Prognostic value of blood cell count-derived ratios in BRAF-mutated metastatic melanoma

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Background. The treatment and prognosis of metastatic melanoma have changed during the last decade to include immunotherapy or targeted therapy as standard therapeutic options for *BRAF*-mutated melanoma. However, predictive and/or prognostic markers are lacking, especially in clinical situations where several options are available. The aim of this study was to determine the association of pre-therapeutic blood cell count-derived ratios (BCDR) with survival in patients with *BRAF*-mutated metastatic melanoma.
Methods. We evaluated the prognostic role of BCDR in therapy-naïve patients with *BRAF*-mutated metastatic melanoma treated with immune checkpoint inhibitors or targeted therapy. The impact of BCDR on survival was analysed using univariate and multivariate Cox proportional hazard models.
Results. We enrolled 46 patients treated with BRAF inhibitors and 20 patients who received anti-PD-1 checkpoint inhibitors. The median progression-free survival (PFS) and overall survival (OS) were 8.3 and 18.2 months, respectively, with no statistical difference between groups. The objective response rate was 39% (30% in the anti-PD-1 and 44% in the targeted therapy groups). Baseline BCDR values were associated with improved PFS and OS in the immunotherapy group. Only the platelet-to-lymphocyte ratio (PLR) was associated with OS and PFS in the targeted therapy group. Independent prognostic indicators for PFS were lactate dehydrogenase, PLR and the lymphocyte-to-monocyte ratio (LMR) and those for OS were LMR, toxicity and the number of initial metastases.
Conclusion. BCDR had a substantial prognostic value in patients with *BRAF*-mutated metastatic melanoma treated with immune checkpoint inhibitors. However, a prognostic role for BCDR seemed less apparent in patients treated with targeted therapies.

Key words: melanoma, immunotherapy, targeted therapy, prognostic factors

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INTRODUCTION

Treatment of advanced/metastatic melanoma has dramatically changed during the last decade with the introduction of the immune-checkpoint inhibitors anti-CTLA-4 in 2010 (ref.[1]) and later anti-PD-1 (ref.[2,3]). The subsequent introduction of selective inhibitors of the B-Raf proto-oncogene *BRAF* (BRAFi), such as vemurafenib, dabrafenib, and encorafenib[4-6], and their combination with mitogen-activated protein kinases (MEK) inhibitors, such as cobimetinib, trametinib, and binimetinib[6-8], have opened up further targeted treatment possibilities for a group of melanoma patients with *BRAF* mutations. These new therapeutics have significantly improved the prognosis of metastatic melanoma, but both immunotherapy and targeted approaches have their advantages and disadvantages.

The main benefit of targeted therapy lies in its rapid onset of action and robust objective response. However, a high percentage of treatment failure occurs due to acquired resistance[9]. By contrast, immunotherapy is effective regardless of the *BRAF* mutational status[10]; however, it carries a possible risk of irreversible immune-related adverse events. Unfortunately, to date, no randomised clinical trials have answered the question regarding the optimal sequence of BRAF/MEK inhibitor therapy and immunotherapy. Furthermore, no predictive biomarkers have emerged to aid in the decision-making process in the treatment of *BRAF*-mutated melanoma.

Several studies conducted in recent years have identified different ratios derived from blood cell counts that can provide reliable prognostic information[11-13]. However, these studies, and especially those involving patients with melanoma, are usually performed in patients undergoing immunotherapy[14-18]. In general, these studies are also limited to investigations of the neutrophil-lymphocyte ratio (NLR), with little emphasis on the platelet-lymphocyte ratio (PLR), the lymphocyte-monocyte ratio (LMR) or

the systemic inflammation index (SII) as prognostic indicators in advanced/metastatic melanoma. Even less is known about the association of all these blood cell count-derived parameters in melanoma patients treated with BRAF inhibitors.

The aim of the present study was therefore to determine whether pre-therapeutic blood cell count-derived ratios are associated with PFS and OS in patients with *BRAF*-mutated melanoma treated either with immunotherapy or with BRAF/MEK inhibitors.

METHODS

Patient selection

This retrospective cohort study included all treatment-naïve patients with metastatic malignant melanoma harbouring a *BRAF* gene mutation and undergoing treatment either with anti-PD1 therapy or BRAF +/- MEK inhibitors at the University Hospital Hradec Kralove between October 2012 and October 2020. All included patients had available haematological values. Patients were treated until disease progression or unacceptable toxicity.

The study was conducted following the principles of the Declaration of Helsinki. Because of the retrospective nature of the study, patient consent for inclusion was waived. No additional investigations, apart from routine laboratory tests, were performed.

Variables

For all patients, complete blood count (white blood cell count [WBC], platelets count [PLT] absolute lymphocyte count [ALC], absolute neutrophil count [ANC], absolute monocyte count [AMC]) and clinical data were collected before the treatment initiation and again at twelve weeks after the start of therapy. The collected characteristics included age, sex, adjuvant treatment and metastatic characteristics (such as location and number of affected organs and serum lactate dehydrogenase [LDH] levels). Histopathologic studies of the primary tumour (histology and Breslow thickness [mm]) were recorded.

