictor for disease

recurrence [30]. In our study, NLR as a continuous variable was also associated with higher risk of progression and shorter RFS. In univariate analysis, this effect also remained for the dichotomized value of NLR (cut-off at 3.5), but after adjustment for confounding factors in the multivariate analysis, the dichotomized value did not show a significant association with RFS. These differences could be explained by differences in the patient cohort. While both studies investigated stage III melanoma patients, we excluded patients who received adjuvant therapy with ICI or targeted therapy. Furthermore, we only included patients with microscopic SLN metastasis and therefore excluded stage III melanoma patients with macroscopic metastasis or only cutaneous metastases, who are known to have an inferior prognosis.

Lino-Silva et al. were the first to analyze NLR in stage I-III patients with localized melanoma. They described an NLR $\geq$ 2.0 as a prognostic marker for shorter OS, although in subgroup analysis this only remained significant for stage II patients. Their group also found an association of NLR $\geq$ 2.0 with lymph node metastasis and recurrence [16]. We found high continuous NLR values in our multivariate analysis to be significantly associated with shorter RFS and OS in our study population. Furthermore, we found a high dNLR (not assessed by Lino-Silva et al.) to have an even more significant and stronger association with a shorter OS than a high NLR.

The largest study that assessed the prognostic value of the baseline NLR in stage I-III melanoma patients was performed by Robinson et al. They included 1077 patients with negative SLN, 274 with microscopic and 138 with macroscopic SLN metastasis. The NLR increased with tumor stage and was lowest in patients without lymph node metastasis, higher in patients with microscopic lymph node metastasis and highest in those with macroscopic metastasis. They did not find a correlation between NLR and recurrence, but found an association between the tumor burden at first diagnosis and NLR [13]. In our study, NLR was an independent prognostic factor for RFS and OS in the multivariate analysis, which included the diameter of the SLN metastasis (marker for tumor burden).

Contrary to most studies and to our results, Wade et al. reported a low baseline NLR and PLR to be associated with shorter OS and MSS in patients with stage I-III melanoma. Since this association was only detectable in multivariate analysis, but not in univariate analysis, they suggested that in low tumor stages the elimination of confounding factors is even more important. Interestingly, we also experienced that NLR and dNLR only showed a significant effect on OS after adjusting for confounding factors. As opposed to the study by Wade et al., in our stage III cohort with microscopic SLN metastasis, a high NLR or high dNLR predicted a shorter OS. A possible reason for Wade et al.'s opposing results compared to ours, and those of other studies investigating melanoma with stage III disease, could be the high number of patients with stage I melanoma (890 patients compared to 184 patients with stage II and 274 patients with stage III melanoma) they included [15].

Recently, several studies were published analyzing the predictive value of the dNLR. These were mainly performed in advanced melanoma patients (stage IV and not operable stage III). Ferrucci et al. and Capone et al. retrospectively analyzed advanced melanoma patients who were treated with ICI (ipilimumab or nivolumab). Ferrucci and colleagues found a high dNLR and absolute neutrophil count to be independently associated with an increased risk of death and disease progression. Capone et al. described an NLR > 5.0 and dNLR > 3.0 at baseline to be associated with shorter OS and shorter progression-free survival (PFS) [20,21]. Our study supports these findings, although we used different cut-off values and analyzed patients with resectable stage III melanoma. We identified a dNLR $\geq$ 2.0 to be the strongest and most significant independent predictor for shorter OS. Interestingly, the dNLR was not associated with RFS even though studies have shown similar prognostic values for NLR and dNLR in cancer patients, suggesting that the lymphocyte fraction is accurately estimated by the result of the absolute neutrophil count subtracted from the absolute leukocyte count in the denominator of dNLR [31]. The dNLR is, however, not exclusively determined by neutrophil and lymphocyte values but also by all other white blood count subtypes in the denominator, with monocytes accounting

for the other main fraction. As these have been reported to be lower in patients with malignancies [14,17,32,33], they might have led to an inaccurate dNLR calculation.

While several retrospective studies in stage IV melanoma patients showed a significant association of the LMR with OS [17,32,33], there are few reports about the role of the LMR in localized cutaneous melanoma so far. In our study, a low LMR was an independent prognostic marker for shorter RFS. Similarly, Wang et al. identified an LMR $\leq$ 7.38 and surgery as factors significantly associated with worse PFS and OS in a univariate analysis in patients with mucosal melanoma. In their multivariate analysis, significance was only maintained for OS [14]. In our study, there was no significant association between LMR and OS. Interestingly, and opposed to these findings, Wade et al. revealed an association of a low LMR (and high NLR) with a regression of the primary melanoma for stage I-III patients [15]. In addition, Gandini et al. found patients with distant metastases to have higher absolute leukocyte, neutrophil and monocyte counts and lower lymphocyte counts in comparison to stage I-III patients. There was no association of peripheral blood cell counts with OS in localized or regionally metastasized melanoma patients. Only the blood values of stage IV patients with distant metastasis showed an association with OS. They suggest that a high neutrophil, low monocyte and low lymphocyte count are stronger predictors for shorter OS in advanced tumor stages compared to earlier tumor stages [34]. While we detected an association between a high relative lymphocyte count and low LMR with RFS, we did not find any association between a high monocyte or lymphocyte count or LMR with OS.

We found CRP to be the strongest independent prognostic parameter for RFS in our studied cohort. Few studies have been published on CRP as a prognostic marker for progression in melanoma patients. Fang et al. showed that CRP (as continuous baseline variable and as dichotomized value <10 mg/L, $\geq$10 mg/L) was associated with shorter OS and MSS in any melanoma stage. In a stage III/VI subgroup analysis, this association remained. CRP >10 mg/L in stage I/II patients was also associated with progression. All these findings remained significant after adjustment for confounding factors [22]. As opposed to Fang et al., we found a CRP baseline level of >3.0 mg/L to be a strong predictor for shorter RFS, but not for shorter OS. Most likely, these differences occurred as Fang et al. analyzed stage III and stage IV patients together in one subgroup, while we focused on a specific stage III melanoma cohort. In published studies so far, the defined cut-off value for CRP was often 10 mg/L. Compared to these, our cut-off value of 3.0 mg/L is rather low. Possibly, the lower cut-off value emerged because the performing laboratory in our study routinely reports quantitative CRP levels down to the lower detection limit of 0.3 mg/L. CRP values originating from other laboratories are sometimes simply reported as being below the detection limit for inflammation, which is <5 mg/L. Since our determined optimal cut-off value of 3.0 mg/L is below this detection limit, we suggest quantifying CRP also in the low range beneath 5 mg/L.

In addition to the independent assessment of the predictive value of single blood parameters, we assessed CRP, LMR and NLR in different combinations for their ability to predict disease progression. Doing so, we detected that a combined score of high CRP and low LMR was the strongest and most significant prognostic factor for melanoma recurrence when compared to all investigated parameters.

A limitation of this study is its retrospective nature and that it was conducted as a single-center study. To better assess the effects of the analyzed parameters on OS, a larger patient cohort with more death events would have been needed. Nevertheless, the strictly uniform patient cohort (only stage III patients with microscopic disease) and the fact that all important known prognostic parameters were included in this study are a strength compared to other studies which included patients from stage I–III or stage III patients with microscopic and macroscopic disease, as well as patients with only cutaneous metastases. In addition, RFS is an accepted surrogate marker for OS and is the most commonly used primary endpoint in prospective randomized adjuvant trials with melanoma patients. While not being able to exclude patients who received interferon-α as adjuvant therapy, we

excluded patients who received adjuvant PD-1-inhibitors or BRAF/MEK-inhibitor therapy, which (unlike interferon-α) have been shown to have a strong effect on RFS in randomized phase III trials. In addition, to account for possible effects of interferon-α, we included treatment with interferon-α as a control variable in our multivariate analysis.

