Absolute and relative differential blood count predicts survival of AJCC stage I-II melanoma patients scheduled for sentinel lymph node biopsy

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ABSTRACT

Background/Objectives: Elevated neutrophil-to-lymphocyte ratio (NLR) in peripheral blood is associated with poor overall survival (OS) in metastatic melanoma patients receiving immunotherapy. However, the impact of peripheral blood cells in patients undergoing sentinel lymph node biopsy (SLNB) is still unclear. This study was intended to characterize the impact of peripheral blood leukocytic cells on overall survival (OS) in melanoma patients undergoing SLNB.

Methods: A total of 1412 AJCC stage I-II melanoma patients scheduled for SLNB at a single institution in the period 2010–2015 with available perioperative blood tests were randomly assigned to two independent cohorts. Associations of peripheral blood leukocytes with OS were analysed using Kaplan–Meier estimator and multivariate Cox proportional hazards model.

Results: NLR >4.26, absolute neutrophil count >5800/µL, relative neutrophil count >69.7% and relative lymphocyte count ≤17.5% were significantly associated with reduced OS in both cohorts. Absolute monocytes >810/µL, absolute eosinophils ≤200/µL, relative monocytes >6.6%, relative eosinophils ≤2.7% and relative basophils ≤0.6% were significantly associated with reduced OS in one cohort each. On multivariate analysis, a combined score including absolute levels of neutrophils, lymphocytes, monocytes and eosinophils was significantly associated with OS in both cohorts. The hazard ratio of patients with a risk score of 3–4 was 5.42 (95% confidence interval: 1.52–19.42, P = 0.0094) in cohort 1 and 9.42 (2.06–43.06, P = 0.0038) in cohort 2, respectively.

Conclusions: We conclude that peripheral blood leukocytes are independently associated with OS in stage I-II melanoma patients and should be considered as prognostic markers in these patients. Eosinophils and basophils deserve more attention in future investigations.

Key words: AJCC stage I-II, basophils, differential blood count, eosinophils, melanoma, neutrophil-to-lymphocyte ratio, sentinel lymph node biopsy.

INTRODUCTION

Melanoma is an aggressive form of skin cancer, related to early metastasis and rapid disease progression, once metastasized.1 It is well known that cancer can be associated with altered or defective myelopoiesis. Systemic inflammation and local inflammatory microenvironmental factors determine tumorigenesis, metastasis and disease progression.2–5 In metastasized stage IV melanoma, there is a clear association between increased levels of neutrophils or an increased neutrophil-to-lymphocyte ratio (NLR) and disease progression upon chemotherapy.6–9 In recent years, there is growing evidence that peripheral blood mononuclear cells are associated with response to immune checkpoint inhibitors and survival.10–14 In addition to neutrophils, monocytes and NLR, the absolute number and increasing levels of eosinophils seem to play an important role in the early phase of immunotherapy-induced anti-cancer response.15

However, there is little knowledge of the prognostic impact of differential blood counts in patients with...
localized stage I-II cutaneous melanoma. The aim of this study was to evaluate the prognostic relevance of differential blood cell counts in melanoma patients with localized disease undergoing curative total excision of their primary melanoma and diagnostic sentinel lymph node biopsy (SLNB).

PATIENTS AND METHODS

Patients

Medical records from 1475 consecutive patients with newly diagnosed cutaneous melanoma in AJCC stage I-II scheduled for SLNB at the Department of Dermatology, University Hospital Tubingen, Germany, were retrospectively reviewed. From these, 65 patients were excluded due to unavailability of a perioperative blood test. The 1412 patients with complete data were randomly assigned to two cohorts (703 patients in cohort 1, and 709 patients in cohort 2) based on odd or even ID number. This approach was used to create an identification cohort and a validation cohort. All patients were primarily diagnosed between 1 January 2010 and 51 December 2015. Follow-up was done prospectively at our outpatient clinics starting at the date of their first diagnosis up to 12 July 2018. Patients were staged according to the AJCC 7th Edition guidelines and were treated and followed up according to the German national guideline. Patients were included in the study if they had a histopathologically confirmed diagnosis of cutaneous melanoma, if they were rendered eligible for SLNB, and if a complete blood count was performed perioperatively. In all patients, the blood draw was performed on the day after wide local excision with or without SLNB. Patients with disease recurrence of a previously resected melanoma were excluded. This study conforms to the Helsinki Declaration of 1975 and successive amendments. Approval to conduct this study was obtained from the ethics committee of University of Tubingen, Germany (reference number 286/2018BO2).