NLR was calculated as NLR=ANC/ALC. PLR was calculated as PLR=PLT/ALC. LMR was calculated as LMR=AMC/ALC, and the systemic immune-inflammation index was calculated as SII=ANC×PLT/ALC. Changes in NLR, PLR, LMR, and SII were assessed at week 12 as an increase (yes vs no) from the initial value.

Treatments and radiologic assessments

Drugs were administered orally at the recommended dosages (dabrafenib: 150 mg b.i.d; Vemurafenib: 960 mg b.i.d.; trametinib: 2 mg/day; cobimetinib: 60 mg/day, nivolumab 3 mg/kg or 240mg every 14 days and pembrolizumab 200 mg every 21 days).

Tumours were assessed at baseline and then every 12 to 16 weeks. The clinical response was classified according to the Response Evaluation Criteria in Solid Tumours v1.1 (RECIST).

Progression-free survival (PFS) was calculated from the first cycle of treatment to disease progression, as documented by imaging, death (event) or last follow-up (censored). Overall survival (OS) was calculated from the first cycle of treatment to death (event) or last follow-up (censored). The cut-off date for analysis was 4 January 2021.

Statistical analysis

Descriptive statistics were used to summarise patient and treatment characteristics. The chi-square test or Fisher´s exact test and Mann-Whitney test were used to compare patient baseline characteristics and the parameters between the patients treated with anti-PD1 and BRAF +/- MEK inhibitors.

Univariate and multivariate Cox proportional hazard models were used to investigate associations of NLR, PLR, LMR and SII (or their increase in time from weeks 1 to 12) with survival (PFS and OS), adjusted for baseline characteristics (therapy, gender, age, time to metastatic disease, histology, presence of initial metastatic disease, number of metastatic sites, LDH and presence of toxicity). Results were presented as hazard ratios (HR) with 95% confidence intervals (CIs). The OS and PFS were assessed using Kaplan-Meier estimates.

Optimal thresholds for NLR, PLR, LMR and SII were determined in part based on accessible literature reviews[11-13] and were correlated to optimal cut-off points based on results from the ROC curve. The NLR, PLR, LMR and SII cut-off values were 3, 2, 169 and 800, respectively.

A *P* value of 0.05 or less was considered statistically significant.

RESULTS

Patient characteristics

Among the 120 patients treated for metastatic melanoma in the first-line setting, 66 patients harboured *BRAF* mutations, and all those patients were included in our study. The majority of those patients (46 patients) were treated with either BRAFi (vemurafenib, dabrafenib – 23 patients) or a combination of BRAFi and MEK inhibitors (vemurafenib, cobimetinib, dabrafenib, trametinib – 23 patients). Twenty patients received anti-PD-1 checkpoint inhibitors (nivolumab, pembrolizumab) in the first-line setting.

The baseline patient characteristics are summarised in Table 1. The median age at first treatment was 66.5 years in both groups, and 56% of patients were male. The most frequent histological type was nodular melanoma. A total of 17 patients (26%) had metastatic disease at initial diagnosis. Most of the patients (52%) had two or three afflicted organs, with lymph nodes (47 patients), subcutaneous tissue (35 patients) and lungs (28 patients) as the three leading sites. Comparison of immunotherapy and targeted therapy revealed an imbalance only in the number of involved metastatic sites, which was higher in the subgroup of patients treated with BRAF +/- MEK inhibitors (see Table 1).

Table 1. Basic characteristic of patients.