In summary, although a number of studies assess the prognostic value of different blood parameters such as NLR in melanoma patients, this study is the first to systematically comparatively analyze the prognostic value of different ratios of blood parameters and CRP in melanoma patients with microscopic SLN metastasis. This uniform patient cohort is a strength of this study since different tumor stages may confound the results in other studies that assessed some, but not all of these parameters. Our analysis shows that the prognostic value of CRP for RFS is much higher in this patient cohort than that of the widely discussed NLR.

5. Conclusions

Altogether, we detected strong associations of NLR, LMR and CRP with RFS, which were independent of other known prognostic parameters in our cohort of stage III melanoma patients with microscopic SLN metastasis. CRP was the parameter with the strongest association with RFS compared to the other parameters. Nevertheless, the combination of CRP and LMR was associated with the strongest potential to predict progression. Therefore, we propose to consider these variables when assessing a patient's risk of disease recurrence, the necessity for closer monitoring, or for an adjuvant therapy. Nevertheless, further studies in larger, prospectively collected patient cohorts are required to validate our findings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers15030702/s1, Table S1a: Baseline tumor characteristics (primary; n = 138); Table S1b: Baseline tumor characteristics (lymph node metastasis; n = 138); Table S2a: Overview of results of all blood variables in univariate and multivariate Cox regression analysis for recurrence-free survival (RFS) with applied exclusion criteria; Table S2b: Overview of results of all blood variables in univariate and multivariate Cox regression analysis for overall survival (OS) with applied exclusion criteria; Table S3: Univariate Cox regression analysis with covariates for recurrence-free survival and overall survival; Table S4: Univariate and multivariate Cox regression analysis with combined blood values of NLR, LMR and CRP for recurrence-free survival; Figure S1: Kaplan-Meier survival curves showing overall survival for covariates of the multivariate Cox regression model.

Author Contributions: Conceptualization: C.F. and V.A.S.S.; methodology: C.F.; formal analysis: V.A.S.S.; investigation: C.F. and V.A.S.S.; data curation: C.F. and V.A.S.S.; writing: C.F. and V.A.S.S.; review and editing: S.H., G.H.-G., W.J. and C.M.; visualization: C.F. and V.A.S.S.; supervision: C.F. All authors have read and agreed to the published version of the manuscript.

Funding: C.F. was funded by the Köln Fortune Program of the University of Cologne (project numbers 498/2020 and 175/2022). S.H. received funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, HO 6389/2-2, 'KFO 337'—405344257. We acknowledge support for the Article Processing Charge from the DFG (German Research Foundation, 491454339). This research did not otherwise receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Cologne (protocol code 20-1584; 27.01.2021) for studies involving humans.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All relevant data from this study can be found in the manuscript or in the provided Supplementary Material.

Acknowledgments: We thank all patients who participated in this study.

Conflicts of Interest: All authors declare no conflicting interests affecting this study. Conflicts outside the submitted work are that C.F. has been on the advisory board or received honoraria from Bristol-Myers-Squibb, Immunocore and Novartis, and received travel grants from Bristol-Myers-Squibb, Novartis and Pierre Fabre.

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3.2. Anhang zur Publikation

cancers

MDPI

Supplementary Materials

C-Reactive Protein and Lymphocyte-to-Monocyte Ratio Predict Recurrence in Stage III Melanoma Patients with Microscopic Sentinel Lymph Node Metastasis

Table S1a: Baseline tumor characteristics (primary; n=138).

	All patients n=138 (100%)
Site of primary	
Head and neck	4 (2.9)
Trunk	51 (37.0)
Upper extremity	20 (14.5)
Lower extremity	63 (45.7)
Type of primary	
SSM	39 (28.3)
NMM	63 (45.7)
ALM	6 (4.3)
LMM	0 (0.0)
other	24 (17.4)
unknown	6 (4.3)
Tumor thickness (Breslow)	
<1mm	3 (2.2)
≥1<2mm	36 (26.1)
≥2<4mm	53 (38.4)
≥4mm	46 (33.3)
Median in mm (IQR)*	3.1 (1.6-4.5)
Presence of ulceration	
No	74 (53.6)
Yes	64 (46.4)
Mitosis rate > 1/mm²	
No	5 (3.6)
Yes	95 (68.8)
Unknown	38 (27.5)
*IQR = Interquartile range (Q1 – Q3).	

Table S1b: Baseline tumor characteristics (lymph; node metastasis; n=138).

	All patients n=138 (100%)
Average resected number of SLN	
1	69 (50.0)
2	38 (27.5)
3	15 (10.9)
≥4	16 (11.5)
Number of affected SLN	
1	108 (78.3)
2	22 (15.9)
3	8 (5.8)
Largest diameter of SLN metastasis (in mm)	
<1 (0.09-0.99)	
≥1 <2 (1.00-1.99)	41 (29.7)
≥2 <3 (2.00-2.99)	28 (20.3)
≥3 <4 (3.00-3.99)	19 (13.8)
≥4	14 (10.1)
Unknown	26 (18.8)
Median in mm (IQR)*	10 (7.2)
	1.3 (0.5-3.0)
Capsule invasion of SLN metastasis	
No	122 (88.4)
Yes	15 (10.9)
Unknown	1 (0.7)
Presence of satellite metastasis	
No	130 (94.2)
Yes	8 (5.8)
CLND	
No	66 (47.8)
Yes	72 (52.2)
-no additional metastasis in CLND	58 (61.7)
-additional metastasis in CLND	36 (38.3)
*IQR = Interquartile range (Q1 – Q3).	

Table S2a: Overview of results of all blood variables in univariate and multivariate Cox regression analysis for recurrence-free survival (RFS) with applied exclusion criteria.

Blood values (continuous parameters)	**Univariate Cox regression analysis** n	HR (95% CI) p-value	**Multivariate Cox regression analysis*** n	HR (95% CI) p-value	**Exclusion criteria**
NLR	125	**1.236 (1.064-1.437) 0.006**	114	**1.340 (1.050-1.711) 0.019**	Not excluded
dNLR	125	1.254 (0.975-1.612) 0.077	114	1.223 (0.957-1.563) 0.107	1)
LMR	125	**0.689 (0.564-0.841) <0.001**	114	**0.608 (0.422-0.877) 0.008**	Not excluded
PLR	125	**1.005 (1.001-1.008) 0.008**	114	**1.282 (1.001-1.643) 0.049**	4)**
Platelet count	138	1.001 (0.998-1.003) 0.634	126	1.069 (0.860-1.327) 0.548	1)
Absolute leucocyte count	138	1.004 (0.923-1.092) 0.933	126	1.093 (0.865-1.380) 0.434	1)
Absolute lymphocyte count	125	**0.659 (0.458-0.948) 0.025**	114	0.089 (0.606-1.080) 0.150	1) and 4)**
Relative lymphocyte count	125	**0.959 (0.928-0.990) 0.011**	114	**0.758 (0.583-0.985) 0.038**	3) and 4)
Absolute neutrophile count	125	1.032 (0.943-1.130) 0.492	114	1.141 (0.912-1.426) 0.248	1)
Relative neutrophile count	124	1.023 (0.993-1.054) 0.137	113	1.175 (0.904-1.527) 0.229	1)
Absolute eosinophile count	124	0.602 (0.067-5.394) 0.650	113	0.959 (0.713.1.288) 0.779	1)
Relative eosinophile count	124	0.929 (0.761-1.133) 0.467	113	0.875 (0.650-1.177) 0.376	1)
Absolute monocyte count	125	2.637 (0.911-7.632) 0.074	114	1.200 (0.939-1.534) 0.145	1)
Relative monocyte count	125	**1.178 (1.069-1.299) <0.001**	114	**1.332 (1.049-1.692) 0.019**	3) and 4)***
LDH	53	**1.008 (1.002-1.013) 0.010**	50	1.277 (0.759-2.148) 0.358	1) and 2)
CRP	138	**1.065 (1.026-1.105) <0.001**	126	**1.457 (1.214-1.747) <0.001**	Not excluded

Exclusion criteria:

1) Not significant in univariate or multivariate Cox regression analysis.
2) Patient number n<100.
3) Relative blood values, if there are absolute values provided.

