AALBORG
UNIVERSITY

Perioperative examination of inflammatory markers in relation to sentinel lymph node biopsy in patients with melanoma; a pilot study

Author: Karoline A. Kristjansen[a]
Main supervisor: Lene Birk-Sørensen[b]
Project supervisor: Louise Bønnelykke-Behrndtz[c]
Secondary supervisor: Louise Bønnelykke-Behrndtz[c]

a: Student number 20175407, project number 4e22au5, Stud.med., 5. Semester candidate, Aalborg University.
b: Department of Plastic and Breast Surgery, Aalborg University Hospital, Denmark
c: Department of Plastic and Breast Surgery, Aarhus University Hospital, Denmark

Word count: 3507

Perioperative examination of inflammatory markers in relation to sentinel lymph node biopsy in patients with melanoma; a pilot study

Karoline A. Kristjansen[1], Louise Bønnelykke-Behrndtz[2] and Lene Birk-Sørensen[1]
[1]Department of Plastic and Breast Surgery, Aalborg University Hospital
[2]Department of Plastic and Breast Surgery, Aarhus University Hospital

Abstract

Introduction: Sentinel lymph node biopsy (SLBN) is essential in staging melanoma and for properly selecting patients for adjuvant immunotherapy. However, subsequent inflammation due to surgical injury and wound healing is theorized to potentially aid malignant progression, by improving conditions for remaining tumor cells, and may therefore effect prognosis. We want to test if an association between SLNB and a systemic inflammatory response can be made. A systemic inflammatory response will be measured by neutrophil-to-lymphocyte ratio (NLR), an indicator for systemic inflammation and established prognostic factor in serval malignancies. Supplementary markers for inflammation will also be assessed.

Methods: We conducted a prospective uncontrolled longitudinal pilot study. In total, 20 patients diagnosed with melanoma and undergoing SLBN were included. Perioperative blood samples were collected prior to SLNB, 2 hours and 6 hours postoperatively. Blood samples were assessed for inflammatory cells (Neutrophil granulocytes, lymphocytes, eosinophil granulocytes, basophil granulocytes and Metamyelo.+Myelo+Promyelocytes) with particular interest in NLR, supplementary pro-inflammatory cytokines (IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α and IFN-γ) and additional acute phase reactants (CRP and LDH).

Results: NLR increases significantly from 1.94 (95% CI:1.5:2.3) preoperatively to 9.5 (95% CI:7.5:11.6) 2 hours postoperatively (mean diff. 7.6 95% CI:-9.68:- 5.54) (p<0.0001). NLR increases further 6 hours postoperatively to 16.04 (95% CI:9.89:22.19) (mean diff. 6.47 95% CI:-11.49:-1.46) (p=0.0151). Remaining granulocytes decreases postoperatively. No perioperative changes in acute phase reactants are found. Among supplementary pro-inflammatory cytokines, mean IL-6 increases from baseline to 2 hours postoperatively (p<0.0001), along with mean IL-10 (p=<0.0001). While TNF-α (p= 0.0064) (p=0.0026) and IFN-γ (p=0.0003) (p=0.0125) decreases both at 2 and 6 hours postoperatively respectively. Remaining pro-inflammatory cytokines show nonsignificant changes.

Conclusion: SLNB induces a moderate postoperative systemic inflammatory response measured by NLR. This finding emphasizes the need for further investigation on perioperative inflammatory response, as inflammation may impact micrometastasis. In prospect, research on perioperative inflammation and prognosis may represent a target for optimizing treatment.

Background

Melanoma is a common malignancy increasing globally. In Danish population the number of new events has been increasing 6% annually since 2015 (1–3). Melanoma is the most fatal skin cancer and is therefore a great challenge to public health (4). Regional lymph node involvement is of highly prognostic value in early-stage melanoma. Thus, sentinel lymph node biopsy (SLNB), in which sentinel node is identified, excised, and microscopically assessed, has become widely acknowledged as an integral part of staging and properly allocating patients to correct oncologic treatment (5).

Tissue injury secondary to surgery induces an inflammatory response as an intrinsic part of wound healing (6). Correlations between the extent of surgical injury and inflammatory response has been identified (7). Inflammation is widely recognized as a hallmark of cancer (8), and has been shown to contribute to tumorigenesis and progression of malignant tumors by pro-tumoral effects of neutrophils and suppression of anti-tumoral effects of lymphocytes (9–12).

The relationship between pro-tumoral neutrophils and anti-tumoral lymphocytes is characterized as neutrophil-to-lymphocyte ratio (NLR), a marker of inflammation and a prognostic factor in several malignancies, including melanoma (13–15)A skewed NLR may represent a window of opportunity for neoplastic cells to take advantage of the pro-tumoral effects of increased neutrophils and decreased lymphocytes, resulting in growth and dissemination. Literature has not yet reported whether surgical injury from SLNB results in a systemic inflammatory response.

Aim and Hypothesis

This study aims to examine if SLNB induces a systemic inflammatory response by evaluating inflammatory cells with particular interest in NLR and supplementary plasma levels of pro-inflammatory cytokines and acute phase reactants. We hypothesize that SLNB induces a systemic inflammatory response with increased plasma levels of NLR and secondary inflammatory cells, pro-inflammatory cytokines and acute phase reactants when comparing preoperative and postoperative plasma levels.

Methods

Study Design

Prospective monocenter uncontrolled longitudinal study conducted at Department of Plastic and Breast Surgery, Aalborg University Hospital, Denmark.

Study Population

Patients (n=20) diagnosed with malignant melanoma and scheduled for SLNB at Aalborg University Hospital was consecutively included from June 2021 to October 2021.