Baseline Characteristics	All patients		Patients with anti-PD-1 therapy		Patient with BRAFi +/- MEKi therapy		*P*
	n=66	(%)	n=20	(%)	n=46	(%)	
Age (median [range]); years	66.5 [35–80]		66.5 [35–80]		66.5 [34–86]		0.91*
Gender							1.0†
Male	37	(56)	11	(55)	26	(56)	
Female	29	(44)	9	(45)	20	(44)	
Histology type of primary melanoma							0.16†
Nodular	35	(53)	10	(50)	25	(54)	
Superficial	8	(12)	5	(25)	3	(7)	
Acral	2	(3)	1	(5)	1	(2)	
Mucosal	1	(2)	0	(0)	1	(2)	
Unknown or not evaluable	20	(30)	4	(20)	16	(35)	
Breslow thickness of primary melanoma (median [range];mm)	3.6 [0.2–12]		3.8 [0.2–12]		3.5 [0.7–25]		0.87*
Braf mutation V600							0.68†
E	51	(77)	15	(75)	36	(78)	
K	12	(18)	5	(25)	7	(16)	
A	2	(3)	0	(0)	2	(4)	
R	1	(2)	0	(0)	1	(2)	
Adjuvant therapy							0.027†
YES	15	(23)	1	(5)	14	(30)	
NO	51	(77)	19	(95)	32	(70)	
Initial metastatic disease							0.55†
YES	17	(26)	4	(20)	13	(28)	
NO	49	(74)	16	(80)	33	(72)	
Time to metastatic disease (median [range];months)	18.2 [0–171.5]		19.0 [0–168.6]		16.0 [0–171.5]		0.99‡
Number of initial metastatic sites							0.006†
1	16	(24)	10	(50)	6	(13)	
2 to 3	34	(52)	8	(40)	26	(57)	
4 and more	16	(24)	2	(10)	14	(30)	
Site of metastases							
Lung	28	(42)	6	(30)	22	(48)	0.28†
Liver	16	(24)	2	(10)	14	(30)	0.12†
Lymphnode	47	(71)	11	(55)	36	(78)	0.077†
Subcutaneous tissue	35	(53)	10	(50)	25	(54)	0.79†
Brain	7	(11)	0	(0)	7	(15)	0.092†
Bones	12	(18)	2	(10)	10	(22)	0.32†
Renal	5	(8)	0	(0)	5	(11)	0.31†
Spleen	3	(5)	1	(5)	2	(4)	1.00†
Other	11	(17)	3	(15)	8	(17)	1.00†
LDH elevation							0.33†
YES	43	(65)	12	(60)	31	(68)	
NO	14	(21)	6	(30)	8	(17)	
Not evaluable	9	(14)	2	(10)	7	(15)	
LDH (median, [range];ukat/L)	4.7 [2.5–20.6]		4.7 [2.9–20.6]		4.8 [2.5–19.4]		0.42*
Toxicity							0.06†
YES	38	(58)	8	(40)	30	(65)	
NO	28	(42)	12	(60)	16	(35)	
Treatment after failure							0.25†
YES	21	(32)	4	(20)	17	(37)	
NO	45	(68)	16	(80)	29	(63)	
Following therapy							
BRAF + MEK inhibitors	4	(18)	4	(100)	0	0	
Ipilimumab	7	(33)	0	(0)	7	(41)	
Chemotherapy	2	(10)	0	(0)	2	(12)	
Nivolumab + ipilimumab	2	(10)	0	(0)	2	(12)	
Nivolumab	6	(29)	0	(0)	6	(35)	
Best achieved response							0.23†
CR	9	(14)	4	(20)	5	(11)	
PR	17	(26)	2	(10)	15	(33)	
SD	16	(24)	6	(30)	10	(22)	
PD	24	(36)	8	(40)	16	(34)	

*Mann-Whitney Test, †Fisher exact test, ‡Logrank test; CR, Complete response; PR, Partial response; SD, Stable disease; PD, Progressive disease; BRAFi, B-Raf proto-oncogene inhibitor; MEK, Mitogen-activated protein kinases; PD-1, Program death 1.

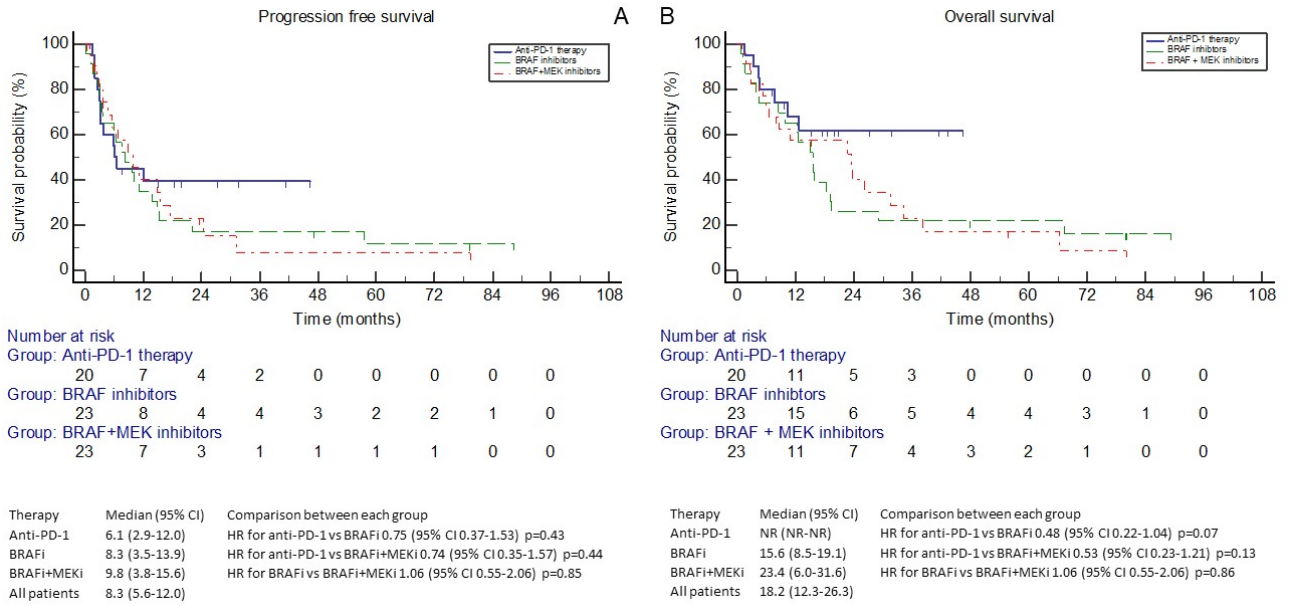

Fig. 1. Progression free survival (A) and Overall survival (B) based on therapy.
BRAFi, BRAF inhibitors; MEKi, MEK inhibitors; HR, hazard ratio; CI, confidential interval.