4) High pairwise Pearson correlation (r ≥0.7) between blood values. In this case, the weaker parameter regarding exclusion criteria 1-3 was dropped.

* Note: Single blood value in multivariate Cox regression analysis with following co-variates: Age, AJCC-stage, capsule invasion, adjuvant interferon-α, size of biggest SLN metastasis.

** High correlation with NLR.

*** High correlation with LMR.

Note: Significant results are **bold.**

Table S2b: Overview of results of all blood variables in univariate and multivariate Cox regression analysis for overall survival (OS) with applied exclusion criteria.

Blood values (continuous parameter)	**Univariate Cox regression analysis** n	HR (95% CI) p-value	**Multivariate Cox regression analysis*** n	HR (95% CI) p-value	**Exclusion criteria**
NLR	124	1.174 (0967-1.426) 0.105	114	**1.396 (1.004-1.941) 0.047**	4)**
dNLR	124	1.287 (0.945-1.753) 0.109	114	**1.410 (1.024-1.942) 0.035**	Not excluded
LMR	124	0.818 (0.642-1.042) 0.103	114	0.769 (0.504-1.256) 0.326	1)
PLR	125	1.003 (0.998-1.007) 0.306	114	1.148 (0.804-1.639) 0.446	1)
Platelet count	136	1.000 (0.997-1.003) 0.977	126	1.103 (0.818-1.488) 0.518	1)
Absolute leucocyte count	136	1.044(0.943-1.155) 0.407	126	**1.334 (1.022-1.742) 0.034**	Not excluded
Absolute lymphocyte count	124	0.777 (0.482-1.251) 0.299	114	0.935 (0.639-1.370) 0.732	1)
Relative lymphocyte count	124	0.966 (0.926-1.008) 0.109	114	0.710 (0.492-1.023) 0.066	1)
Absolute neutrophile count	124	1.081 (0.975-1.200) 0.140	114	**1.404 (1.086-1.815) 0.010**	Not excluded
Relative neutrophile count	123	1.027 (0.987-1.068) 0.187	113	1.399 (0.951-2.057) 0.088	1)
Absolute eosinophile count	123	0.060 (0.002-1.757) 0.103	113	9.864 (0.564-1.323) 0.500	1)
Relative eosinophile count	123	0.745 (0.548-1.012) 0.059	113	0.764 (0.501-1.165) 0.211	1)
Absolute monocyte count	124	1.538 (0.358-6.620) 0.563	114	1.125 (0.808-1.569) 0.485	1)

Relative monocyte count	124	1.050 (0.915-1.206) 0.486		114	0.939 (0.679-1.299) 0.704	1)
LDH	51	**1.027 (1.014-1.041) <0.001**		48	**4.877 (1.550-15.342) 0.007**	2)
CRP	136	1.019 (0.958-1.084 0.548		126	1.216 (0.928-1.594) 0.157	1)

Exclusion criteria:

5) Not significant in univariate or multivariate Cox regression analysis.
6) Patient number n<100.
7) Relative blood values, if there are absolute values provided.
8) High pairwise Pearson correlation (r ≥0.7) between blood values. In this case, the weaker parameter regarding exclusion criteria 1-3 was dropped.

* Note: Single blood value in multivariate Cox regression analysis with following co-variates: Age, AJCC-stage, capsule invasion, adjuvant interferon-α, size of biggest SLN metastasis.
** High correlation with dNLR.
Note: Significant results are **bold.**

Table S3. Univariate Cox regression analysis with covariates for recurrence-free survival and overall survival.

	Univariate Cox analysis	
Variable (Reference bold)	**Recurrence-free survival** HR (95% CI) p-value	**Overall survival** HR (95% CI) p-value
Age (n=55; n=83) >65 years vs **≤65 years**	**1.985 (1.261-3.125)** **0.003**	**3.060 (1.657-5.650)** **<0.001**
AJCC-stage (n=36, n=32, n=70, n=0) IIIB vs **IIIA** IIIC vs **IIIA** IIID vs **IIIA**	2.247 (0.992-5.087) 0.052 **4.303 (2.114-8.759) <0.001** /	1.656 (0.589-4.658) 0.339 **2.685 (1.111-6.490)** **0.028** /
Capsule invasion (n=15; n=122) yes vs **no**	1.677 (0.858-3.277) 0.130	**4.394 (2.120-9.109)** **<0.001**
Adjuvant interferon-α		

(n=45; n=92)				
yes vs **no**	0.692	(0.422-1.133)	**0.329**	**(0.156-0.692)**
	0.143		**0.003**	
Size of biggest SLN metastasis				
(n=66; n=62)				
> 1mm vs **≤ 1mm**	**1.849**	**(1.139-3.004)**	**3.444**	**(1.674-7.086)**
	0.013		**<0.001**	
Gender				
(n=67; n=71)				
female vs **male**	0.721	(0.458-1.136)	0.672	(0.364-1.239)
	0.158		0.203	
Ulceration				
(n=64, n=74)				
yes vs **no**	**2.390 (1.505-3.794)**	**<0.001**	**2.044**	**(1.108-3.770)**
			0.022	
Mitotic rate				
(n=95; n=5)				
>1/mm² vs **≤ 1/mm²**	0.260 (0.036-1.881)	0.182	0,045 (0.000-64.245)	0.403
Number of affected SLN				
(n=108; n=22; n=8)				
2 vs **1**	0.784	(0.401-1.534)	0.493	(0.175-1.385)
3 vs **1**	0.477		0.179	
	1.921	(0.873-4.227)	0.621	(0.149-2.579)
	0.104		0.512	
Satellite metastases				
(n=8; n=130)				
yes vs **no**	0.683	(0.249-1.873)	0.968	(0.298-3.138)
	0.459		0.956	
CLND				
(n=72; n=66)				
yes vs **no**	**1.710**	**(1.072-2.729)**	1.356	(0.728-2.525)
	0.024		0.337	
Note: Significant results are **bold.**				

Table S4. Univariate and multivariate Cox regression analysis with multiple blood value cut-offs of NLR, LMR and CRP for recurrence-free survival.