Inclusion criteria:

- Adult (>18 years of age)
- Diagnosed with invasive cutaneous melanoma
- Eligible for SLNB (Melanoma stage ≥ T1b)
- Obtained signed informed consent

Exclusion criteria:

- Pregnancy

Demographics and Clinical Characteristics

Data concerning age, sex, specifics regarding SLNB procedure including surgery time, number of glands excised, the anatomic location and number of anatomic locations involved in the SLNB procedure and pathology results including Breslow thickness, melanoma subtype, status of ulceration

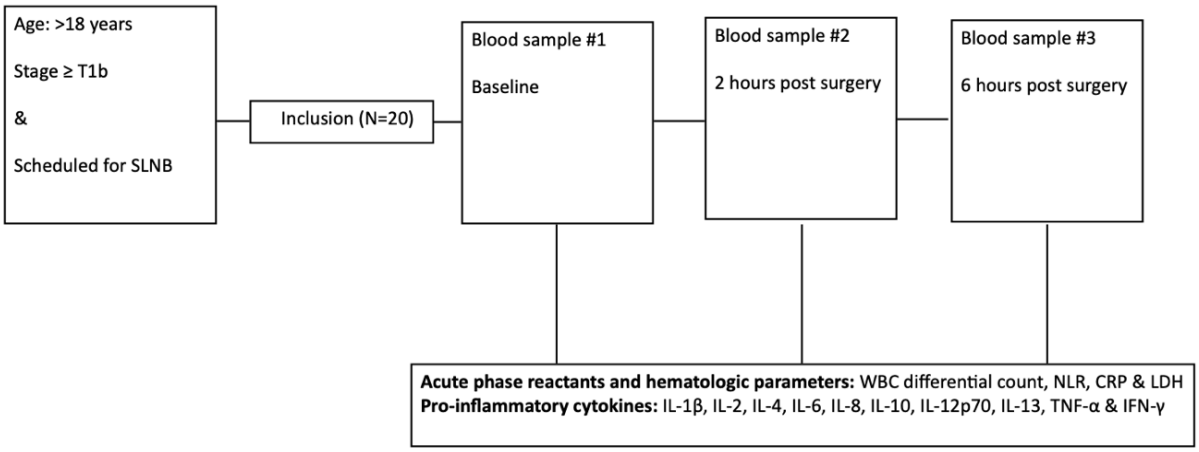

Figure 1: Study Design. Eligible patients will be included until n=20. Patients will have blood sample #1 collected on the day and SLNB and prior to any medical interventions, blood sample #2 will be collected 2 hours post surgery and blood sample #3 will be collected 6 hours post surgery. All blood samples will be analyzed by the mentioned parameters.

and sentinel node status is retrieved from medical records.

Measurements of inflammatory parameters

Once included, perioperative blood samples are collected on the day of SLNB on following time points:

- Preoperative (Baseline)
- 2 hours postoperatively (+/- 15 min)
- 6 hours postoperatively (+/- 15 min)

Baseline sample is collected preoperatively and prior to lymphoscintigraphy to prevent possible interference from radioactive tracer. See **Figure 1.**

Analyses of blood samples include hematologic parameters, pro-inflammatory cytokines, and acute phase reactants.

Hematologic parameters: White blood cell differential count. Values of NLR will be categorized in groups according to severity of inflammation.

- Group 1 (No inflammation): NLR <3
- Group 2 (Mild inflammation): NLR 3-9
- Group 3 (Moderate inflammation): NLR 9-18
- Group 4 (Severe inflammation): NLR >18

Pro-inflammatory cytokines: IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α and IFN-γ.

Acute phase reactants: CRP, LDH.

Blood samples are collected by trained medical staff at Aalborg University Hospital, Dept. of Clinical Biochemistry. CRP, LDH and leukocyte differential count is analyzed at Dept. of Clinical Biochemistry, Aalborg University Hospital. Multiplex panel for pro-inflammatory cytokines is analyzed at Dept. of Biochemistry, Aarhus University Hospital.

Statistical analysis

Baseline characteristics are tabulated, and frequencies are reported. Distribution of all data is tested. Paired t-test is applied on normally distributed data, while Wilcoxon signed-rank test is used when data is not normally distributed. Regression analyses is performed evaluating changes in mean over time. Data is presented with mean, SD and p-values <0.05 are considered statistically significant. The statistical analysis and illustrations are performed using Stata software, Stata/MP 17.0 for Mac (Statacorp, Texas, USA).

Ethics

An informed written and signed consent is obtained prior to inclusion, and before any study related initiatives are taken. The health ethics committee of Northern Jutland, Denmark rules approval non applicable. The Northern Jutland Region and the Danish Data Protection Agency is notified before initiation. All data is anonymized and collected in RedCap in accordance with health data regulations.

Results

Demographics and Clinical Characteristics

Twenty patients are included in the study; demographics and clinical characteristics are illustrated in **Table 1.** Sex is evenly distributed with 10 male and 10 female patients. Mean age is 60.25 years ±15.41. Breslow thickness is reported in intervals according to TNM-classification with 6 patients (30%) with a melanoma thickness of 0.8-1.0 mm equivalent to stage T1b, 8 patients (40%) with melanoma thickness of 1.1-2.00 mm equivalent to T2, 6 patients (30%) with melanoma thickness of 2.1-4.0 mm equivalent to T3 and non with melanoma thickness of >4.00 mm equivalent to T4. Superficial spreading is the most frequent with 13 (65%) of patients. Ulceration is present in 3 (15%) of cases. Mean time of surgery is 85.3 minutes ±43.01, mean number of glands excised is 3.1 ±1.997. SLNB is performed on the head and neck in 3 patients (15%), the axilla in 9 patients (45%) and the inguinal region in 8 patients (40%). The number of anatomical locations undergoing SLNB is predominantly 1 occurring in 16 (80%) of the patients, 2 in 2 (10%) of the patients and 3 different locations in 2 (10%) of patients. The vast majority of patients are sentinel node negative (95%) but 1 (5%) patient has microscopic lymphatic spread.

Table 1. Demographics and characteristics (N=20)

Variable		No. (%)
Sex		
	Male	10 (50.0)
	Female	10 (50.0)
Age (Mean±SD)		60.25 (15.41)
Ulceration		
	Present	3 (15.0)
	Absent	17 (85.0)
Breslow thickness		
	0.8-1.0 mm	6 (30.0)
	1.1-2.0 mm	8 (40.0)
	2.1-4.0 mm	6 (30.0)
	>4.0 mm	0
Melanoma subtype		
	Superficial spreading	13 (65.0)
	Nodular	4 (20.0)
	Desmoplastic	1 (5.0)
	Lentigo maligna	1 (5.0)
	Unclassified	1 (5.0)
Surgery time in minutes (Mean±SD)		85.3 (43.01)
Number of glands excised		
	1	6 (31.6)
	2	1 (5.3)
	3	7 (37.8)
	4	1 (5.3)
	5	2 (10.5)
	6	0 (0)
	7	1 (5.3)
	8	1 (5.3)
Sentinel node status		
	Negative	19 (95.0)
	Positive	1 (5.0)
Location		
	Head and neck	3 (15.0)
	Axilla	9 (45.0)
	Inguinal region	8 (40.0)
Number of locations		
	1	16 (80.0)
	2	2 (10.0)
	3	2 (10.0)