Blood counts

Absolute leukocytes and relative numbers of neutrophils, lymphocytes, monocytes, eosinophils and basophils were extracted from patients’ records. Absolute numbers of the former named blood cells were calculated as the result of absolute leukocytes multiplied by the corresponding relative percentage and divided by 100% (e.g. absolute lymphocyte count \[ALC=\text{leukocytes} \times \text{relative lymphocyte count} \times |\%/100\%|\]). Ratios (neutrophil-to-lymphocyte ratio; NLR, and lymphocyte-to-monocyte ratio; LMR) were calculated as the ratios of the corresponding absolute blood counts, that is \[NLR=\text{ANC}/ALC\] and \[LMR=\text{ALC}/\text{AMC}\].

Statistical analysis

Overall survival (OS) was calculated from wide local excision of the primary melanoma to death (event) or last follow-up (censored). Distant metastasis-free survival was defined as the time between wide local excision of the primary melanoma to first occurrence of distant metastatic disease (event) or last follow-up (censored). Estimates of OS and distant metastasis-free survival were conducted with the Kaplan–Meier method. Associations of blood counts with OS or distant metastasis-free survival were tested with log-rank test (dichotomized variables) or with Cox regression (continuous variables). Hazard ratios (HR) together with 95% confidence intervals (95% CI) were calculated with Cox regression. Cut-off points to dichotomize variables were calculated utilizing R ‘maxstat’ package which makes use of \(P\) value minimization. All analyses were carried out with R, version 5.4.0, and the ‘survival’ and ‘maxstat’ packages. All reported tests were two-sided, and \(P\)-values < 0.05 were considered significant.

RESULTS

Patient characteristics

Between January 2010 and December 2015, 1412 patients fulfilled inclusion criteria and were randomly assigned to two cohorts (705 patients in cohort 1, and 709 patients in cohort 2) as described in the methods section. SLNB was performed successfully in 671 patients in cohort 1, and in 680 patients in cohort 2. Reasons for failure of SLNB in these 61 patients were either insufficient labelling of the draining lymph node, non-detection of the sentinel node intraoperatively or abort of the SLNB in case of risky situs conditions with sentinel nodes directly adjacent to major vessels or nervous structures. Interestingly, 56% (cohort 1) and 69% (cohort 2) of these cases had their primary melanoma located in the head and neck area (head, neck and face) and these patients were of significantly older median age (cohort 1: 75 vs. 65 years, cohort 2: 69 vs. 62 years) (data not shown). Table 1 lists detailed clinicopathologic factors and their association with overall survival (OS) in both cohorts. Median follow-up for survivors was 42.6 months (interquartile range: 51.4 to 65.4 months).

Survival analysis and associations with differential blood counts

Univariate survival analysis of the absolute and relative blood counts analysed as dichotomized based on optimized cut-off values, neutrophil-to-lymphocyte ratio (NLR) > 4.26 (cohort 1: \(P = 0.012\), cohort 2: \(0.0005\)), absolute neutrophil count (ANC) > 5800/µL (\(P = 0.019\), \(P = 0.001\)), relative neutrophil count (RNC) > 69.7% (\(P = 0.005\), \(P = 0.001\)) and relative lymphocyte count (RLC) ≤ 17.5% (\(P = 0.0013\), \(P = 0.0005\)) were significantly associated with reduced OS in both cohorts (Table 2). Total leukocytes > 7400/µL (\(P = 0.0057\), \(P = 0.088\)), absolute lymphocyte count (ALC) ≤ 1220/µL (\(P = 0.12\), \(P = 0.0076\)), absolute monocyte count (AMC) > 810/µL (\(P = 0.0004\), \(P = 0.38\)), absolute eosinophil count (AEC) ≥ 200/µL (\(P = 0.055\), \(P = 0.042\)), relative monocyte count (RMC) > 6.6% (\(P = 0.22\), \(P = 0.047\)), relative eosinophil