Blood value combinations			
Univariate Cox analysis		**Multivariate Cox analysis ***	
Event number	HR (95% CI) p-value	Event number	HR (95% CI) p-value
NLR (≥3.5) + CRP (>3.0)		NLR (≥3.5) + CRP (>3.0)	
1 (n=45) vs **0** (n=71) events	**2.673 (1.616-4.421) <0.001**	1 (n=39) vs **0** (n=67) events	**2.370 (1.352-4.156) 0.003**
2 (n=9) vs **0** (n=71) events	**4.131 (1.872-9.117) <0.001**	2 (n=8) vs **0** (n=67) events	**4.838 (2.009-11.652) <0.001**
1 (n=45) vs **2** (n=9) events	0.647 (0.298-1.402) 0.270	1 (n=39) vs **2** (n=8) events	0.490 (0.200-1.203) 0.119
LMR (<3.5) + CRP (>3.0)		LMR (<3.5) + CRP (>3.0)	
1 (n=57) vs **0** (n=51) events	**3.365 (1.870-6.054) <0.001**	1 (n=53) vs **0** (n=48) events	**3.239 (1.710-6.135) <0.001**
2 (n=17) vs **0** (n=51) events	**6.386 (3.119-13.076) <0.001**	2 (n=13) vs **0** (n=48) events	**7.700 (3.436-17.255) <0.001**
1 (n=57) vs **2** (n=17) events	**0.527 (0.289-9.60) 0.036**	1 (n=53) vs **2** (n=13) events	**0.421 (0.212-0.835) 0.013**
NLR (≥3.5) + LMR (<3.5)		NLR (≥3.5) + LMR (<3.5)	
1 (n=36) vs **0** (n=63) events	**2.133 (1.217-3.738) 0.008**	1 (n=32) vs **0** (n=59) events	**2.216 (1.211-4.055) 0.010**
2 (n=26) vs **0** (n=63) events	**2.614 (1.463-4.671) 0.001**	2 (n=23) vs **0** (n=59) events	**2.160 (1.113-4.189) 0.023**
1 (n=36) vs **2** (n=26) events	0.816 (0.448-1.488) 0.507	1 (n=32) vs **2** (n=23) events	1.026 (0.521-2.020) 0.941
NLR (≥3.5) + LMR (<3.5) + CRP (>3.0)		NLR (≥3.5) + LMR (<3.5) + CRP (>3.0)	
1 (n=41) vs **0** (n=48) events	**3.054 (1.612-5.788) <0.001**	1 (n=39) vs **0** (n=45) events	**3.398 (1.709-6.755) <0.001**
2 (n=28) vs **0** (n=48) events	**4.167 (2.148-8.083) <0.001**	2 (n=23) vs **0** (n=45) events	**3.515 (1.647-7.500) 0.001**
3 (n=8) vs **0** (n=48) events	**6.059 (2.456-14.945) <0.001**	3 (n=7) vs **0** (n=45) events	**7.690 (2.789-21.202) <0.001**
1 (n=41) vs **3** (n=8) events	0.504 (0.218-1.165) 0.109	1 (n=39) vs **3** (n=7) events	0.442 (0.173-1.128) 0.087
2 (n=28) vs **3** (n=8) events	0.688 (0.293-1.617) 0.391	2 (n=23) vs **3** (n=7) events	0.457 (0.167-1.251) 0.128

*Single blood value in multivariate Cox regression analysis with following covariates: Age, AJCC-stage, capsule invasion, adjuvant Interferon-α, size of biggest SLN metastasis.

Note: Significant results are **bold.**

Supplementary figure

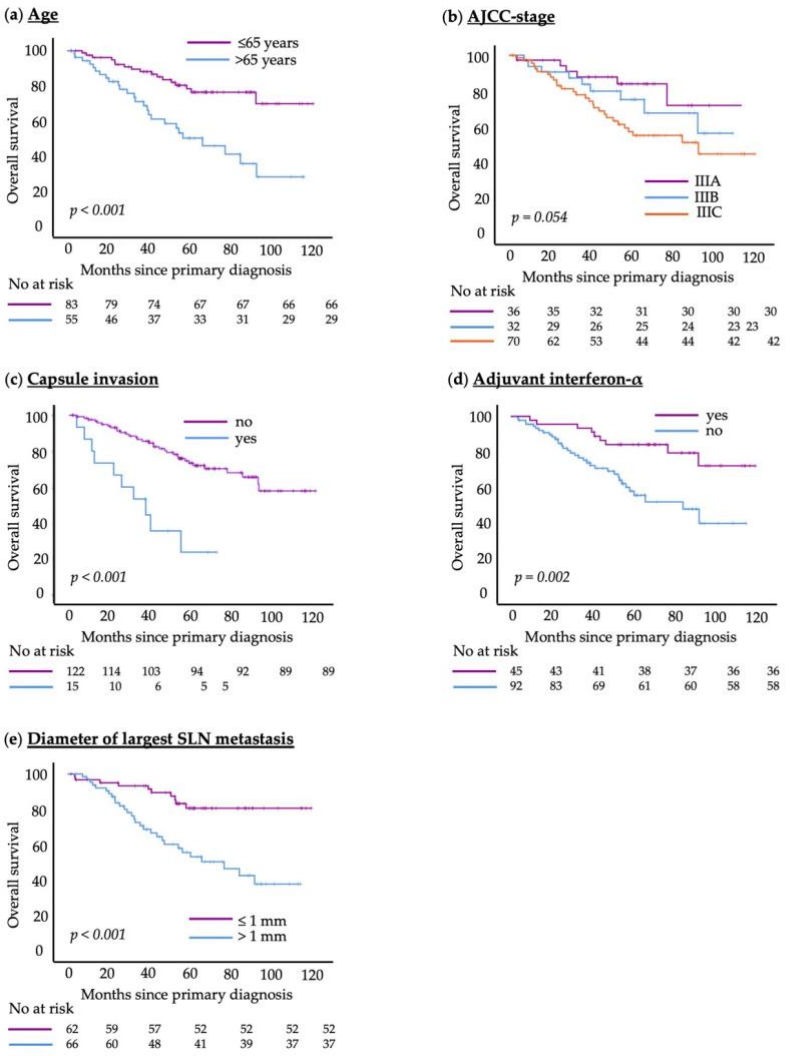

Figure S1. Kaplan-Meier survival curves showing overall survival for covariates of the multivariate Cox regression model: (**a**) patient age; (**b**) AJCC-stage; (**c**) capsule invasion of sentinel lymph node (SLN) metastasis; (**d**) adjuvant interferon-α therapy; (**e**) diameter of largest SLN metastasis. The log-rank test was used to compare between groups; $p < 0.05$ was considered significant.

4. Diskussion

Reliable prognostic factors are essential to identify the potential risk of recurrence and the survival probability. In this retrospective study, I evaluated and compared the prognostic value of various commonly monitored blood parameters in stage III melanoma patients with microscopic sentinel lymph node (SLN) metastasis. Endpoints were recurrence-free survival (RFS) and overall survival (OS). To the best of my knowledge, this represents the first study to systematically compare the prognostic potential of ten distinct blood cell types, four of their ratios, the enzyme lactate dehydrogenase (LDH), and C-reactive protein (CRP), specifically in stage III melanoma patients with microscopic SLN metastasis. My results show that neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and CRP provide valuable prognostic information about RFS independent of other known well-established prognostic factors. In my study, CRP showed the greatest prognostic potential, while the combination of high CRP and low LMR had even stronger prognostic power for recurrence in this study cohort. Regarding OS, my analysis showed that high derived neutrophil-to-lymphocyte ratio (dNLR) and high NLR were significantly associated with reduced OS.

4.1. Adjuvante Therapie: Ja oder nein?

Good prognostic factors could clarify whether a stage III melanoma patient with SLN metastasis after R0 resection has a high risk of disease recurrence and needs adjuvant therapy to reduce this risk. In patients with low risk of recurrence, close monitoring could be sufficient. Because adjuvant systemic therapy with immune checkpoint inhibitors (ICIs) or targeted signal transduction inhibitors can have serious and sometimes long-term side effects, the indication for treatment after resection should be carefully considered [9,10]. On the other hand, if a patient is likely to relapse, he or she may benefit from adjuvant therapy with ICIs or BRAF/MEK inhibitors despite the occurrence of side effects [28,29]. To assess a uniform patient cohort and to avoid any bias in the course of disease in my study population, I deliberately excluded patients who had undergone these adjuvant therapies. This reduced the number of patients in my cohort from 183 to 138. I did not exclude patients who received interferon-α because the effect of interferon-α on recurrence, and especially on overall survival in stage III melanoma patients, is much smaller than the effect of ICIs or signal transduction inhibitors [29]. The number of study participants would have decreased by another 45 patients to 93, as 45 patients received interferon-α. Nevertheless, I found a significant correlation of adjuvant interferon-α therapy with OS in my cohort using univariate Cox regression analysis. Also, Kaplan-Meier survival curves showed significantly better overall survival in patients treated with adjuvant interferon-α. There was no correlation with relapse-free survival. Therefore, I included interferon-α as a covariate in my multivariate Cox regression analysis model. After controlling

for other confounders, interferon-α lost its previously observed univariate association with overall survival in the multivariate Cox regression analysis.