Table 2. Neutrophil-to-lymphocyte ratio over time

	Baseline No. (%)	2 hours Postoperatively No. (%)	6 hours Postoperatively No. (%)
Normal (NLR = 1-3)	18 (90%)	1 (5%)	0
Mild stress (NLR = 3-9)	2 (10%)	9 (45%)	3 (20%)
Moderate stress (NLR = 9-18)	0	9 (45%)	8 (53%)
Severe stress (NLR > 18)	0	1 (5%)	4 (27%)

Perioperative change in NLR

The mean NLR in plasma increases from 1.94 (95% CI:1.5:2.3) at baseline to 9.5 (95% CI:7.5:11.6) 2 hours postoperatively (mean diff. 7.6 95% CI:-9.68:-5.54) (p<0.0001) and further up to 16.04 (95% CI:9.89:22.19) 6 hours postoperatively (mean diff. 6.47 (95% CI:-11.49:-1.46) (p=0.0151). In regression models the mean NLR increases significantly over time (p<0.0001) with regression coefficients from baseline to 2 hours postoperatively 3.2 (95% CI:2.5:4.0), to 6 hours postoperatively (coef. 5.5 (95% CI:4.6:6.3). NLR over time illustrates that preoperatively 18 (90%) patients have a level of NLR <3 reflecting no evident systemic inflammation, whilst 2 (10%) patients have a level of NLR 3-9 reflecting mild systemic inflammation, as shown in **Table 2**. Two hours postoperatively 1 (5%) patient persists to show no sign of inflammation with NLR <3. Mild, moderate, and severe inflammation is shown by 9 (45%), 9 (45%) and 1 (5%) of patients respectively 2 hours postoperatively. NLR levels at 6 hours postoperatively shows no patients to reflect systemic inflammation with NLR <3, 3 (20%) shows mild systemic inflammation, 8 (53%) patients show moderate inflammation and 4 (27%) shows severe inflammation with NLR >18. **Figure 2** illustrates how the mean NLR at baseline reflects no inflammation in the population with a value < 3. 2 hours postoperatively the mean increases to 9.5, as previously mentioned, reflecting a moderate response in our population. Lastly, the NLR mean continues to increase at 6 hours postoperatively to 16, continuing to represent a moderate response, yet approximating the limit for severe inflammatory response at 18.

Perioperative change in inflammatory cells and acute phase reactants

The mean neutrophil count increases from 3.7 10^9/l (95% CI:3.1:4.3) at baseline to 6.9 10^9/l (95% CI:6.1:7.7) 2 hours postoperatively (mean diff. 3.2 10^9/l 95% CI:2.5: 3.9) (p<0.0001) and further up to 9.2 10^9/l (95% CI: 7.8 : 10.5) 6 hours postoperatively (mean diff. 2.3 (95% CI:1.2:3.4) (p=0.0004). In regression models the mean neutrophils increases significantly over time (p<0.0001) with regression coefficients from baseline to 2 hours postoperatively 3.2 (95% CI:2.5:4.0), to 6 hours postoperatively coef. 5.5 (95% CI:4.6:6.3). The mean lymphocyte count in plasma decreases from 2.0 10^9/l (95% CI:1.8:2.2) at baseline to 0.8 10^9/l (95% CI:0.7:1.0) 2 hours postoperatively (mean diff. 1.2 95% CI: 0.9:1.4) (p<0.0001) and decreases further to 0.7 10^9/l (95% CI: 0.6:0.9) 6 hours postoperatively (mean diff. 0.12 95% CI:0.005: 0.3) (p=0.043). In regression models the mean lymphocytes decreases significantly over time (p<0.0001) with regression coefficients from baseline to 2 hours postoperatively -1.2 (95% CI: -1.4:-1.0) to 6 hours postoperatively (coef. -1.3 (95% CI:-1.5:-1.1). See **Figure 3:** Neutrophils, lymphocytes and NLR.

Perioperative changes in leukocytes, remaining granulocytes, and precursor cells are illustrated in **Figure 4**. The mean eosinophil count decreases from 0.15 10^9/l (95% CI:0.11:0.19) at baseline to 0.03 10^9/l (95% CI:0.002:0.05) 2 hours postoperatively (mean diff. 0.123 95% CI:0.09:0.16) (p<0.0001) and decreases further to 0.021 10^9/l (95% CI:-0.01:-0.5) 6 hours postoperatively (mean diff. 0.01 95% CI:-0.002:-0.02) (p= p=0.0918). In regression models the mean eosinophils decreases significantly over time (p<0.0001) with regression coefficients from baseline to 2 hours postoperatively -0.123 (95% CI:-0.15:-0.09) to 6 hours postoperatively (coef. -0.13 95% CI:-0.16:-0.09). The mean basophil count decreases from 0.05±0.02 10^9/l at baseline to 0.03±0.013 10^9/l 2 hours postoperatively (p=0.0001) and decreases further to 0.02±0.13 10^9/l 6 hours postoperatively (p= 0.0178). In regression models the mean basophils decreases significantly over time (p<0.0001) with regression coefficients from baseline to 2 hours postoperatively -0.01 (95% CI:-0.03:-0.01), to 6 hours postoperatively (coef. -0.03 (95% CI:-0.035:-0.02). The mean Metamyelo.+Myelo+Promyelocyte count increases from 0.02± 0.01 10^9/l at baseline to 0.033±0.02 10^9/l 2 hours postoperatively (p=0.0007) and increases further to 0.05±0.0210^9/l 6 hours postoperatively (p=0.0092).In regression models the mean Metamyelo.+Myelo+Promyelocytes increases significantly over time (p<0.0001) with regression coefficients from baseline to 2 hours postoperatively 0.01 (95% CI:0.005:0.02), to 6 hours postoperatively (coef.0.02 (95% CI:0.02:0.03).

SLNB does not affect CRP or LDH over the perioperative time (data not shown).