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Table 1  Descriptive statistics of clinicopathologic factors and association with overall survival

| Characteristic                          | Cohort 1 | Cohort 2 |
|----------------------------------------|----------|----------|
|                                        | n   | %  | HR (95% CI) | P  | n   | %  | HR (95% CI) | P  |
| **Age, years**                         |      |    |             |    |      |    |             |    |
| ≤50                                    | 178  | 25.3 | 1           |    | 190  | 26.8 | 1           |    |
| 50–60                                  | 124  | 17.6 | 1.15 (0.45–2.90) | 0.77 | 154  | 18.9 | 1.31 (0.58–2.97) | 0.52 |
| 60–70                                  | 168  | 25.9 | 2.25 (1.06–4.77) | 0.055 | 156  | 22.0 | 1.00 (0.45–2.31) | 0.99 |
| >70                                    | 253  | 33.1 | 3.54 (1.76–7.12) | 0.0004 | 229  | 32.3 | 2.66 (1.37–5.17) | 0.004 |
| **Histopathological subtype**          |      |    |             |    |      |    |             |    |
| Basal 474                               | 69.0 | 1   | <0.0001     |    | 472  | 68.0 | 1           |    |
| Lentiginous 511                         | 51.0 | 1   | 4.19 (2.02–6.70) |    | 222  | 32.0 | 4.05 (2.44–6.68) |    |
| Acral 215                               | 1.31 | 0.35 | 2.28 (0.88–5.94) | 0.00 | 56   | 5.8  | 1.15 (0.47–5.27) | 0.46 |
| **Localization**                       |      |    |             |    |      |    |             |    |
| Extremities 295                         | 41.7 | 1   | 4.19 (2.02–6.70) | 0.00 | 550  | 46.5 | 1           |    |
| Trunk 289                               | 41.1 | 1   | 1.74 (1.05–2.89) | 0.052 | 267  | 57.7 | 1.60 (0.91–2.79) | 0.10 |
| Head and Neck 78                       | 11.1 | 0.85 | 0.55 (0.35–2.08) | 0.72 | 72   | 10.2 | 2.09 (1.02–4.52) | 0.045 |
| Face 43                                 | 6.1  | 1.59 (0.65–5.88) | 0.51 | 40   | 5.6  | 1.42 (0.49–4.12) | 0.52 |
| **Sentinel node status**               |      |    |             |    |      |    |             |    |
| Negative 605                            | 89.9 | 1   | <0.0001     |    | 588  | 86.5 | 1           |    |
| Positive 68                             | 10.1 | 4.58 (2.57–7.47) | 0.0016 | 50   | 7.1  | 2.76 (1.22–6.24) | 0.015 |
| Adjuvant therapy                        |      |    |             |    |      |    |             |    |
| None 511                                | 1    |    |             |    | 501  | 1    |             |    |
| Interferon-alpha 189                    | 0.78 | 0.46 (0.15–1.51) |    | 206  | 0.92 | 1.00 (0.54–1.56) | 0.76 |
| Other 2                                 | 5.42 | 0.75 (0.39–9.27) | 0.09 | 2    | NA   | 1.00 |             |    |

ALM, acrolentiginous melanoma; CI, confidence interval; HR, hazard ratio; LMM, lentigo maligna melanoma; n, number of patients; NM, nodular melanoma; P, P value; SSM, superficial spreading melanoma.

*51 cases had missing information on ulceration (16 cases in cohort 1, 15 cases in cohort 2).

*178 cases had missing information on Clark level (90 cases in cohort 1, 88 cases in cohort 2).

*61 cases had missing information on sentinel node status (32 cases in cohort 1, 29 cases in cohort 2).