4.2. Etablierte prognostische Parameter

Since January 2018, the eighth AJCC melanoma classification has been implemented, providing a basis for individual prognostic evaluation and therapeutic decision-making [30,31]. It includes information on the primary tumor, lymph node status, and presence of metastasis as the most important prognostic factors. The primary tumor is described by the vertical tumor thickness and the presence of ulceration. The lymph node status includes information about the number of lymph nodes involved and whether they are macroscopically detectable or not, as well as the presence of satellite metastasis, in-transit metastasis, or microsatellite metastasis. The M-stage (distant metastasis) takes into account the location of distant metastases and the level of LDH in the blood system [6,32]. In addition to the AJCC stage, histopathologic factors such as age, gender, melanoma location [33,34], and melanoma type have been reported in the literature and in clinical practice as prognostic factors [35].

4.3. Reflexion zur Modellbildung und zum statistischen Denken

The statistical approach for this study was developed in close collaboration with my doctoral supervisor, Prof. Dr. Cindy Franklin, and Guillermo Hidalgo-Gadea, an external statistical consultant from the Faculty of Psychology at Ruhr University Bochum. Together, we designed a structured, clinically relevant, and transparent analysis strategy. Under the supervision of Mr. Hidalgo-Gadea and Prof. Dr. Cindy Franklin, I conducted and interpreted the final statistical analysis using IBM SPSS Statistics (Version 27)**.**

As a first step, we performed an exploratory analysis to become familiar with the available patient and disease data. This included univariate Cox proportional hazards regression and Kaplan–Meier survival analyses, applied to both RFS and OS. This approach enabled us to identify which clinical and histopathological variables—such as patient age or the mitotic rate of the primary tumor—showed associations with survival outcomes and were thus candidates for further modeling.

Following this, we focused on a set of 16 routinely collected blood parameters that were identified from the literature as potentially prognostic. To reduce these to a core group suitable for multivariate analysis, we applied four predefined exclusion criteria, which were selected based on clinical reasoning, statistical robustness, and the goal of model parsimony. We acknowledge that these decisions reflect a deliberate methodological approach and that other analysts may have made different selections depending on their priorities and interpretation of the data. A more detailed discussion of the four exclusion criteria can be found in Section 4.5.

('Analysierte Blutwerte').

After applying these criteria, the following parameters were retained for further analysis: for RFS – NLR, LMR, and CRP; for OS – dNLR, absolute leukocyte count, and absolute neutrophil count. To enhance the clinical usability of these variables, we determined cut-off values for each of the remaining six blood markers using receiver operating characteristic (ROC) curves, a commonly used method in medical statistics to identify clinically meaningful thresholds based on the trade-off between sensitivity and specificity[36]. This approach was chosen in consultation with our statistical advisor. Meaningful cut-off values could be determined for four of the six tested blood parameters. For RFS, cut-off values were identified for NLR, LMR, and CRP, while for OS, only dNLR yielded a usable threshold. The AUC (area under the curve) for absolute leukocyte and neutrophil counts was approximately 0.5, indicating no meaningful discriminatory power, so no cut-offs were defined for these. In cases where ROC curves did not yield a clear cut-off point, we additionally used Kaplan–Meier analyses to explore the effect of various potential thresholds. This served both as a confirmation of ROC-based values and as a tool to visualize and evaluate clinical interpretability when clear ROC-based thresholds were not available.

A hierarchical multivariate Cox regression model was then created to assess the independent prognostic value of the blood parameters. The following covariates were included in all models: patient age at diagnosis, AJCC stage, capsule invasion of the sentinel lymph node and adjuvant therapy with interferon-α. Each blood parameter was added individually to a base model with these covariates to evaluate its added value. Cox regression is a method used to evaluate the effect of variables on the time to an event, like recurrence or death. Multivariate Cox regression allows controlling for other factors, thereby identifying variables with independent prognostic value.

In my study, I analyzed several variables, including established prognostic factors, in univariate Cox regression analysis to examine their impact on RFS and OS. This was done to comprehend my data and to build a sophisticated multivariate Cox regression model. Using this model, I assessed and compared the independent prognostic potential of different blood values on RFS and OS. I chose AJCC stage as a covariate for this multivariate Cox regression model because it incorporates all the aforementioned information about primary tumor type, lymph node status, and distant metastasis status. Furthermore, it exhibited significant correlations with both RFS and OS in the univariate Cox regression analysis. However, a significant difference was observed only when comparing AJCC stage IIIA versus stage IIIC. AJCC stage IIIA versus stage IIIB showed no significant difference in multivariate analysis. Additionally, since my study focused on stage III melanoma patients with microscopic SLN metastasis, I selected lymph node capsule invasion and the size of the largest SLN metastasis as covariates. This information, among other factors, is crucial in deciding whether to perform

or offer complete lymphadenectomy in patients with positive SLN. Complete lymph node dissection is now being performed less frequently after several studies showed no benefit on OS [4,37,38]. In my exploratory analysis, capsule invasion showed a significant correlation only with OS, not with RFS. In contrast, the size of the largest SLN metastasis was significant for both RFS and OS. Other variables, such as ulceration of the primary, could have been considered, as this variable was also significant for RFS and OS in the univariate exploratory analysis. However, I could not include too many variables in the final multivariate Cox regression model due to the model's transparency. Therefore, I excluded variables included in the AJCC stage, such as ulceration of the primary tumor. Mitotic rate is no longer part of the classification in the eighth revision of the AJCC staging system, mainly because the information given by histopathologists is often not very precise[32]. In fact, in my study, mitotic rate > or ≤ 1mm² did not significantly affect RFS or OS in univariate Cox regression analysis. Therefore, I did not include it in the multivariate Cox regression model. In addition to the prognostic factors listed above, in the literature, older patient age, male gender, and melanoma location on the trunk or head and neck are associated with poorer prognosis of melanoma patients[33,34]. In my study, I did not find a significant gender difference, concerning RFS and OS but a difference in age > 65 years versus ≤ 65 years at first diagnosis for both, RFS and OS. Therefore, I included age as a covariate in the model. As described above, I also included whether a patient received interferon-α as adjuvant therapy after SLN dissection, as this might affect the patient's outcome.

To test whether each blood parameter improved the performance of the multivariate model, we used ROC analysis again, comparing models with and without the blood parameter. Finally, we explored combined use of blood parameters for RFS. Since only one marker (dNLR) remained for OS, no combinations were tested there. For RFS, combinations of NLR, LMR, and CRP were evaluated in both univariate and multivariate Cox regression to assess whether certain combinations provided greater predictive power than individual markers. This approach reflects a growing interest in composite biomarkers, as individual parameters may only partially capture the complexity of systemic inflammation and immune status in melanoma patients.

4.4. Immunsystem und maligne Tumoren

The immune system can play an essential role in tumorigenesis, disease progression and overall survival [39-41]. These mechanisms are extremely complex, and I can only give a brief overview of some important aspects in my thesis regarding the potential prognostic value of specific blood cells and components and their ratios. First, I will discuss how the immune system can play a role in tumor formation. Secondly, I will explore what happens to the immune system after cancer has developed—when a cell has mutated and is dividing uninhibitedly—

and how the knowledge of this process can aid in the search for new prognostic blood values in cancer patients.