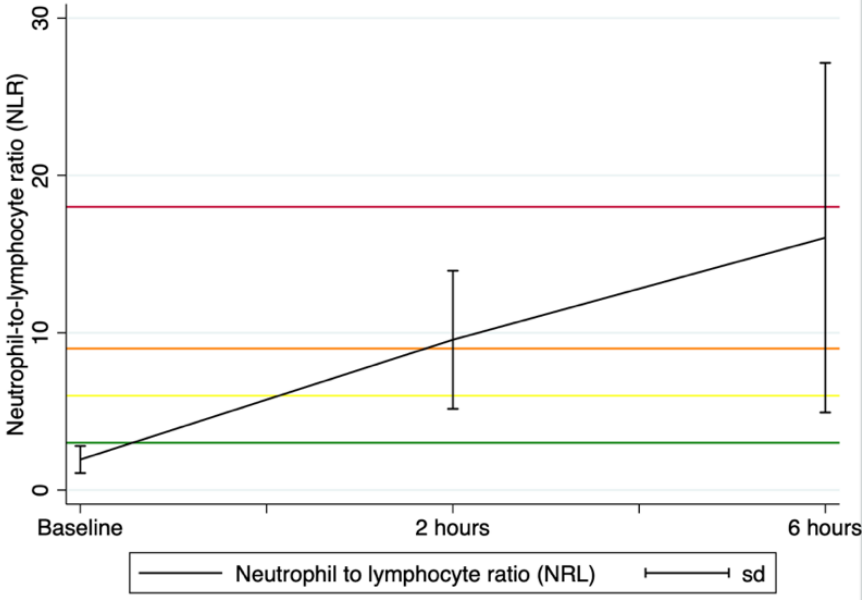

Figure 2: NLR illustrated with mean and SD at baseline, 2 hours post surgery and 6 hours post surgery. Horizontal lines indicate level of stress: NLR <3 = No inflammation (green), NLR 3-6 = Mild (Yellow), NLR 6-9 = Moderate (Orange), NLR >18 Severe (Red).

Perioperative change in pro-inflammatory cytokines

Peri-operative changes in pro-inflammatory cytokines are illustrated in **Figure 5.** The mean IL-6 in plasma increases from 1.15± 0.61 pg/ml at baseline to 2.31±1.65 pg/ml 2 hours postoperatively (p<0.0001) and at 6 hours postoperatively the mean value is 2.20±2.17 pg/ml (p=0.232). The mean IL-10 in plasma increases from 0.46 ±0.46 pg/ml at baseline to 2.12±2.55 pg/ml 2 hours postoperatively (p=<0.0001) at 6 hours postoperatively IL-10 decreases to 1.27±0.92 pg/ml (p=0.233). The mean TNF-α in plasma decreases from 3.21±1.12 pg/ml at baseline to 2.67±0.84 pg/ml 2 hours postoperatively (p= 0.0064) and decreases further to 2.18±0.79 pg/ml at 6 hours postoperatively (p= 0.0026). The mean IFN-γ in plasma decreases from 9.50±8.56 pg/ml at baseline to 3.81±3.91 pg/ml 2 hours postoperatively (p=0.0003) and decreases further to 2.29±3.44 pg/ml 6 hours postoperatively (p=0.0125).

SLNB does not affect IL-1β, IL-2, IL-4, IL-8, IL-12p70, IL-13 over the perioperative time (data not shown).

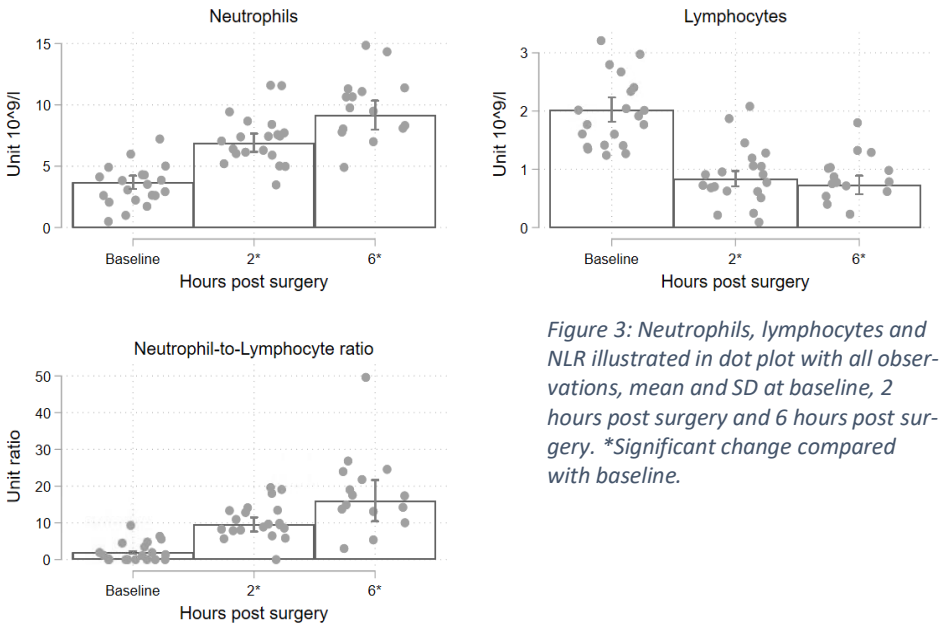

*Figure 3: Neutrophils, lymphocytes and NLR illustrated in dot plot with all observations, mean and SD at baseline, 2 hours post surgery and 6 hours post surgery. *Significant change compared with baseline.*

Discussion

In this prospective study we investigate perioperative change in different parameters in plasma to assess the systemic inflammatory impact of SLNB. We demonstrate an association between SLNB and systemic inflammatory response, as several parameters show a clear tendency, while others are more nondefinitive. Importantly, we find the majority of our population to not have increased baseline NLR prior to SLNB, while NLR increases to a possibly pathological level postoperatively. It is unclear how long the increased level of NLR persists in patients after SLNB, as there is no decline in our measurements. The increase in neutrophils stands in contrast to the lymphocytes and remaining granulocytes; eosinophils and basophils, which are depleted postoperatively. Metamyelo.+Myelo+Promyelocytes, a precursor to neutrophils, are mobilized and increases in accordance with neutrophils. The engagement between inflammatory cells and cytokines is complex, and only a minor description of some effects is mentioned (16). The primary target of IL-6, which significantly increases 2 and 6 hours postoperatively, is to produce acute phase protein e.g. CRP (16,17). However, no significant change in CRP or LDH is observed. IL-10 has inhibitory properties on lymphocytes and increases in the initial postoperative blood sample, while lymphocytes decreases both 2 and 6 hours postoperatively (16). TNF- α exerts its effects primarily on neutrophils, promoting adhesion (18). We find TNF-α to significantly decrease postoperatively. IFN-γ, which mainly targets lymphocytes and macrophages, decreases postoperatively (19).