*4 cases with adjuvant treatment in the Combi-AD trial. Two patients received dabrafenib plus trametinib. One patient received placebo plus placebo, and 1 patient received either the combination of dabrafenib plus trametinib or placebo, but was not unblinded until the end of follow-up.

stage-specific analysis was conducted in all patients in a combined cohort with n = 1412 to increase statistical power. In AJCC stage I, AMC ≥ 5800/mL (P = 0.046), AMC ≥ 810/µL (P = 0.040), RNC ≥ 69% (P = 0.0086) and RBC ≤ 6% (P = 0.027) were significant prognostic factors for reduced OS (Table 5). Regarding stage I and stage II patients only, meaning patients with negative sentinel node, total white blood count (WBC), NLR, ANC, ALC, AMC, AEC, RNC, RLC, REC and RBC were significantly associated with OS with the highest HRs for both absolute and relative eosinophils (Table S2).
Combination of the four absolute blood cells counts that were significantly associated with OS in univariate analysis (ANC, ALC, AMC and AEC) yielded a score ranging from 0 (i.e. no risk factors present, ANC ≤ 5800/µL, ALC > 1220/µL, AMC ≤ 810/µL and AEC > 200/µL) to 4 (i.e. all risk factors present). The resulting absolute differential blood count score subdivided the cohort of patients into four subgroups with significantly different OS probability in both cohorts (both \( P < 0.0001 \)) (Fig. 1). In cohort 1, patients with a total score of 0 had a 95.4% (95% CI 90.3%–100%) 5-year survival rate compared to 88.2% (84.2%–92.4%) in patients with only one risk factor, 84.9% (78.7%–91.6%) in patients with two risk factors and 66.2% (51.9%–84.5%) in patients with three or four risk factors present. In cohort 2, 5-year survival rates were 95.4% (89.2%–100%), 90.5% (86.5%–94.5%), 84.0% (77.6%–90.9%) and 70.4% (57.5%–86.2%), respectively. A combined score of the relative differential blood cell counts also qualified as a suitable measure to stratify significantly differing subgroups (both \( P < 0.0001 \)) (Fig. 2). Five-year survival rates in cohort 1 were 100% (100%–100%), 90.9% (86.7%–95.4%), 86.0% (81.6%–90.5%) and 65.7% (51.9%–83.2%), respectively, and 100% (100%–100%), 92.7% (88.6%–97.0%), 85.5% (80.9%–90.5%) and 71.7% (59.3%–86.8%), respectively, in cohort 2.

### Calculation of a comprehensive blood count risk score

Combination of the four absolute blood cells counts that were significantly associated with OS in univariate analysis (ANC, ALC, AMC and AEC) yielded a score ranging from 0 (i.e. no risk factors present, ANC ≤ 5800/µL, ALC > 1220/µL, AMC ≤ 810/µL and AEC > 200/µL) to 4 (i.e. all risk factors present). The resulting absolute differential blood count score subdivided the cohort of patients into four subgroups with significantly different OS probability in both cohorts (both \( P < 0.0001 \)) (Fig. 1). In cohort 1, patients with a total score of 0 had a 95.4% (95% CI 90.3%–100%) 5-year survival rate compared to 88.2% (84.2%–92.4%) in patients with only one risk factor, 84.9% (78.7%–91.6%) in patients with two risk factors and 66.2% (51.9%–84.5%) in patients with three or four risk factors present. In cohort 2, 5-year survival rates were 95.4% (89.2%–100%), 90.5% (86.5%–94.5%), 84.0% (77.6%–90.9%) and 70.4% (57.5%–86.2%), respectively. A combined score of the relative differential blood cell counts also qualified as a suitable measure to stratify significantly differing subgroups (both \( P < 0.0001 \)) (Fig. 2). Five-year survival rates in cohort 1 were 100% (100%–100%), 90.9% (86.7%–95.4%), 86.0% (81.6%–90.5%) and 65.7% (51.9%–83.2%), respectively, and 100% (100%–100%), 92.7% (88.6%–97.0%), 85.5% (80.9%–90.5%) and 71.7% (59.3%–86.8%), respectively, in cohort 2.