According to Aggarwal et al., only 5%–10% of all cancers are caused by the inheritance of mutated genes and somatic mutations, whereas 90%–95% can be linked to lifestyle and environmental factors. These external factors include smoking, diet, obesity, and (chronic) infections[42]. Chronic infections can lead to chronic inflammation, which is pro-tumoral. For example, hepatitis B or C infection can lead to hepatocellular carcinoma, and persistent Helicobacter pylori infection is associated with gastric cancer and the presence of mucosa-associated lymphatic tissue (MALT) lymphoma [40]. While on the one hand, cancer can be induced by persistent inflammation, on the other hand, solid tumors can recruit immune and inflammatory cells into the tumor microenvironment (TME) and cause chronic inflammation [40,41]. Under normal circumstances, and especially in early tumor stages, this immune response eliminates tumorigenesis at initiation. This is called immune surveillance [41]. However, the generated TME with a large variety of immune cells is in a delicate balance between pro- and anti-tumoral effects [43]. For example, a high infiltration of neutrophils correlates with a worse prognosis for several types of cancer. They secrete cytokines, chemokines, reactive oxygen species, reactive nitrogen species, nitric oxide, and matrix metalloproteinases [44,45] among others, which enhance tumor angiogenesis, tumor growth, and metastasis [46]. Although neutrophils are often reported to be pro-tumoral (mainly in late tumor stages), they can also exert anti-tumoral effects (especially in early tumor stages) [39,40,47]. Lymphocytes on the other hand are recognized for their primarily anti-tumoral effects [48,49]. Cytotoxic T cells (CTLs) can identify tumor cells through their presented antigens and eliminate them using secreted molecules such as perforins and granzymes or by binding their death ligands and inducing apoptosis [50]. T helper cells (Th cells) play a crucial role in orchestrating immune responses against cancer by secreting cytokines that activate other immune cells, aiding in the elimination of tumor cells [51]. Regulatory T cells (Tregs), on the other hand, suppress immune responses, which can lead to the promotion of tumor growth [52,53].

The leukocytes described above, along with other immune and inflammatory cells, are recruited by the tumor, surrounding tissue, and previously recruited immune cells [40]. The TME and the balance of inflammatory cells, proteins, and enzymes in the blood of a cancer patient can provide insight into the nature of the malignancy. Therefore, they have been subject to several studies. Following, I will discuss some of these studies and compare them to my study, in which I analyzed several blood values for their potential prognostic effect on RFS and OS.

4.5. Analysierte Blutwerte

Several studies are investigating new serum biomarkers for prognostic purposes, but there is no consensus on their clinical utility. The only serum biomarker currently used as a prognostic

factor for melanoma patients with distant metastasis (stage IV) is LDH. LDH is included in the AJCC melanoma classification where it helps to estimate the prognosis of stage IV melanoma patients [32]. It is also known as a negative predictive marker for PFS in melanoma patients treated with ICI [54]. In my study, I assessed the LDH serum level within a maximum of 15 days prior to the sentinel lymph node biopsy (SLNB). It showed a significant correlation with RFS and OS in univariate Cox regression analysis. However, I excluded it from my further analyses because I only had data from 53 patients (38% of the cohort), which would have significantly reduced the study size.

The blood values that I examined were those of leukocytes in their entirety, as well as different subtypes of leukocytes (lymphocytes, neutrophils, eosinophils, and monocytes), platelets, the C-reactive protein (CRP), lactate dehydrogenase (LDH), and the ratios of some of these. These included the neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-to-lymphocyte ratio (dNLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR).

A manual parameter selection was applied to these blood parameters, guided by a set of statistical and clinical exclusion criteria. This approach was taken to ensure parsimony of the multivariate model, as a parsimonious model is more likely to generalize well to new data, and to reduce multicollinearity in order to ensure that each parameter in the model provides unique and independent information. Separate exclusion criteria were applied for the prognostic potential of the aforementioned blood values for RFS and for OS. Firstly, blood variables were excluded if they were not significant for the mentioned endpoints in univariate Cox or multivariate Cox regression analysis. Secondly, as previously stated, LDH was excluded from the analysis due to the limited availability of data. This was done to ensure that the model was built on a more complete dataset. Nevertheless, there is a possibility that the exclusion of a specific parameter may result in a less complete understanding of the prognostic factors involved. Thirdly, when absolute blood counts were available, relative counts were excluded, as absolute values are regarded as providing superior diagnostic support. have been suggested to provide better diagnostic support [55]. However, this may not always be the case, and valuable information provided by relative values might be overlooked. The fourth and final reason for excluding a blood variable from further investigation was when there was a high pairwise Pearson correlation ($r \geq 0.7$) between two blood values. In this case, the weaker parameter regarding the first three exclusion criteria 1-3 was dropped. After applying the four clinical and statistical exclusion criteria in terms of RFS, the following values remained and were further investigated for their potential prognostic value for recurrence: neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and C-reactive protein (CRP). Following the application of the exclusion criteria for OS, the derived neutrophil-to-lymphocyte ratio (dNLR), the absolute leukocyte count and the absolute neutrophil count were identified as remaining values and were subjected to further investigation.

To enable the clinical application of these blood values as a biomarker, I determined cut-off values for these blood parameters by comparing sensitivity and specificity using Receiver-operating characteristic curve (ROC) curves. I assessed the influence of various potential values using Kaplan-Meier estimates and further analyzed their effects through univariate and multivariate Cox proportional hazards models. This approach enabled me to dichotomize the parameters, classifying them into two groups based on their respective cut-off points. However, it is important to acknowledge that contrasting sensitivity and specificity to establish cut-off values can be somewhat subjective: different researchers might prioritize different aspects of the ROC curves and thus come to slightly different thresholds. For instance, the development of the CRP cut-off value underlines this challenge. There, the ROC analysis did not yield a clear cut-off value, with potential values ranging between 3.0 and 5.0. Ultimately, the cut-off value was set at 3.0, as this provided the most balanced distribution between the two groups. This decision, while methodologically sound, reflects the inherent subjectivity in selecting cut-off values. By using this method, I have ensured that the selected cut-off points are clinically relevant and practical to use despite the subjective elements involved. This approach enhances the utility of these blood parameters in routine clinical practice, allowing better patient stratification and more personalized treatment strategies.

In my study, I collected blood samples, which were drawn within 15 days prior to SLNB. The timing of sampling is critical, as it can influence inflammatory markers, which may correlate with tumor burden in lymph nodes or the primary tumor. Analyzing blood samples taken after the primary tumor removal and before the SLNB, I controlled for the potential confounding factor of the primary tumor. By including the diameter of the SLN in my multivariate analysis, I aimed to control for this potential confounder. In the following, I will discuss the NLR, LMR, CRP and dNLR more closely and compare my results to already published information on the topic.

4.5.1. NLR

The NLR is the ratio of neutrophil granulocytes to lymphocytes. Neutrophils in blood and TME of cancer patients are often reported to be pro-tumoral [44,45] while lymphocytes are primarily described to have an anti-tumoral effect [48,49]. Therefore, an increased NLR is hypothesized to be associated with a poor prognosis in melanoma patients in most studies. [16,18,56]. Nevertheless, some studies have found that high numbers of neutrophils or a high NLR are associated with a favorable prognosis. This was particularly reported for early-stage melanoma [39,40,47].

Several studies have analyzed the prognostic significance of NLR in advanced melanoma, as well as its predictive value in patients undergoing treatment with ICIs or BRAF/MEK inhibitors. However, there is a limited body of research on the prognostic relevance of NLR and other

blood ratios in early-stage melanoma, particularly in patients who have not received adjuvant ICIs or signal transduction inhibitors. For example, Ma et al. conducted a retrospective study on 107 stage III melanoma patients to investigate the prognostic value of NLR on melanoma recurrence. Their research revealed a strong association between NLR ≥ 2.5 and shorter disease-free survival (DFS) [56].In my study, I also observed that a higher NLR, when treated as a continuous variable, was linked to an increased risk of progression and reduced RFS. This association remained significant in the univariate analysis. However, after adjusting for confounding variables in the multivariate Cox regression analysis, the dichotomized value (with a 3.5 cut-off) no longer demonstrated a significant association with RFS. Additionally, Ma et al., compared the full white blood count (WBC) in the low and high NLR groups. The study found that the white blood cell count was lower in the group with a lower NLR and a better prognosis. However, my study did not compare the blood values between the two NLR groups, above and below the cut-off of 3.5 [56]. In my analysis on the prognostic potential of the absolute leukocyte count on RFS and OS, I found it to be significantly associated with OS after adjusting for confounding factors, though. The observed differences could be attributed to slight differences in the patient cohorts. Both my study and Ma et al.'s focused on stage III melanoma patients, while mine exclusively included patients with microscopic SLN metastasis. Consequently, patients with cutaneous metastases or macroscopic metastasis in stage III, who are known to have a worse prognosis, were excluded from the study. Furthermore, patients who had undergone adjuvant therapy with ICIs or targeted therapy were excluded from the study.