The median of follow-up was 19.1 (range 0.7–89.2) months. At the time of analysis, 23 patients were still alive (13 in the immunotherapy group and 10 in the targeted therapy group), and eight patients (6 in the immunotherapy group and 2 in the targeted therapy group) were still on therapy. The most frequent reason for therapy discontinuation was disease progression (12 patients in immunotherapy and 44 patients in targeted therapy group) and ten patients had to stop due to toxicity (2 in immunotherapy and 8 in the targeted therapy group).

The median PFS and OS for all patients were 8.3 (95% CI: 5.6–12.0) and 18.2 (95% CI: 12.3–26.3) months, respectively. Comparison of the three basic therapeutic approaches (anti-PD-1 versus BRAF monotherapy and BRAF plus MEK inhibition) revealed no statistical differences in PFS or OS. However, the median OS for anti-PD-1 has not been reached, and a statistical trend was apparent for better OS in the immunotherapy group than in the BRAFi monotherapy group (Fig. 1).

Overall, nine patients (14%) had a complete response (CR) (four in the anti-PD-1 group and five in the BRAF/MEK therapy) and 17 patients (26%) had a partial response (two in the anti-PD-1 group and fifteen in the targeted therapy group). Sixteen patients (24%) had stable disease and 24 patients (36%) had progressive disease (PD) Table 1. The objective response rate was 39% (30% in the anti-PD-1 therapy group and 44% in the targeted therapy group).

Association of blood cell count-derived ratios and their changes with PFS and OS

We transformed the continuous data into a dichotomous form by employing cut-off values. The distribution and frequency of the elevated and decreased cut-off values and their medians for every blood cell count-derived ratio are shown in Table 2. A significant difference was detected between the immunotherapy group and the targeted therapy group regarding the frequency of lower and higher cut-off values for LMR and a borderline significant difference was detected for PLR. No differences were noted between patients with increases in NLR, PLR, LMR and SII levels from the first to the twelfth week. The increase in the baseline levels at the twelfth week had no impact on PFS or OS.

The NLR, PLR, LMR and SII values were strongly associated with the OS in both treatment groups. The baseline values under (NLR, PLR and SII) or above (LMR) the cut-off values were associated with improved survival. In the immunotherapy group, all the factors were significantly associated with PFS. However, PLR was the only factor that showed a statistically significant association with PFS in the subgroup of patients treated with targeted therapy (Fig. 2a-d).

No statistical differences were found between the blood count-derived ratios in the anti-PD-1 and BRAF/MEK therapy groups. However, selection of the patients with worse prognosis based on blood count-derived ratios (i.e. those with NLR, PLR and SII above the cut-off values and with LMR below the cut-off value) resulted in a doubling of the median PFS in favour of targeted therapy over immunotherapy. By contrast, comparison of the two therapeutic approaches with low cut-off levels for NLR, PLR and SII and high LMR levels indicated that better results were achieved with immunotherapy.

Blood cell count-derived ratios and their prognostic impact on survival

The results of Cox univariate and multivariate analyses are presented in Tables 3 and 4. All blood cell count-derived ratios had a statistically significant impact on PFS and OS according to the univariate analysis. We then performed a multivariate analysis for PFS and OS that

Table 2. Blood cell count–derived ratio characteristics.

	Patients with anti-PD-1 therapy		Patient with BRAFi +/- MEKi therapy		*P*
	n=20	(%)	n=46	(%)	
NLR ≥ 3					0.17†
YES	7	(35)	27	(59)	
NO	12	(60)	19	(41)	
Not evaluable	1	(5)	0	(0)	
PLR ≥ 160					0.054†
YES	6	(30)	28	(61)	
NO	13	(65)	18	(39)	
Not evaluable	1	(5)	0	(0)	
LMR ≥ 2					0.04†
YES	17	(85)	28	(61)	
NO	2	(10)	18	(39)	
Not evaluable	1	(5)	0	(0)	
SII ≥ 800					0.29†
YES	7	(35)	24	(52)	
NO	12	(60)	22	(48)	
Not evaluable	1	(5)	0	(0)	
Increase of NLR [1st and 12th week]					0.16†
YES	11	(55)	16	(35)	
NO	7	(35)	25	(54)	
Not evaluable	2	(10)	5	(11)	
Increase of PLR [1st and 12th week]					0.57†
YES	9	(45)	16	(35)	
NO	9	(45)	25	(54)	
Not evaluable	2	(10)	5	(11)	
Increase of LMR [1st and 12th week]					0.58†
YES	8	(40)	22	(48)	
NO	10	(50)	19	(41)	
Not evaluable	2	(10)	5	(11)	
Increase of SII [1st and 12th week]					0.39†
YES	8	(40)	13	(28)	
NO	10	(50)	28	(61)	
Not evaluable	2	(10)	5	(11)	

*Mann-Whitney Test, †Fisher exact test; LMR, Lymphocyte-to-monocyte ratio; NLR, Neutrophil-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; SII, Systemic inflammation index; BRAFi, B-Raf proto-oncogene inhibitor; MEK, Mitogen-activated protein kinases; PD-1, Program death 1.

included all blood cell count-derived ratios, together with age, gender, type of therapy, number of initial metastases, time to metastatic disease and LDH baseline levels. The presence of toxicity was added as another variable in the OS analysis. This analysis identified only elevated LDH level as an independent prognostic factor for inferior PFS, but it was not a prognostic factor for OS. The other independent parameters associated with PFS were PLR and LMR; NLR showed a borderline statistical significance (HR 2.13; 95% CI: 0.99–4.56; *P*=0.05). The only hematologic parameter associated with OS was LMR (HR 0.31; 95% CI: 0.13–0.76; *P*=0.01). Our analysis revealed toxicity and the number of initial metastases as the only other independent factors associated with OS (Table 4).