### Multivariate analysis

The absolute differential blood count score was further assessed utilizing multivariate Cox regression analysis accounting for age, sex and the established pathologic markers Breslow tumour thickness, ulceration and pathologic SLN status (positive vs. negative). Age as a
continuous variable (cohort 1: \( P = 0.0018 \), cohort 2: \( P = 0.056 \), Breslow tumour thickness as a continuous variable \( P < 0.0001, P = 0.00026 \), presence of ulceration \( P = 0.0051, P = 0.0023 \), positive sentinel node (both \( P < 0.0001 \) and an absolute differential blood count score of 5 or 4 points \( P = 0.0094, P = 0.0058 \) were significantly associated with reduced OS (Table 4; multivariate analysis considering blood cell subtypes is summarized in Table S4). Moreover, patients with an absolute differential blood count score of at least 2 showed significantly impaired OS also in stage I-II patients with negative sentinel node (Table S5).

**DISCUSSION**

In this study, we ascertained a significant association of several subtypes of peripheral blood leukocytes with overall survival of melanoma patients without clinical evidence of metastatic disease, independent of well-established histopathological markers.
By searching the register of scheduled wide local excisions plus sentinel lymph node biopsies from 2010 to 2015, we were able to identify 1475 consecutive patients. Among these patients, complete blood count with white blood cell differential was available for 1412 patients (703 in cohort 1 and 709 in cohort 2). The survival rates of patients included in our study were slightly higher than those reported in the melanoma staging system, reflecting the improvement in systemic melanoma therapy in the recent past.1 However, the recognized and well-established markers primary tumour thickness, ulceration and histopathological status of the sentinel lymph node remained strong prognostic factors.

In contrast to previous studies showing prognostic significance in stage III or stage IV, the herewith presented results show that peripheral blood cell counts are also associated with survival in localized disease as early as in stage I and stage II.6,9,16 A small number of studies recently demonstrated that peripheral blood cell markers, namely neutrophil-to-lymphocyte ratio (NLR) and absolute monocyte count (AMC), play a prognostic role in high-risk stage II and/or stage III melanoma.16–18 However, these studies did not reflect the typical distribution of patients undergoing SLNB. For instance, stage III was overrepresented with 45.2%, 56.5% and 49.5%, respectively, meaning over one third to one half of patients had macro or micro metastases in the sentinel lymph node.16–18 Moreover, these studies included high percentages of high-risk melanomas with a median Breslow tumour thickness of 5.0 mm (Q1-Q3: 1.0–7.0 mm)17 or excluded patients with pT2a or lower.18

Different from previous studies, we analysed blood cell counts in stage I, stage II and disease-free stage III separately and observed associations with survival in each disease stage.16,18 Especially the relative lymphocyte count (RLC) with a threshold of ≤17.5% was significantly associated with impaired OS in stage I (P = 0.057), stage II (P = 0.0075) and stage III (P = 0.0064) patients. These data are the first to show an impact of peripheral blood cells on survival even in localized low-risk stage I melanoma with survival rates greater than 95%. Interestingly, in stage I patients only 12% or 29% of deaths occurred within the first year or within the first two years since diagnosis, respectively, showing a long-term rather than a short-term impact of peripheral blood cells. Associations of differential blood count with distant metastasis-free survival were calculated to account for possible confounders of altered OS according to peripheral leukocytes such as older age, myeloproliferative diseases or other types of cancer that typically affect OS, but not distant metastasis-free survival. The results for distant metastasis-free survival showed that the investigated blood cells are directly related to melanoma-specific outcome rather than depicting only the patients’ general health status.

In patients with metastatic melanoma receiving immunotherapy, high levels of neutrophils or the NLR or low levels of lymphocytes have been demonstrated as poor prognostic factors.10,15,14,19 Total leukocytes, monocytes and neutrophils have been found to be higher in non-responders to the CTLA-4 antibody ipilimumab.19 Moreover, during the first cycles of immunotherapy, the frequency of monocytic myeloid-derived suppressor cells (moMDSC) increases in non-responders, reflecting the strong general

Figure 2 Overall survival in stage I-III patients expressed by Kaplan–Meier estimator according to relative differential blood count score (DBC) in cohort 1, and in cohort 2. HR, hazard ratio; P, P value.