In a retrospective analysis of 742 melanoma patients, Lino-Silva et al. analyzed the NLR in patients with stage I to stage III melanoma. They were the first to explore the use of NLR in localized melanoma stages. The study revealed that an NLR ≥ 2.0 can serve as a prognostic marker for reduced OS. However, in the subgroup analysis, this relationship was found to be significant only for stage II patients. Furthermore, the study identified a correlation between NLR ≥ 2.0 and both lymph node metastasis and recurrence [18]. In my study population, high continuous NLR values were significantly associated with shorter RFS and OS in the multivariate analysis. In order to identify a suitable cut-off value for the NLR in terms of its prognostic potential for RFS, a ROC curve analysis was conducted, contrasting sensitivity and specificity. Additionally, Kaplan-Meier survival analysis was employed to assess the effects of various cut-off values. The Kaplan-Meier analysis demonstrated significant results for cut-off values ranging from 2.8 to 3.8 (in 0.1 steps). One exception was the cut-off value of 3.0, which was not significant with a p-value of p=0.052. The cut-off values of 3.3 and 3.4 demonstrated the greatest significance with p-values of p=0.012. To facilitate the clinical use of the NLR, I rounded the cut-off value up to 3.5. The decision to round the cut-off value to 3.5 was made

with the intention of enhancing the clinical utility of the NLR by providing a simpler and more memorable threshold. Had the initial cut-offs been adhered to, different results may have been obtained. Ultimately, the dichotomized NLR with a cut-off of 3.5 demonstrated no significance in the multivariate Cox regression analysis for RFS. In contrast, a high derived NLR (dNLR), which was not evaluated by Lino-Silva et al., demonstrated a stronger and more significant association with reduced OS than elevated NLR.

Robinson et al., conducted the most extensive study to date on the value of baseline NLR as a potential prognostic factor in stage I-III melanoma patients. The study comprised 1077 patients with negative SLN, 274 with microscopic and 138 with macroscopic SLN metastasis. However, no significant link was found between NLR and recurrence risk. The authors reported a relationship between the baseline NLR and the volume of melanoma at presentation, providing potential prognostic information about occult metastasis. In their study, NLR levels increased with tumor stage, being lowest in patients without lymph node metastasis, higher in those with microscopic metastasis, and highest in cases of macroscopic metastasis [16]. In my study, multivariate analysis showed a significant association between NLR and both RFS and OS. Additionally, in my study, I found the size of the largest SLN metastasis to be associated with RFS and OS in univariate analysis and that a large SLN metastasis was associated with worse overall survival, even after adjusting for confounding factors such as the AJCC-stage or the dNLR in multivariate analysis. My multivariate Cox regression model incorporated the diameter of SLN metastasis as an indicator of tumor burden at the time of diagnosis and still found NLR to be an independent prognostic predictor for recurrence and long OS, irrespective of the SLN diameter.

As previously mentioned, some studies have reported contradictory findings regarding neutrophils, lymphocytes, and their ratio. For example, Wade et al. reported worse OS and melanoma-specific survival (MSS) for patients with a baseline NLR below 2.5 and a baseline platelet-to-lymphocyte ratio (PLR) below 100, which is contrary to most studies and my results. Their study focused on early-stage melanoma patients with stages I-III. Wade et al. suggested that eliminating confounding factors is critical in low tumor stages, as this association was evident only in the multivariate analysis and not in the univariate analysis. However, there is no consensus on confounding factors, which can lead to differing results. They propose that high inflammatory blood values in early stages may represent the host inflammatory response to melanoma, similar to Pęczek et al.'s description of the natural immune response to a new tumor trying to eliminate it in its early stage [41].

However, it is still necessary to discuss and understand the precise response mechanism in each stage. It is worth noting that NLR and dNLR only showed a significant effect on OS after adjusting for confounding factors. Unlike the findings of Wade et al., my analysis of the stage III cohort with microscopic SLN metastasis demonstrated that elevated NLR or dNLR was

associated with shorter OS, rather than lower levels. These opposing results may be due to the different tumor stages investigated. In this study, I focused on patients with stage III melanoma. It is worth noting that Wade et al., included patients with stage I-III melanoma, but the majority of their sample (890 patients) were in stage I. Only 184 patients with stage II and 274 patients with stage III melanoma were included [39].

4.5.2. dNLR

The dNLR is a variation of the NLR. Its predictive potential on recurrence, survival, and response to adjuvant therapy was explored in studies with advanced melanoma patients. Ferrucci et al. retrospectively examined ICI-treated advanced melanoma cases and found a significant association between high levels of dNLR (≥3.0) and high levels of absolute neutrophil counts (≥ 7500) with increased risk of death and disease progression. Patients with both high dNLR and high absolute neutrophil count had a worse prognosis. The patients received either ipilimumab or nivolumab. According to Ferrucci and colleagues these biomarkers may therefore be useful in predicting response to ICI treatment. [22]. In a retrospective analysis of 97 ICI-treated advanced melanoma cases, Capone et al. also examined inflammatory markers. In the univariate analysis, the continuous variables of the absolute neutrophil count, NLR, dNLR, and LDH were found to be significantly associated with OS. However, in the multivariate analysis, only NLR and LDH (continuous variables) maintained this significance. Concerning cut-off values, the study showed that patients with stage IV melanoma and an NLR ≥ 5.0 or a dNLR ≥ 3.0 had significantly worse OS and PFS than those with low NLR and dNLR [23].
My study offers partial support for these findings, although I identified different cut-off values and focused on operable stage III melanoma patients who did not receive ICI treatment. I found that a dNLR of ≥ 2.0, compared to < 2.0, was one of the strongest independent predictors of shorter OS. Interestingly, in my analysis, the dNLR as a continuous value was not associated with RFS, although the NLR was. The difference can be explained by the fact that I assessed the time till recurrence without ICI and most patients after progression received ICI which influenced OS. Considering this, my results are very similar to those of Ferruci et al.

It should be noted though, that there is no clear consensus in the literature on how to calculate the dNLR. Ferruci and his team calculated the dNLR using the same method as I did: dNLR = absolute neutrophil count / (absolute leukocyte count – absolute neutrophil count) [22]. This is the most commonly used method to my knowledge [57]. Capone et al., calculated the dNLR by subtracting the absolute lymphocyte count from the absolute leukocyte count and dividing the absolute neutrophil count by this result (dNLR = absolute neutrophil count / (absolute leukocyte count - absolute lymphocyte count)) [23].

The NLR, on the other hand, is calculated as the ratio of the absolute neutrophil count to the absolute lymphocyte count. Therefore, the dNLR is influenced not only by neutrophil and lymphocyte counts but also by all the other white blood cell subtypes, with monocytes forming a significant portion. As monocyte levels are often lower in patients with malignancies, this may have resulted in an inaccurate calculation of the dNLR.