Wound healing is widely acknowledged to consist of four continuous and overlapping phases; hemostasis, inflammation, proliferation, and remodeling (20). These processes are initiated when the skin is disrupted regardless of the etiology, thus a surgical wound does not differ vastly from other mechanical tissue injuries (21,22). In this context, the accentuated effects of neutrophils manifest during the inflammatory and proliferative phases. In the inflammatory phase, neutrophils are recruited to the wound and exert several effects (23), including the release of toxic substances and reactive oxygen species, with the intent of eradicating any bacteria (24). During the proliferative phase, neutrophils trigger the release of extracellular matrix modifying enzymes and vascular endothelial growth factor aiding in angiogenesis (25).

The abovementioned processes are essential in wound healing yet constitute potentially adverse

effects in the setting of cancer, and specifically remaining malignant cells after surgical procedures (25,26). These effects have been shown in zebra larvae, where neutrophils aid proliferation of preneoplastic cells in response to surgical injury (27). The mechanisms of promoting tumor proliferation include uptake of neutrophil elastase by cancerous cells, thereby degrading insulin receptor substrate-1 and subsequently favoring tumor proliferation (28). In addition, supplementing the microenvironment of the tumor with bioactive factors promoting healing is central (9). These factors include extracellular matrix modifying enzymes, which may facilitate further invasion and release of VEGF, hereby providing increased angiogenesis (25). Additional adverse effects are seen by the release of reactive oxygen species, which are potentially mutogenic to adjacent cancer cells (9,26). Pro-inflammatory cytokines IL-1β, IL -6, IL -8 and TNF- α are identified as facilitators of local and systemic spread of malignancy through inflammation(17). Contrarily, anti-tumoral lymphocytes constitute a crucial part of tumor surveillance and destruction (11). However, lymphocytes can be suppressed by neutrophils in the setting of systemic inflammation (29,30). The adverse effects of surgical injury and acute wound healing are seen in animal studies, where complete primary tumor excision in mice is associated with increased systemic metastatic burden when comparing with a control group not undergoing surgery (31).

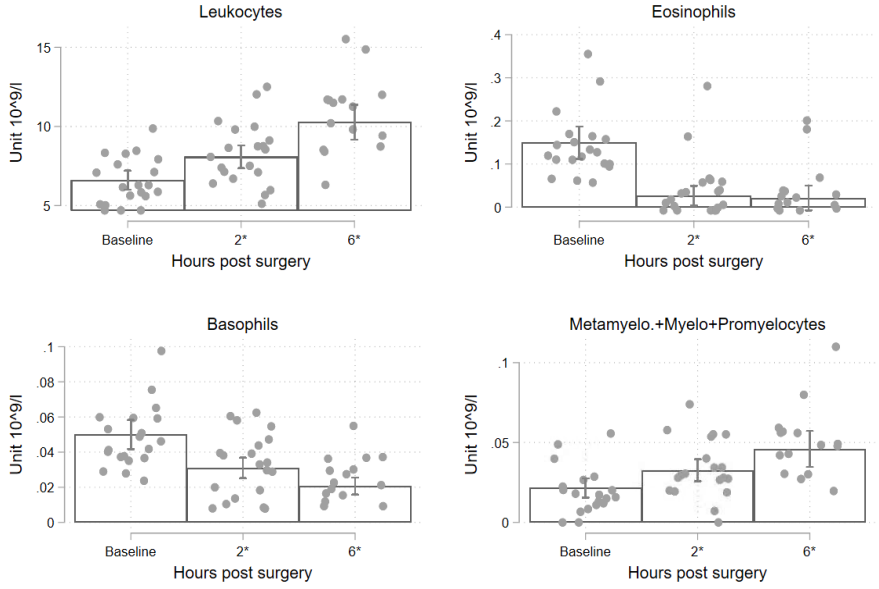

*Figure 4: Leukocytes, Eosinophils, Basophils and Metamyelo.+Myelo+Promyelocytes illustrated in dot plot with all observations, mean and SD at baseline, 2 hours post surgery and 6 hours post surgery. *Significant change compared with baseline.*

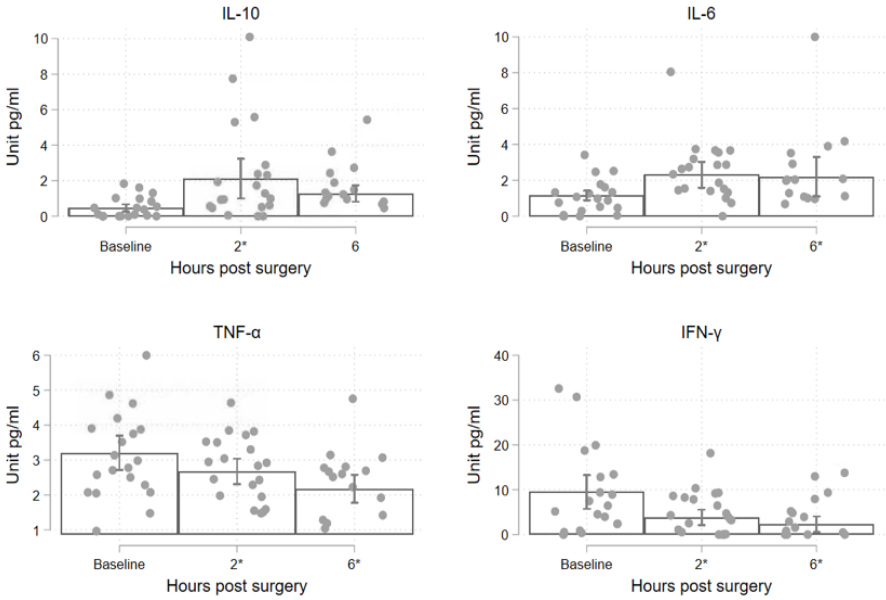

*Figure 5: Pro-inflammatory cytokines illustrated in dot plot with all observations, mean and SD at baseline, 2 hours post surgery and 6 hours post surgery. *Significant change compared with baseline.*

Prolonged inflammation can result in a chronic wound (32). Neutrophils participate in protracted

healing in addition to aiding pre-neoplastic and malignant cells (27). The ramifications of this is examined in studies investigating the effect of postoperative complications on prognosis and recurrence (33). An association between postoperative infection, recurrence and prognosis is documented in colorectal cancer, where a meta-analysis with 21 studies. This includes prospective nonrandomized, randomized, and retrospective trials with a total of 21,902 patients included. The analysis finds anastomotic leakage after restorative surgery to have a negative prognostic impact on local recurrence and cancer specific survival (34). Increased recurrence is also seen in patients with postoperative infection after excision of supraglottic tumor (35). The same tendency is documented in gastric cancer, where postoperative infection is associated with increased recurrence and decreased disease-free survival (36). Furthermore, literature uncovers some of the underlying mechanisms of the adverse impact of postoperative infection through gene regulation in peripheral blood leukocytes. Genes involved in coding for antitumoral components are downregulated, while genes involved in coding for pro-tumoral components are upregulated (37).