DISCUSSION

In recent years, the range of therapeutic options for melanoma has been dramatically improved and has moved metastatic melanoma from a fatal disease to, in some cases, a chronic disease. Today, the standard therapeutic strategy is to use either immunotherapy or, in the case of *BRAF* mutant melanoma, targeted therapy[19].

Although these approaches have shown promising results, a substantial portion of patients still do not respond well. Furthermore, these therapeutics have significant toxicity. Modern oncology tries to identify patients most likely to benefit from these types of therapies but, unfortunately, we do not have many predictive markers for melanoma therapy outcomes. To date, the only validated predictive marker is the *BRAF* mutation[20]. However, we have several prognostic biomarkers for OS which might

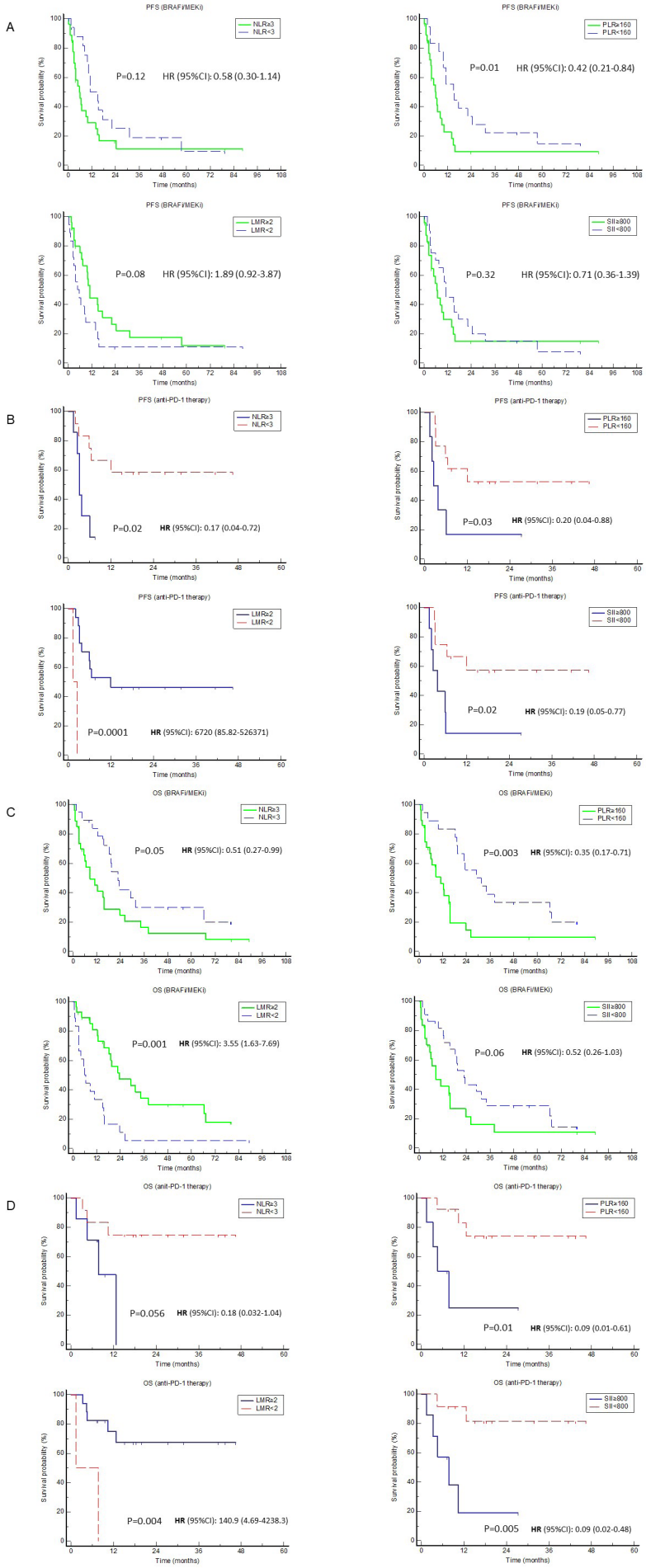

Fig. 2. Progression free survival for each blood cell count-derived ratio in patients with targeted therapy (A) and immunotherapy (B) and Overall survival for each blood cell count-derived ratio in patients with targeted therapy (C) and immunotherapy (D).
PFS, progression free survival; OS, overall survival; NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; LMR, Lymphocyte-to-monocyte ratio; SII, Systemic inflammation index; HR, Hazard ratio; CI, confidential interval; BRAFi, BRAF inhibitors; MEKi, MEK inhibitors.

Table 3. Univariate analysis.