4.5.3. LMR

Similar to the previously discussed inflammatory markers, several retrospective studies have been published on the prognostic and predictive potential of LMR in advanced melanoma patients. These studies have shown a significant association between an increased LMR and better OS [19,58-60]. However, there is a limited number of studies examining the role of the LMR in localized cutaneous melanoma. In my research, a low LMR was identified as an independent prognostic factor for shorter RFS. Similarly, in univariate analysis, Wang et al. observed that a LMR ≤ 7.38, along with surgery, was significantly linked to poorer PFS and OS in patients with mucosal melanoma. However, in their multivariate analysis, this only remained significant for OS[17]. Intriguingly, my study did not find a significant association between the LMR and OS but between the LMR and RFS. Contrary to the findings of Wang et al., and my own results, Wade et al., described a low LMR (and high NLR) that correlated with primary melanoma regression in stage I-III patients [39]. These differing results could be explained by the fact that Wade et al.'s study population includes different low-stage melanoma patients (stage I-III), compared to my study, which only included patients with microscopically detectable stage III melanoma. Gandini et al., observed higher leukocyte counts, neutrophil counts, and monocyte counts, as well as lower lymphocyte counts in patients with distant metastases compared to stage I-III patients. Having a closer look at these stage VI patients, they described a significant association of leukocyte counts, neutrophil counts and monocyte counts, NLR and LMR with OS also after adjusting for confounding factors (age, sex, metastatic stage, presence of brain metastasis and treatment). They suggested that advanced tumor stages may have stronger predictors for shorter OS, such as high neutrophil, low monocyte, and low lymphocyte counts, than earlier tumor stages [60]. My findings indicate an association between an elevated relative lymphocyte count and decreased LMR with shorter RFS, but I did not observe any associations of these with OS. This could be due to various factors. For one, it might be possible that a high relative lymphocyte count and low LMR are biologically related to disease progression and recurrence risk but may not be strong enough to impact OS. Additionally, limitations in sample size and the duration of follow-up could contribute to the observed differences. Furthermore, the presence of other confounding factors, not accounted for in the analysis or measured in the study (for example the type of systemic therapy after progression) could mask the relationship between LMR/ relative lymphocyte count and OS.

4.5.4. CRP

In my studied cohort, CRP emerged as the most significant independent prognostic factor for RFS. The current body of literature regarding the utilization of CRP as a prognostic biomarker for melanoma progression is limited. Fang et al. demonstrated a significant association between CRP levels - both as a continuous baseline variable and when dichotomized into categories of <10 mg/L and ≥10 mg/L - with reduced OS and MSS across all stages of melanoma. This association persisted in the stage III/VI subgroup analysis. In the subgroup analysis of stage I/II, there was an association between CRP ≥10 mg/L and progression. In my study, a baseline CRP level greater than 3.0 mg/L was identified as a strong predictor of shorter RFS, but not for OS, which contrasts with the findings of Fang et al. This discrepancy may arise from Fang et al. combining patients with stage III and stage IV melanoma into a single subgroup, whereas my analysis specifically focused on a cohort of stage III melanoma patients with microscopic SLN metastasis who had not received adjuvant therapy with ICIs or BRAF/MEK inhibitors. As already discussed for the LMR in the previous paragraph the lack of a significant association between CRP and OS, compared to CRP and RFS, in my study may also be attributed to factors within the study itself. The reason could be the study's limitations in sample size and follow-up period. Furthermore, confounding factors not measured and integrated into the study, such as the influence of subsequent systemic therapies could have affected the relationship between CRP and OS.

Previous studies commonly used a CRP cut-off value of 10 mg/L. However, I used a lower cut-off-value of 3.0 mg/L due to our laboratory's policy of reporting quantitative CRP levels down to 0.3 mg/L. CRP is primarily used as a detector for acute inflammatory processes, and some laboratories only report whether CRP is above or below the inflammation detection limit of 5.0 mg/L. I recommend the quantification of CRP levels even in the low range below 5.0 mg/L, as my identified optimal cut-off of 3.0 mg/L is below the standard detection limit for inflammation. Yoshida et al. conducted a study analyzing the effect of CRP on the adaptive immune system in patients with metastatic melanoma. They found that high serum CRP levels were significantly associated with shorter survival in their cohort. The authors investigated patients who underwent ICI therapy with nivolumab and ipilimumab. They suggest that high CRP levels create an immunosuppressive environment in melanoma. Furthermore, researchers not only consider CRP as a prognostic or predictive marker but also propose blocking CRP as a therapeutic strategy to enhance the effectiveness of immune checkpoint therapies in cancer [61]. I focused on the hypothesis that CRP could be a potential prognostic factor for patient outcomes, aiding in treatment planning and risk stratification. Therefore, I excluded patients who received ICI therapy. In my cohort, patients with high CRP levels showed an association with worse RFS. If CRP is a prognostic marker with no active effect on recurrence, or actively influences patient outcome and therefore might be a potential therapeutic target, is unclear.

4.6. Kombinationen von Blutwerten

The objective of this study was to evaluate the prognostic significance of various blood parameters, including CRP, LMR, and NLR, both individually and in combination, for predicting disease progression in melanoma patients. My analysis demonstrated that a composite score incorporating elevated CRP levels and reduced LMR values served as the most robust and statistically significant prognostic factor for melanoma recurrence. It is noteworthy that a combination of the three ratios (high CRP plus high NLR plus low LMR) was also significantly associated with a reduced RFS in multivariate analysis, but the score of high CRP plus low LMR alone showed a slightly higher hazard ratio (HR). The higher HR of high CRP plus low LMR and the fact that NLR showed no additional value to predict recurrence suggests that determining CRP and LMR levels before SLNB could be a practical parameter to provide valuable insight into the likelihood of disease progression in melanoma patients.

4.7. Stärken und Limitationen

This study has limitations due to its retrospective design and its conduct at a single center. To more accurately evaluate the impact of the analyzed parameters on overall survival (OS), a larger-scale patient cohort with a longer follow-up period and therefore a higher incidence of death events would have been necessary. However, the study is notable for its meticulous focus on a uniformly staged patient cohort, limited specifically to stage III patients with microscopic sentinel lymph node (SLN) metastasis and no confounding adjuvant systemic therapies such as immune checkpoint inhibitors (ICIs) or BRAF/MEK inhibitors. This distinguishes it from other studies that encompass patients across melanoma stages I-III or those with stage III melanoma exhibiting both microscopic and macroscopic disease, as well as patients with only cutaneous metastases and those who have undergone various adjuvant therapies. Additionally, this study's strength lies in the inclusion of all relevant prognostic parameters in the multivariate analysis. It is worth noting that the study has a limitation in that it did not exclude patients who received adjuvant therapy with interferon-α. However, patients who underwent treatment with adjuvant PD-1 inhibitors or BRAF/MEK inhibitors, which are known to significantly influence RFS, were excluded. Potential interferon-α effects were considered in the study design by incorporating its treatment as a control variable in the multivariate Cox regression analysis, though.

4.8. Zusammenfassung und Schlussfolgerung

Taken together, this study demonstrated strong correlations of the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and C-reactive protein (CRP) with recurrence-free survival (RFS) in a cohort of stage III melanoma patients with microscopic sentinel lymph node (SLN) metastasis. These associations remained independent of other well-established prognostic parameters in my cohort. A significant finding of this study was that CRP exhibited the strongest association with RFS compared to the other parameters evaluated. Despite the unique strength of CRP, this study advocates the combined consideration of CRP and LMR, as this combination demonstrated the greatest potential for predicting melanoma progression. Consequently, I propose the inclusion of these variables in the comprehensive assessment of a patient's risk of recurrence to guide decisions regarding closer monitoring or the potential need for adjuvant therapy. However, recognizing the imperative need for scientific rigor, I emphasize the necessity for further investigation in more extensive, prospectively gathered patient populations to corroborate and strengthen the robustness of my findings. This will, in turn, enhance our understanding of prognostic biomarkers in melanoma patients presenting with microscopic SLN metastasis.

This study is unique in that it is the first to compare the prognostic potential of several blood parameter ratios in addition to CRP in melanoma patients with microscopic SLN metastasis. The study's approach is robust, emphasizing a uniform patient cohort, which sets it apart from prior research. Coherence is crucial in mitigating potential confounding factors introduced by varied tumor stages, which may have influenced outcomes in other studies which included different tumor stages. Additionally, this study assessed and compared more biomarkers than previous studies. Furthermore, this analysis revealed that CRP –especially in combination with the LMR- has a greater prognostic value for recurrence-free survival (RFS) than the extensively studied neutrophil-to-lymphocyte ratio (NLR) in this specific patient cohort.

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