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ORIGINAL ARTICLE

Assessment of peripheral blood cells parameters as a valuable tool in patients with neuroendocrine neoplasms

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Abstract

OBJECTIVES: Neuroendocrine neoplasms (NENs) are an extremely heterogeneous medical entity, representing a diagnostic and therapeutic challenge. Chronic inflammation, as is the case with other malignancies, plays a crucial role in NEN carcinogenesis.

DESIGN: The complete blood count (CBC) is a reliable tool for monitoring patients with cancer. Quantifying the absolute count of neutrophils (N), lymphocytes (L), platelets (P), and the ratios that derive from these parameters (neutrophil-to-lymphocyte ratio – NLR, platelet-to-lymphocyte ratio – PLR, and inflammatory systemic index – SII calculated as N×P/L) proved their prognostic and predictive value in numerous malignancies.

MATERIALS AND METHODS: We aimed to investigate the utility of these hematological parameters in 31 patients with NENs of various locations. Our study included the comparative analysis of pre-treatment hematological markers in NEN patients versus 21 age and gender matched healthy individuals. Additionally, for 26 out of the 31 patients included we analyzed and compared the inflammatory markers before and after treatment initiation.

RESULTS: The results revealed a statistically significant higher median value of N, NLR, PLR and SII in the NENs group in comparison with the values obtained in the control group and higher values of N, NLR and SII in the pretreatment group. Furthermore, we observed a higher mean value of the post-treatment P in the pancreatic NENs as opposed to the values obtained for other tumor locations.

CONCLUSIONS: The current study emphasizes the importance of the evaluation of CBC in the NENs setting thus adding value to prognostic models that can be useful for risk stratification and medical decision-making.

Abbreviations:

5-HIAA	- 5-hydroxyindolacetic acid
BC	- blood count
CBC	- complete blood count
CgA	- chromogranin A
Du-NENs	- duodenal neuroendocrine neoplasms
ENETS	- European Neuroendocrine Tumor Society
F-NENs	- functional neuroendocrine neoplasms
G-NENs	- gastric neuroendocrine neoplasms
GEP-NENs	- gastroenteropancreatic neuroendocrine neoplasms
L	- lymphocytes
Lu-NENs	- lung neuroendocrine neoplasms
LWR	- lymphocyte-to-white blood cell ratio
MPV	- mean platelet volume
MTC	- medullary thyroid carcinoma
N	- neutrophils
NENs	- neuroendocrine neoplasms
NF-NENs	- non-functioning neuroendocrine neoplasms
NLR	- neutrophil-to-lymphocyte ratio
P	- platelets
P-NENs	- pancreatic neuroendocrine neoplasms
PHEO/PGL	- pheocromocytoma/paraganglioma
PLR	- platelet-to-lymphocyte ratio
Pr-NENs	- prostatic neuroendocrine neoplasms
Si-NENs	- small intestine neuroendocrine neoplasms
SII	- inflammatory systemic index
SSA	- somatostatin analogs
SSTR	- somatostatin receptors
Up-NENs	- unknown primary neuroendocrine neoplasms
WBC	- white blood cells

INTRODUCTION

Neuroendocrine neoplasms (NENs) are a rare, heterogeneous type of cancer that originates mainly in the epithelial cells of the gastrointestinal tract and bronchopulmonary system (Spada & Fazio 2016). Despite numerous improvements made in the last decades in terms of diagnostic techniques and therapy, we are still facing a pressing need to identify new biomarkers to better stratify these patients. Following the collection of epidemiological data for several European countries and the US over the past 20 years, the incidence of NENs is estimated at 1-5 per 100,000 inhabitants (Darba & Marsa 2019). The often relatively indolent growth rate, in addition to their characteristic of being mostly non-functioning tumors, could explain why patients are diagnosed late, when there is already systemic dissemination (Basuroy *et al.* 2012). Among the most significant prognostic factors used in clinical practice for guiding the therapeutic approach in patients with NENs, the grading, the site of origin and tumor diameter are the best established (Zeng *et al.* 2013). Furthermore, it seems that tumor microenvironment, and the systemic inflammatory response in particular, play an important part in patients' outcome (Colotta *et al.* 2009).