	Progression free survival			Overall survival		
Variable	HR	95% CI	*P*	HR	95% CI	*P*
Age						
<65 / ≥65	1.02	0.58 to 1.84	0.92	0.84	0.45 to 1.58	0.59
Initial metastatic disease						
Yes vs. No	1.48	0.78 to 2.83	0.23	1.93	0.99 to 3.76	0.054
SII level						
≥800 vs. <800	1.83	1.02 to 3.26	0.04	2.54	1.38 to 4.68	0.003
Gender						
Female vs. Male	1.12	0.63 to 1.99	0.69	0.85	0.46 to 1.57	0.61
Number of initial metastases						
≥3 vs. <3	2.05	1.14 to 3.69	0.02	2.80	1.51 to 5.18	0.001
PLR level (cutoff 160)						
≥160 vs. < 160	2.48	1.37 to 4.48	0.003	3.27	1.73 to 6.17	0.0003
NLR level (cutoff 3)						
≥3 vs. <3	2.36	1.30 to 4.29	0.005	2.63	1.41 to 4.93	0.003
Therapy						
BRAFi +/- MEKi vs. anti-PD-1	1.34	0.69 to 2.58	0.39	2.09	0.92 to 4.71	0.077
LMR level (cutoff 2)						
≥2 vs. <2	0.47	0.26 to 0.85	0.01	0.29	0.15 to 0.53	0.0001
LDH level baseline						
≥ 3,75 vs. <3,75	1.12	1.04 to 1.19	0.001	1,16	1.08 to 1.25	<0.0001
Histology						
nodular vs other	0.81	0.45 to 1.43	0.46	0.94	0.51 to 1.72	0.84
Time from diagnosis to metastasis						
< 12 vs. ≥ 12 months	1.10	0.62 to 1.97	0.75	1.21	0.66 to 2.22	0.55
Toxicity						
Yes vs. No	0.57	0.32 to 1.02	0.06	0.49	0.26 to 0.92	0.03
Change in SII						
Yes vs. No	1.25	0.66 to 2.38	0.49	1.31	0.66 to 2.61	0.44
Change in PLR						
Yes vs. No	1.12	0.60 to 2.08	0.72	1.35	0.70 to 2.58	0.37
Change in NLR						
Yes vs. No	1.28	0.69 to 2.38	0.44	1.14	0.59 to 2.18	0.69
Change in LMR						
Yes vs. No	0.82	0.44 to 1.52	0.53	0.81	0.42 to 1.55	0.52

NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; LMR, Lymphocyte-to-monocyte ratio; SII, Systemic inflammation index; HR, Hazard ratio; CI, Confidential interval; LDH, Lactate dehydrogenase; BRAFi, B-Raf proto-oncogene inhibitor; MEK, Mitogen-activated protein kinases; PD-1, Program death 1.

be informative and helpful in planning melanoma therapy. One of the earliest discovered and now the most used and well-established biomarker is LDH (ref.[20]).

An urgent need exists for predictive or at least prognostic markers in situations where multiple treatment options are available. This is especially true if the treatment choices include immunotherapy and targeted therapy, and it becomes even more prominent in countries where the use of drugs is limited or regulated. Therefore, the search must continue for new markers that will allow treatment of patients with the most effective therapy. The parameters obtained from peripheral blood are logical candidates as biomarkers to determine the possible outcome and to monitor treatment effectiveness. Their clear advantage lies in the simplicity of obtaining the required sample. However, the ease of obtaining a sample is often nullified by the difficulty in interpreting the results.

Parameters such as NLR, PLR, LMR and SII are markers associated with the inflammatory process, and inflammation plays a key role in the pathophysiology of many diseases, including cancer[21]. Patients with advanced cancer usually show changes in peripheral blood cell composition that are suggestive of an expansion in the myeloid component (neutrophils, platelets, and monocytes) and a reduction in the lymphoid compartment[22]. These changes in peripheral blood reflect the situation in the tumour environment.

A common feature of cancer-associated chronic inflammation is neutrophilia, and its role in cancer pathogenesis is rather negative. Neutrophils have both

Table 4. Multivariate analysis.

	Neutrophil to lymphocyte ratio						Platelet to lymphocyte ratio					
	Progression free survival			Overall survival			Progression free survival			Overall survival		
Variable	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
NLR level (cut-off 3) ≥3 vs. <3	2.13	0.99 to 4.56	0.05	1.73	0.72 to 4.16	0.22	–	–	–	–	–	–
PLR level (cut-off 160) ≥160 vs. < 160	–	–	–	–	–	–	2.08	1.00 to 4.31	0.05	2.17	0.99 to 4.68	0.05
Age <65 vs. ≥65	0.92	0.46 to 1.84	0.81	0.50	0.22 to 1.13	0.09	1.09	0.53 to 2.26	0.82	0.51	0.23 to 1.15	0.11
Gender Female vs. Male	1.86	0.91 to 3.83	0.09	1.55	0.68 to 3.56	0.30	1.77	0.88 to 3.54	0.11	1.43	0.69 to 2.98	0.34
Number of initial metastases ≥3 vs. <3	1.83	0.85 to 3.92	0.12	2.93	1.26 to 6.82	0.01	1.91	0.90 to 4.03	0.09	2.61	1.15 to 5.95	0.02
Therapy BRAFi +/- MEKi vs. anti-PD-1	0.72	0.32 to 1.62	0.43	1.48	0.59 to 3.73	0.41	0.85	0.39 to 1.85	0.67	1.69	0.68 to 4.23	0.26
LDH level baseline ≥ 3.75 vs. <3.75	3.45	1.29 to 9.21	0.01	2.78	0.98 to 7.91	0.06	3.42	1.28 to 9.09	0.01	2.34	0.83 to 6.59	0.11
Time from diagnosis to metastasis < 12 vs. ≥ 12 months	1.00	0.99 to 1.01	0.99	0.99	0.99 to 1.01	0.68	1.00	0.99 to 1.01	0.49	1.00	0.99 to 1.01	0.82
Toxicity Yes vs. No	–	–	–	0.24	0.11 to 0.53	0.0004	–	–	–	0.23	0.10 to 0.52	0.0004