Abovementioned studies find an increased risk of recurrence and decrease in survival associated with postoperative infection. When reviewing studies investigating noninfectious and mixed postoperative complications, associations between complications and recurrence and prognosis is also established. In breast cancer, delayed wound healing is linked to increased loco-regional recurrence (38). Another study finds postoperative wound complications in breast cancer to be associated with increased risk of systemic recurrence (39). Association between postoperative complications and recurrence is also documented in esophageal and lung cancer (40,41). In lung cancer, survival is also negatively associated with postoperative complications (42). Linking non-infectious and mixed postoperative complications may imply inflammation, the common denominator, to play a major part in increased recurrence of malignancy and poorer prognosis.

When evaluating the severity of postoperative complications, a meta-analysis with 18,611 patients from 14 studies finds an association between severity of complications in patients undergoing surgery for colorectal cancer and a negative impact on disease free and overall survival (43). This finding is supported in lung cancer patients where complications is established as a negative predictor for long time survival, and especially major infections show a strong negative impact (42). Postoperative fever is also an established as a risk factor for increased recurrence in breast cancer patients (44). Severity of complications and postoperative fever may represent measures for systemic inflammatory response.

A meta-analysis containing 11 retrospective studies and 1 prospective study assessing prognostic value of NLR in patients with melanoma stage I-IV finds a poorer overall survival and progression-free survival in patients with increased NLR. The risk for poorer overall survival is HR=2.23, 95% CI=1.64 to 3.04, P<.001, while the risk for poorer progression-free survival is HR=2.19, 95% CI=1.78 to 2.69, P<.001 (45). Another meta-analysis including 5 retrospective studies and 2 prospective studies assessing the prognostic value of NLR in patients treated with immune checkpoint inhibitors, finds poorer overall and progression-free survival in patients with melanoma with a HR of 2.18 (CI: 1.66 to 2,84) and 1.73 (CI:1.33 to 2.25), respectively (46).

In stage I-III melanoma several retrospective studies find association between increased NLR and poorer overall survival and/or disease-free survival (47–50), while one study finds increased NLR in localized melanoma to be associated with better over survival and disease-free survival (51). Robinson et al finds no association between increased NLR and recurrence but finds increased risk of positive SLNB in patients with increased NLR (52). If associations between increased NLR in early-stage melanoma and positive SLNB or metastasis are to be investigated and established, we may be able to deselect these patients from undergoing SLNB and avoiding potentially adverse effects. The prognostic value of NLR is also present in metastatic melanoma, where an association to increased risk of death is established (53–57)in addition to increased risk of progression (58–60). Some studies find an association to both increased risk of death and recurrence (61–63).

NLR is a marker for systemic inflammation and has prognostic value in patients with melanoma. The predictive value to forecast status of sentinel lymph node remains unclear. We show perioperative increase in NLR levels in association to SLNB, therefore future studies might take interest in how to optimize selection of patients undergoing SLNB and how to alter the operative systemic inflammatory response. If the perioperative inflammatory response can be managed optimally, treatment and ultimately prognosis can be improved for patients.

Limitations

This study is a prospective uncontrolled longitudinal pilot study. No power calculations have been made prior to initiation. The study is susceptible to sampling bias, as the study is non-randomized, and the study population did not resemble the target population on characteristics appearing in the demographics table.

Additionally, the study is subject to confounding as information on co-morbidities and medication use are not considered. Lastly, this study provides an effect size, which can be utilized in future research by aiding power calculations and contributing to generating hypothesis and objectives.

Conclusion

SLNB generates a measurable systemic inflammatory response, as we found NLR to significantly reflect a postoperative stress response. This may give rise to adverse effects on tumor cells by pro-tumoral neutrophils and suppression of anti-tumoral lymphocytes. Since inflammation is suggested to impact micrometastasis further investigation of the perioperative inflammatory response is needed, however, our findings may suggest an unexplored opportunity for optimizing treatment and prognosis for patients diagnosed with melanoma undergoing SLNB.

References

1. Garbe C, Keim U, Eigentler TK, Amaral T, Katalinic A, Holleczek B, et al. Time trends in incidence and mortality of cutaneous melanoma in Germany. Journal of the European Academy of Dermatology and Venereology. 2019 Jul 1;33(7):1272–80.
2. Arnold M, Singh D, Laversanne M, Vignat J, Vaccarella S, Meheus F, et al. Global Burden of Cutaneous Melanoma in 2020 and Projections to 2040. . JAMA Dermatol. 2022 May;495–503.
3. Hölmich L. Dansk Melanom Database (DMD) Årsrapport 2021. 2022 Jun.
4. Bay C, Kejs AMT, Storm HH, Engholm G. Incidence and survival in patients with cutaneous melanoma by morphology, anatomi-