Cancer, chronic inflammation, and the underlying relationship between the two have been extensively discussed in the literature (Grivennikov *et al.* 2010; Sethi *et al.* 2012). The first to describe the link between cancer and inflammation was Virchow in the 19th century (Berkovic *et al.* 2014). As personalized medicine in oncology is becoming more and more appealing, Berkovic and colleagues publishes in 2014 an interesting paper that presents a potential model that could explain the role of cytokines in gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) tumorigenesis (Berkovic *et al.* 2014). Thus, chronic inflammation contributes to cancer and a tumor can sustain the inflammatory processes involved in neoplastic growth and development (Zhou *et al.* 2018). There are still unanswered questions regarding the exact mechanism by which chronic inflammation promotes tumor development, however, the state of the art supports the fact that tumor growth, angiogenesis and metastasis are related to inflammatory mediators (de Visser *et al.* 2006; Okayasu 2012).

Systemic inflammation can be easily quantified by evaluating the ratio between neutrophils, platelets and lymphocytes. This offers an incredible advantage, as inflammatory markers are readily available and inexpensive. In addition, the initial evaluation of patients, regardless of the diagnosis, will include a full blood count (BC) as a prerequisite investigation before and after surgery or any type of systemic therapy.

Different studies have reported that peripheral blood neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have a potential clinical value in solid tumors such as ovarian and colon cancer (Absenger *et al.* 2013; Raungkaewmanee *et al.* 2012). This does not come as a surprise, considering that neutrophils and platelets are not only responders to infections and, respectively, involved in thrombosis and homeostasis, but also an important source of angiogenic and growth factors (Catani *et al.* 2020; Singel & Segal 2016). Additionally, the regulation of apoptosis and the suppression of proliferation and tumor cell migration are mechanisms coordinated by lymphocytes (Zhao *et al.* 2020).

In oncological patients, surveillance at shorter intervals may be necessary, and even more so in biologically and clinically heterogeneous tumors as NENs. Furthermore, in the case of metastases, the periodic evaluation is even more critical, and routine tests like the CBC can be extremely useful in stratifying patients into risk groups. We therefore aimed to investigate the potential role of inflammatory markers before and after treatment in the context of patients with neuroendocrine tumors of different primary sites.

MATERIALS AND METHODS

This retrospective case-control study included 31 patients with NENs evaluated in the Endocrinology and Oncology departments of Elias Hospital between August 2003 and May 2018. Eligibility criteria included all patients over the age of 18 years for whom we were able to obtain data on the BC at the time of diagnosis, prior to any line of treatment (surgery or systemic). Patients showing clinical evidence of infection or inflammatory conditions or those diagnosed with other

type of cancer other than NENs or with mixed tumors were excluded from our study group. For the appropriate comparison group of inflammatory markers prior to any line of treatment, 21 age and gender matched healthy individuals were chosen.

The second part of our study evaluated the comparative analysis of inflammatory markers obtained before and after the initiation of treatment. Five patients were excluded (due to wait-and-see treatment strategy – N = 2, chemotherapy – N = 1, and lack of history of inflammatory markers at the first evaluation after treatment initiation – N = 2). Clinical information including general patients demographics (age, sex) and follow-up data were gathered from medical records. Neutrophils, lymphocytes, platelets and total white blood cell count were determined with a Hematology Analyzer. We also calculated the related ratios: NLR, PLR, lymphocyte-to-white blood cell ratio (LWR). In addition, we included in our analysis the mean platelet volume (MPV) and the inflammatory systemic index (SII) that was calculated using the formula: neutrophil count × platelet count/ lymphocyte count (Yan *et al.* 2020).

The study received the approval of the Ethics Committee of Elias Hospital. Informed consent was obtained from patients included in the study, according to the Helsinki Declaration.

Statistical analysis

For data analysis we used SPSS statistical software for Windows version 25 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD for continuous variables that showed parametric distribution and median with IQR variation interval for those with nonparametric distribution. The corresponding percentage value was also used for the categorical variables. Determination of the distribution of continuous variables was performed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Depending on the type of distribution of continuous variables, parametric tests (t-test comparing 2 independent samples) and non-parametric tests (Mann-Whitney test) were used. We applied the Chi-square test ($\chi 2$) to compare the percentages of categorical variables and the Fisher test for large and small groups, respectively. For the analysis of the relationship between the continuous variables with normal distribution we used the Pearson correlation coefficient and for the variables with abnormal distribution we analyzed the Spearman's Rho correlation coefficient. Statistical significance was indicated by a p value < 0.05. The value of the correlation coefficient is segregated into 4 categories, namely: weak correlation (coefficient between 0-0.25), moderate correlation (0.25-0.5), good correlation (0.5-0.75) and very good correlation with a coefficient ranging between 0.75 and 1.