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Table 4. Multivariate analysis – continuation.

	Lymphocyte to monocyte ratio						Systemic infalammation index					
	Progression free survival			Overall survival			Progression free survival			Overall survival		
Variable	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
LMR level (cut-off 2) ≥2 vs. <2	0.38	0.16 to 0.91	0.03	0.31	0.13 to 0.76	0.01	–	–	–	–	–	–
SII level (cut-off 800) ≥800 vs. <800	–	–	–	–	–	–	1.38	0.68 to 2.79	0.37	2.14	0.99 to 4.66	0.55
Age <65 vs. ≥65	1.11	0.53 to 2.29	0.79	0.61	0.27 to 1.39	0.24	0.92	0.46 to 1.86	0.83	0.53	0.24 to 1.14	0.11
Gender Female vs. Male	2.18	1.01 to 4.71	0.05	1.91	0.86 to 4.23	0.11	1.59	0.79 to 3.19	0.19	1.52	0.72 to 3.20	0.27
Number of initial metastases ≥3 vs. <3	1.60	0.73 to 3.51	0.24	2.22	0.97 to 5.06	0.06	2.09	0.97 to 4.48	0.06	2.8	1.26 to 6.20	0.01
Therapy BRAFi +/- MEKi vs. anti-PD-1	0.65	0.28 to 1.50	0.32	1.17	0.46 to 3.01	0.74	0.85	0.39 to 1.86	0.68	1.67	0.67 to 4.14	0.27
LDH level baseline ≥ 3.75 vs. <3.75	4.12	1.53 to 11.08	0.005	2.46	0.92 to 6.59	0.07	3.36	1.24 to 9.12	0.02	2.24	0.79 to 6.28	0.13
Time from diagnosis to metastasis < 12 vs. ≥ 12 months	1.00	0.99 to 1.01	0.48	1.00	0.99 to 1.01	0.97	1.00	0.99 to 1.01	0.85	0.99	0.99 to 1.01	0.54
Toxicity Yes vs. No	–	–	–	0.24	0.11 to 0.53	0.0004	–	–	–	0.23	0.10 to 0.49	0.0002

NLR, Neutrophil-to-lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; LMR, Lymphocyte-to-monocyte ratio; SII, Systemic inflammation index; HR, Hazard ratio; CI, Confidential interval; LDH, Lactate dehydrogenase; BRAFi, B-Raf proto-oncogene inhibitor; MEK, Mitogen-activated protein kinases; PD-1, Program death 1.

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tumour-promoting and immune-suppressive roles and, together with the cytokines associated with tumour progression, neutrophils can suppress the activity of cytotoxic T cells and promote metastasis[23]. Some evidence indicates that platelets can guard tumour cells from immune elimination and promote tumour-cell transendothelial migration and metastasis. A variable secretion of growth factors by platelets could further support tumour growth, angiogenesis and metastasis[24].

A similar ambivalent role in carcinogenesis is played by the various subsets of monocytes, which serve as a source of dendritic cells and tumour-associated macrophages (TAMs) (ref.[25]). The TAMs are considered to promote tumorigenesis, thereby driving the negative outcome of various cancers[26]. By contrast, lymphocytes play an essential role in tumour-derived inflammatory responses, as these cells can induce antitumor activity with the help of cytotoxic cell death and the production of anti-proliferative cytotoxins. Relative lymphocytopenia therefore represents a significant decline in the cell-mediated adaptive immune response[27].

The best studied blood cell count-derived ratio associated with metastatic melanoma has been the NLR. Its usefulness has been evaluated mostly for immunotherapy, including both anti-CTLA-4 (ref.[14-16]) and anti-PD-1 therapy[17,18] or for a combination of both drugs[28]. Other parameters (PLR, LMR and SII) and their association with survival are less well studied, particularly in melanoma[29-31], and even fewer data are available regarding the usefulness of these ratios in metastatic melanoma patients treated with BRAF/MEK inhibitors, where again most information pertains to the NLR (ref.[32-34]). Our study confirmed the anticipated results for NLR and provided similar data for the rest of the blood cell count-derived ratios, where values above the cut-off for NLR, PLR and SII predicted reductions in OS and in PFS (ref.[35-37]). Conversely, for LMR, higher values were associated with better PFS and OS (ref.[31]).