cal site and TNM stage: A danish population-based register study 1989-2011. Cancer Epidemiol. 2015 Feb 1;39(1):1–7.
5. Bartlett EK, Karakousis GC. Current staging and prognostic factors in melanoma. Vol. 24, Surgical Oncology Clinics of North America. W.B. Saunders; 2015. p. 215–27.
6. Kohl BA, Deutschman CS, Williams L. The inflammatory response to surgery and trauma. Vol. 12, Current Opinion in Critical Care. 2006.
7. Ioannidis A, Arvanitidis K, Filidou E, Valatas V, Stavrou G, Michalopoulos A, et al. The Length of Surgical Skin Incision in Postoperative Inflammatory Reaction. JSLS. 2018 Oct 1;22(4).
8. Grivennikov SI, Greten FR, Karin M. Immunity, Inflammation, and Cancer. Vol. 140, Cell. 2010. p. 883–99.
9. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Vol. 144, Cell. 2011. p. 646–74.
10. Cachot A, Bilous M, Liu YC, Li X, Saillard M, Cenerenti M, et al. Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. 2021.
11. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. 2018; Available from: http://www.genesdev.org/cgi/doi/10.1101/gad.314617.
12. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci U S A. 2006 Aug 15;103(33):12493–8.
13. Donskov F. Immunomonitoring and prognostic relevance of neutrophils in clinical trials. Vol. 23, Seminars in Cancer Biology. 2013. p. 200–7.
14. Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: Experience in patients with cancer. Vol. 88, Critical Reviews in Oncology/Hematology. 2013. p. 218–30.
15. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. Vol. 106, Journal of the National Cancer Institute. Oxford University Press; 2014.
16. Justiz Vaillant AA, Qurie A. Interleukins. StatPearls; 2022.
17. Diakos CI, Charles KA, Mcmillan DC, Clarke SJ. Review Cancer-related infl ammation and treatment eff ectiveness [Internet]. Vol. 15, www.thelancet.com/oncology. 2014. Available from: www.thelancet.com/oncology
18. Zelová H, Hošek J. TNF-α signalling and inflammation: Interactions between old acquaintances. Vol. 62, Inflammation Research. 2013. p. 641–51.
19. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004 Feb;75(2):163–89.
20. Stupin V, Manturova N, Silina E, Litvitskiy P, Vasin V, Artyushkova E, et al. The effect of inflammation on the healing process of acute skin wounds under the treatment of wounds with injections in rats. J Exp Pharmacol. 2020;12:409–22.
21. Velnar T, Bailey T, Smrkolj V. The Wound Healing Process: an Overview of the Cellular and Molecular Mechanisms. Vol. 37, The Journal of International Medical Research. 2009.
22. Arias JI, Aller MA, Arias J. Surgical inflammation: A pathophysiological rainbow. Vol. 7, Journal of Translational Medicine. 2009.
23. Martin P. Wound Healing-Aiming for Perfect Skin Regeneration. Vol. 276, New Series. 1997.
24. DeCoursey TE, Ligeti E. Regulation and termination of NADPH oxidase activity. Vol.

62, Cellular and Molecular Life Sciences. 2005. p. 2173–93.
25. Deryugina EI, Zajac E, Juncker-Jensen A, Kupriyanova TA, Welter L, Quigley JP. Tissue-Infiltrating Neutrophils Constitute the Major In Vivo Source of Angiogenesis-Inducing MMP-9 in the Tumor Microenvironment. Neoplasia (United States). 2014;16(10):771–88.
26. Bohle B, Pera M, Pascual M, Alonso S, Mayol X, Salvado M, et al. Postoperative intra-abdominal infection increases angiogenesis and tumor recurrence after surgical excision of colon cancer in mice. Surgery. 2010 Jan;147(1):120–6.
27. Antonio N, Bønnelykke-Behrndtz ML, Ward LC, Collin J, Christensen IJ, Steiniche T, et al. The wound inflammatory response exacerbates growth of pre-neoplastic cells and progression to cancer. EMBO J. 2015 Sep 2;34(17):2219–36.
28. Houghton AMG, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, et al. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med. 2010 Feb;16(2):219–23.
29. Grecian R, Whyte MKB, Walmsley SR. The role of neutrophils in cancer. Vol. 128, British Medical Bulletin. Oxford University Press; 2018. p. 5–14.
30. Pillay J;, Kamp VM, van Hoffen E;, Visser T;, Tak T;, Lammers JW. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. Vol. 122, Journal of Clinical Investigation. 2012.
31. Al-Sahaf O, Wang JH, Browne TJ, Cotter TG, Redmond HP. Surgical injury enhances the expression of genes that mediate breast cancer metastasis to the lung. Ann Surg. 2010 Dec;252(6):1037–43.
32. Boniakowski AE, Kimball AS, Jacobs BN, Kunkel SL, Gallagher KA. Macrophage-Mediated Inflammation in Normal and Diabetic Wound Healing. The Journal of Immunology. 2017 Jul 1;199(1):17–24.
33. Beecher SM, O'Leary DP, McLaughlin R, Kerin MJ. The Impact of Surgical Complications on Cancer Recurrence Rates: A Literature Review. Vol. 41, Oncology Research and Treatment. S. Karger AG; 2018. p. 478–82.
34. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: Systematic review and meta-analysis. Ann Surg. 2011 May;253(5):890–9.
35. Rodrigo JP. Prognostic significance of postoperative wound infection on head and neck cancer. 1998.
36. Hayashi T, Yoshikawa T, Aoyama T, Hasegawa S, Yamada T, Tsuchida K, et al. Impact of infectious complications on gastric cancer recurrence. Gastric Cancer. 2015 Apr 1;18(2):368–74.
37. Alonso S, Mayol X, Nonell L, Salvans S, Pascual M, Pera M, et al. Peripheral blood leucocytes show differential expression of tumour progression-related genes in colorectal cancer patients who have a postoperative intra-abdominal infection: a prospective matched cohort study. Colorectal Disease. 2017 May 1;19(5):O115–25.
38. Murthy BL, Thomson CS, Dodwell D, Shenoy H, Mikeljevic JS, Forman D, et al. Postoperative wound complications and systemic recurrence in breast cancer. Br J Cancer. 2007 Nov 5;97(9):1211–7.
39. Beecher SM, O'Leary DP, McLaughlin R, Sweeney KJ, Kerin MJ. Influence of complications following immediate breast reconstruction on breast cancer recurrence rates. British Journal of Surgery. 2016 Mar 1;103(4):391–8.
40. Lerut T, Moons J, Coosemans W, van Raemdonck D, de Leyn P, Decaluwé H, et al. Postoperative complications after transthoracic esophagectomy for cancer of the esophagus and gastroesophageal junction