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immunosuppression in these patients which is at least in part induced by proteins belonging to the group of alarmins like S100A8/A9 and HMGB1.10

The findings of this study support the astonishing circumstance that peripheral cells of the myeloid and lymphoid lineage seem to play a role even in non-metastasized patients without evidence of disease. The complex interplay of the distinct subsets of myeloid immune cells has been shown to affect hallmarks of cancer like angiogenesis.4 Local and systemic inflammations are thought to promote tumorigenesis and disease progression5, and inflammation has been linked with cancer since the time of Virchow who noted leukocytes in neoplastic tissues and put inflammation into context of cancer.20 However, it is still unclear, whether an increased NLR or an increased total number of neutrophils is due to factors produced by the tumour cells or if they simply reflect a proinflammatory state of the host which could be due to a variety of inflammation-associated diseases such as rheumatoid arthritis, metabolic syndrome or other cancers. We speculate that, at least in a portion of patients with surgically resected high-risk melanoma, such shifts of the circulating immune cells could be induced by disseminated remnant tumour cells and their bystanders, thereby causing an upregulation of the innate immune system.21 It is considered ascertained that occult melanoma cells circulating in the blood are present in many melanoma patients and finally lead to disease progression with seeding of metastases.22-26 A proinflammatory state in circulation and in local tissues might facilitate this process. An interesting question is whether the hereby presented findings can only be considered a passive prognostic marker or if they constitute a potential therapeutic target which enables us to develop new strategies to manipulate this proinflammatory state and thereby overcome resistance to immune checkpoint inhibitor treatment or to prevent metastasis.20,27

Recent studies on stage I-III patients focused on NLR or AMC and did not present data on eosinophils16-18 although several studies highlighted the impact of this granulocyte subtype in the setting of immune checkpoint inhibition with CTLA-4 or PD-1 antibodies.10,13,14,28,29 Here, we show that high levels of absolute and relative eosinophils correlate with favourable survival in stage I-III patients without evidence of active metastatic disease. In recent years, mechanisms of eosinophils in cancer rejection have been uncovered showing these cells are not bystander cells but contribute substantially to anti-cancer immune responses. Eosinophils secrete chemoattractants including TNF, CCL5, CXCL9 and CXCL10 which enhance CD8+ T-cell infiltration and initiate macrophage polarization and normalization of tumour vessels which lead to tumour hypoxia.30

Similar results as for eosinophils were observed for relative basophils with higher levels being associated with favourable survival at least in one of the cohorts. Until recently, not much was known about the role of basophils in cancer. However, a recent report uncovered that basophils promote tumour rejection by producing CCL3 and CCL4 chemokines which enhanced CD8+ T-cell infiltration.31 This finding underlines our observation of elevated relative basophil levels in patients with favourable prognosis. Remarkably, a risk score combining the total neutrophils, lymphocytes, monocytes and eosinophils discriminated patients with excellent, intermediate and unfavourable OS. Most importantly, multivariate analysis showed that patients with a score of 2 or 3-4 exhibited a significantly unfavourable OS, which was independent of the covariates age, Breslow tumour thickness, ulceration and status of sentinel lymph node. These results show that blood cells of the routine differential blood count are independent prognostic factors for stage I-II patients undergoing SLNB. Future studies in patients receiving adjuvant therapy must clarify whether these blood cell counts have the quality of predictive

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### Table 4 Multivariate survival analysis of overall survival in the combined cohort

| Characteristic                          | n (%)   | HR (95% CI)   | P     |
|-----------------------------------------|---------|---------------|-------|
| Age†                                     | n     |               |       |
| Gender                                  |        |               |       |
| Female                                  | 595 (45.1) | 1.05 (1.01–1.04) | 0.0002 |
| Male                                    | 725 (54.9) | 1              |       |
| Breslow’s tumour thickness†              |        |               |       |
| Yes                                     | 1220 (95.8) | 1.20 (0.81–1.76) | 0.56  |
| No                                      | 378 (32.3) | 1.11 (1.07–1.16) | <0.0001 |
| Ulceration                              |        |               |       |
| No                                      | 1161 (88.1) | 1              | <0.0001 |
| Yes                                     | 279 (21.9) | 2.50 (1.71–5.65) |       |
| Sentinel Node Status                    |        |               |       |
| Negative                                | 157 (11.9) | 5.50 (2.54–5.21) | <0.0001 |
| Positive                                |        |               |       |
| 0 risk factors                          | 152 (11.5) | 1              |       |
| 1 risk factor                           | 697 (52.9) | 2.13 (0.85–5.57) | 0.11  |
| 2 risk factors                          | 562 (27.5) | 2.82 (1.11–7.16) | 0.029 |
| 3–4 risk factors                        | 107 (8.1) | 7.10 (2.69–18.77) | <0.0001 |