However, the results were not as promising for targeted therapy. This is the first study to address the impact of PLR, LMR and SII on the prognosis of patients treated with targeted therapy for metastatic melanoma. The only factor showing a statistically significant effect on PFS was the PLR. The other parameters, even the NLR, were of no prognostic value. The prognostic impact of targeted therapy on OS was statistically more evident, but still not as convincing as the prognostic impact seen for immunotherapy. In addition, the possibility remains that this association with OS might be biased by subsequent immunotherapy administration following targeted therapy failure. A marked disbalance in the disease burden between the immunotherapy and targeted therapy arm could provide another explanation, as the patients treated with targeted therapy showed a larger number of metastatic sites.

We were unable to find any statistical difference between blood cell count-derived ratios and their association with PFS and OS in the immunotherapy and targeted therapy groups, but we did observe that patients with prognostically unfavourable NLR, PLR, LMR and SII ratios showed better survival outcomes when treated with BRAF/MEK inhibitors than with immunotherapy. Our multivariate analysis identified other factors which could be useful as prognostic factors, such as the PLR and especially the LMR, which kept its significance in the multivariate analysis for both PFS and OS. However, the significance of the NLR as a prognostic factor was not sustained in the multivariate analysis.

The dynamics of the blood cell count-derived parameters can also be useful for predicting survival, as has been shown for metastatic renal cancer[38], where dynamics evaluation proved to be an independent predictive and prognostic tool. However, the results are not that convincing for melanoma, as it is unclear if the absolute change or the relative change in blood cell count-derived ratios should be considered. Some melanoma studies have shown that an increase in the NLR of ≥ 30% after the initiation of therapy is associated with a shortened OS for immunotherapy[14,17], but not for targeted therapy[33]. We did not observe any correlation between the change in blood cell count-derived ratios and survival for either immunotherapy or targeted therapy, even if we correlated these results with the percentage changes from the baseline values (data not shown).

Some aspects make the interpretation of our results challenging, particularly the retrospective nature of the study, the small number of patients and the variability of the cut-offs for each parameter. No validation studies have yet established the best fitting cut-off for NLR, PLR, LMR and SII in melanoma or any other cancer types. This is why these parameters are still not widely used in a daily praxis and why they remain experimental. We have therefore tried to combine a literature review of articles related to the cut-offs of blood cell count-derived ratios with statistical procedures, such as ROC analysis.

The imbalance in the number of metastatic sites between our two cohorts could have been another factor that affected our results. The real-world circumstances, such as the presence of systemic inflammation and the use of corticosteroids, were stated as potential confounding factors[39]. Our patient cohort, however, had used no corticosteroids and showed no signs of inflammation. Nevertheless, all these clinical situations have to be taken into consideration when interpreting the NLR, PLR, LMR and SII results.

Conversely, our study's strength is that it reflects the real-world therapy decision approach. In contrast to other clinical studies on *BRAF* mutant melanomas, we included only treatment naïve patients to eliminate preceding therapy bias. This is the first study to evaluate all four blood cell count-derived ratios at the same time and in the same cohort of melanoma patients. To the best of our knowledge, this is also the first study to evaluate these parameters exclusively in *BRAF* mutant melanomas and to compare their prognostic impact in a cohort of patients treated with immunotherapy and targeted therapy.

CONCLUSION

Our results support a prognostic value of blood cell count-derived ratios (NLR, PLR, LMR and SII) in *BRAF* mutant metastatic melanoma patients treated with immune checkpoint inhibitors. However, the prognostic value of these parameters was less expressed in patients treated with targeted therapies. Therefore, the evaluation of baseline blood cell count-derived ratios should be considered in all patients treated with immunotherapy. Nevertheless, the knowledge of baseline values may aid in the decision-making process when both immunotherapy and targeted therapy are being considered as therapeutic options. Patients treated with BRAF/MEK inhibitors seem to have better prognosis than those on immunotherapy if the initial blood cell count-derived ratios are elevated (NLR, PLR and SII) or decreased (LMR). Larger prospective trials are warranted to make definitive conclusions regarding the prognostic significance of blood cell count-derived ratios in *BRAF*-mutated melanoma and to validate our proposed cut-off levels.

ABBREVIATIONS

ALC, Absolute lymphocyte count; AMC, Absolute monocyte count; ANC, Absolute neutrophil count; BCDR, Blood cell count-derived ratios; BRAF, B-Raf proto-oncogene; BRAFi, B-Raf proto-oncogene inhibitor; CIs, Confidence intervals; CR, Complete response; CTLA-4, Cytotoxic T-lymphocyte antigen 4; HR, Hazard ratio; LDH, Lactate dehydrogenase; LMR, Lymphocyte-to-monocyte ratio; MEK, Mitogen-activated protein kinases; NLR, Neutrophil-lymphocyte ratio; OS, Overall survival; PD, Progressive disease; PD-1, Program death 1; PFS, Progression-free survival; PLR, Platelet-to-lymphocyte ratio; PLT, Platelets count; PR, Partial response; RECIST, Response Evaluation Criteria in Solid Tumours; SD, Stable disease; SII, Systemic inflammation index; TAMs, Tumour-associated macrophages; WBC, White blood cell count.

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