are correlated with early cancer recurrence: Role of systematic grading of complications using the modified clavien classification. Ann Surg. 2009 Nov;250(5):798–806.
41. Rueth NM, Parsons HM, Habermann EB, Groth SS, Virnig BA, Tuttle TM, et al. The long-term impact of surgical complications after resection of stage i nonsmall cell lung cancer: A population-based survival analysis. Ann Surg. 2011 Aug;254(2):368–74.
42. Andalib A, Ramana-Kumar A v., Bartlett G, Franco EL, Ferri LE. Influence of postoperative infectious complications on long-term survival of lung cancer patients: A population-based cohort study. Journal of Thoracic Oncology. 2013;8(5):554–61.
43. McSorley ST, Horgan PG, McMillan DC. The impact of the type and severity of postoperative complications on long-term outcomes following surgery for colorectal cancer: A systematic review and meta-analysis. Vol. 97, Critical Reviews in Oncology/Hematology. Elsevier Ireland Ltd; 2016. p. 168–77.
44. Yan T, Yin W, Zhou L, Jiang Y, Shen Z, Shao Z, et al. Postoperative fever: The potential relationship with prognosis in node negative breast cancer patients. PLoS One. 2010;5(12).
45. Ding Y, Zhang S, Qiao J. Prognostic value of neutrophil-to-lymphocyte ratio in melanoma Evidence from a PRISMA-compliant meta-analysis. Vol. 97, Medicine (United States). Lippincott Williams and Wilkins; 2018.
46. Sacdalan DB, Lucero JA, Sacdalan DL. Prognostic utility of baseline neutrophil-to-lymphocyte ratio in patients receiving immune checkpoint inhibitors: A review and meta-analysis. Vol. 11, OncoTargets and Therapy. Dove Medical Press Ltd.; 2018. p. 955–65.
47. Davis JL, Langan RC, Panageas KS, Zheng J, Postow MA, Brady MS, et al. Elevated Blood Neutrophil-to-Lymphocyte Ratio: A Readily Available Biomarker Associated with Death due to Disease in High Risk Nonmetastatic Melanoma. Ann Surg Oncol. 2017 Jul 1;24(7):1989–96.
48. Ma J, Kuzman J, Ray A, Lawson BO, Khong B, Xuan S, et al. Neutrophil-to-lymphocyte Ratio (NLR) as a predictor for recurrence in patients with stage III melanoma. Sci Rep. 2018 Dec 1;8(1).
49. Blakely AM, Cohen JT, Comissiong DS, Vezeridis MP, Miner TJ. Prognosis and management of thick and ultrathick melanoma. American Journal of Clinical Oncology: Cancer Clinical Trials [Internet]. 2019;42(11):824–9. Available from: https://www.embase.com/search/results?subaction=viewrecord&id=L629358078&from=export
50. Lino-Silva LS, Salcedo-Hernández RA, García-Pérez L, Meneses-García A, Zepeda-Najar C. Basal neutrophil-to-lymphocyte ratio is associated with overall survival in melanoma. Melanoma Res. 2017;27(2):140–4.
51. Wade RG, Robinson A v., Lo MCI, Keeble C, Marples M, Dewar DJ, et al. Baseline Neutrophil–Lymphocyte and Platelet–Lymphocyte Ratios as Biomarkers of Survival in Cutaneous Melanoma: A Multicenter Cohort Study. Ann Surg Oncol. 2018 Oct 1;25(11):3341–9.
52. Robinson et al. (2017). Baseline neutrophil-lymphocyte ratio adds prognostic value to sentinel lymph node biopsy in cutaneous melanoma.
53. Kanatsios S, Melanoma Project M, Li Wai Suen CSN, Cebon JS, Gyorki DE. Neutrophil to lymphocyte ratio is an independent predictor of outcome for patients undergoing definitive resection for stage IV melanoma. J Surg Oncol. 2018 Nov 1;118(6):915–21.
54. Cananzi FCM, Dalgleish A, Mudan S. Surgical management of intraabdominal metastases from melanoma: Role of the neutro-

phil to lymphocyte ratio as a potential prognostic factor. World J Surg. 2014;38(6):1542–50.
55. Zaragoza J, Caille A, Beneton N, Bens G, Christiann F, Maillard H, et al. High neutrophil to lymphocyte ratio measured before starting ipilimumab treatment is associated with reduced overall survival in patients with melanoma. British Journal of Dermatology. 2016 Jan 1;174(1):146–51.
56. Ferrucci PF, Giannarelli D, Gandini S, Cocorocchio E, del Vecchio M, Picasso V, et al. Prognostic relevance of baseline neutrophils and derived neutrophil to lymphocyte ratio for ipilimumab-treated advanced melanoma patients. Journal of Clinical Oncology. 2015 May 20;33(15_suppl):9034–9034.
57. Rosner S, Kwong E, Shoushtari AN, Friedman CF, Betof AS, Brady MS, et al. Peripheral blood clinical laboratory variables associated with outcomes following combination nivolumab and ipilimumab immunotherapy in melanoma. Cancer Med. 2018 Mar 1;7(3):690–7.
58. Teterycz P, Jagodzińska-Mucha P, Cybulska-Stopa B, Mariuk-Jarema A, Kozak K, Koseła-Paterczyk H, et al. High baseline neutrophil-to-lymphocyte ratio predicts worse outcome in patients with metastatic BRAF-positive melanoma treated with BRAF and MEK inhibitors. Melanoma Res. 2018 Oct 1;28(5):435–41.
59. Finon A, Zaragoza J, Maillard H, Beneton N, Bens G, Samimi M, et al. A high neutrophil to lymphocyte ratio prior to BRAF inhibitor treatment is a predictor of poor progression-free survival in patients with metastatic melanoma. European Journal of Dermatology. 2018 Jan 1;28(1):38–43.
60. Fujisawa Y, Yoshino K, Otsuka A, Funakoshi T, Fujimura T, Yamamoto Y, et al. Baseline neutrophil to lymphocyte ratio combined with serum lactate dehydrogenase level associated with outcome of nivolumab immunotherapy in a Japanese advanced melanoma population. Vol. 179, British Journal of Dermatology. Blackwell Publishing Ltd; 2018. p. 213–5.
61. Cocorocchio E, Martinoli C, Gandini S, Pala L, Conforti F, Stucchi S, et al. Baseline neutrophil-to-lymphocyte ratio (NLR) is associated with outcome of patients treated with BRAF inhibitors. Clinical and Translational Oncology. 2020 Oct 1;22(10):1818–24.
62. Bartlett EK, Flynn JR, Panageas KS, Ferraro RA, Jessica JM, Postow MA, et al. High neutrophil-to-lymphocyte ratio (NLR) is associated with treatment failure and death in patients who have melanoma treated with PD-1 inhibitor monotherapy. Cancer. 2020 Jan 1;126(1):76–85.
63. Cassidy MR, Wolchok R, Zheng J, Panageas K, Wolchok JD, Coit DG, et al. Neutrophil to lymphocyte ratio to predict outcome during ipilimumab treatment. Journal of Clinical Oncology. 2016 May 20;34(15_suppl):e21008–e21008.