The absolute differential blood count (1 point each if ANC >5800/µL or ALC ≤1220/µL or AMC >810/µL or AEC ≤200/µL); CI, confidence interval; HR, hazard ratio; n, number of patients; P, P value.

Ninety-four cases were excluded due to missing data for at least one variable.

†Age and Breslow tumour thickness were analysed as continuous variables.
biomarkers. Recent studies showing a prognostic impact of the NLR in patients receiving immunotherapy are promising\textsuperscript{1,2,32,33}, but it is still unclear whether this marker or other blood cell counts possess only prognostic qualities or whether they are suitable for becoming predictive biomarkers for immunotherapy of cancer.

The fact that blood was drawn the morning after the operative procedure of local wide excision (with or without SLNB) can be seen critically. In patients undergoing extensive visceral surgery like pancreaticoduodenectomy (Whipple procedure) or oesophageal resection, leukocyte count rises starting on the first postoperative day.\textsuperscript{34,35} However, compared to these major surgical procedures associated with high perioperative morbidity and significant mortality, the procedure of local wide excision and SLNB is limited in regard of its extent and it is generally well-tolerated. Studies showed that morbidity following SLNB is low with major complications occurring only in rare cases.\textsuperscript{36,37} Importantly, all surgical procedures were performed using slow infusion tumescent anaesthesia at our centre.\textsuperscript{58} None of the patients received general anaesthetic drugs which might affect inflammatory markers. The median leukocyte count in our patients was comparable with preoperative leukocytes in patients undergoing pancreatic or oesophageal surgery.\textsuperscript{34,35} Median leukocyte count was 7.18 G/L, and only 75 of 1412 patients (3.3\%) had elevated leukocytes>11 G/L. Therefore, we conclude that excision and SLNB do not affect perioperative blood tests significantly.

Wide local excision and SLNB are usually performed within 2 to 6 weeks after primary excision with narrow safety margins. Thus, early increases in leukocyte count following primary excision will probably not affect the leukocyte count one day after SLNB. Even in extensive visceral surgery, postoperative leukocyte count reaches normal levels on the fourth postoperative day already.\textsuperscript{34}

In summary, our study shows significant associations of survival of melanoma patients with each leukocytic subtype. Eosinophils and basophils consider more attention in future clinical trials or laboratory studies that aim at uncovering further putative mechanisms. Differential blood count is associated with OS in high-risk melanoma patients as well as in patients with low-risk stage I melanoma. This demonstrates the prognostic relevance independent of disease stage and even in patients without evidence of metastatic disease. The data presented herewith were derived from an unselected, real-life cohort of consecutive melanoma patients scheduled for SLNB which makes them easily transferable to further investigations and to clinical practice. Peripheral blood cell counts should be considered as prognostic markers in stage I-III melanoma patients.

ACKNOWLEDGEMENTS

We thank the whole team of the melanoma unit for their passionate patient care and support in data collection. Open access funding enabled and organized by Projekt DEAL. [Correction added on 8 January, 2021 after first online publication: Projekt DEAL funding statement has been added.]

CONFLICT OF INTEREST

None.

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1. Univariate analysis of DMFS according to differential blood cell counts as dichotomized variables.

Table S2. Univariate analysis of clinicopathologic factors and their association with overall survival in stage I-II patients.

Table S3. Multivariate survival analysis of overall survival of stage I-II patients.

Table S4. Multivariate survival analysis of overall survival in the combined cohort consideringANC, ALC, AMC, AEC, and ABC.