Tab. 1. Demographic and clinical characteristics of 31 patients with NENs for which we analyzed the pretreatment inflammatory markers

PARAMETER	NUMBER OF PATIENTS (%), N = 31
Gender	
Male	17 (54.8%)
Female	14 (45.2%)
Age at diagnosis (years), median (IQR)	57 (30)
Family history of cancer	10 (32.2%)
Tumor location	
Lu-NENs	2 (6.4%)
P-NENs	10 (32.2%)
Si-NENs	2 (6.4%)
Du-NENs	2 (6.4%)
G-NENs	3 (9.6%)
MTC	5 (16.1%)
PHEO/PGL	4 (12.9%)
Pr-NENs	1 (3.2%)
Up-NENs	2 (6.4%)
F-NENs	12 (38.7%)
NF-NENs	19 (61.3%)
Ki-67	
≤2	5 (20%)
3-20	17 (68%)
>20	3 (12%)
Undetermined	6
SSTR	
SSTR2	10 (71.4%)
SSTR5	8 (57.1%)
SSTR2+SSTR5	7 (50%)
Undetermined	17
Metastatic disease at diagnosis	12 (38.7%)
Site of metastasis	
Liver	8 (25.8%)
Lymph node	12 (38.7%)
Bone	4 (12.9%)
Locoregional recurrence	4 (12.9%)
Death rate	19.3%
Type of treatment	
Surgery	22 (70.9%)
Endoscopic resection	1 (3.2%)
SSA	5 (16.1%)
Chemotherapy	1 (3.2%)
"Wait and see" policy	2 (6.4 %)

Lu-NENs – lung neuroendocrine neoplasms, P-NENs – pancreatic neuroendocrine neoplasms, Si-NENs – small intestine neuroendocrine neoplasms, Du-NENs – duodenal neuroendocrine neoplasms, G-NENs – gastric neuroendocrine neoplasms, MTC – medullary thyroid carcinoma, PHEO/PGL – pheocromocytoma/paraganglioma, Pr-NENs – prostatic neuroendocrine neoplasms, Up-NENs – unknown primary neuroendocrine neoplasms, F-NENs – functional neuroendocrine neoplasms, NF-NENs – non-functioning neuroendocrine neoplasms, SSTR – somatostatin receptors, SSA – somatostatin analogs.

Tab. 2. Comparative analyses of inflammatory markers between NENs and the control group

PARAMETER	NENs group (N = 31)	Control group (N = 21)	*p* value
Age (years), median (IQR)	57 (30)	60 (25.5)	0.801
Gender Male Female	 17 14	 7 14	 0.162
Neutrophils, median (IQR) (*10^3/mm^3)	4.8 (2.68)	3.78 (2.11)	**0.035**
Platelets, median (IQR) (*10^3/mm^3)	260 (129)	259 (74.5)	0.780
WBC, median (IQR) (*10^3/mm^3)	7.42 (3.2)	6.74 (2.13)	0.351
Lymphocytes, median (IQR) (*10^3/mm^3)	1.57 (0.75)	1.91 (1.05)	**0.009**
NLR, median (IQR)	3.01 (2.32)	1.84 (1.13)	**0.001**
PLR, median (IQR)	162.96 (93.71)	137.5 (37.98)	**0.003**
SII, median (IQR)	747.5 (669.3)	540.58 (272.45)	**0.001**
LWR, mean ± SD	0.21 ± 0.07	0.30 ± 0.07	**<0.001**
MPV, median (IQR) [fl]	10.4 (1)	10.3 (0.75)	0.647

NENs – neuroendocrine neoplasms, WBC – white blood cells, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – inflammatory systemic index, LWR – lymphocyte-to-white blood cell ratio, MPV – mean platelet volume.

RESULTS

Patients characteristics

In our group of 31 patients with NENs, the median age at diagnosis was 57 years (IQR = 30), with a slightly higher incidence in males N = 17 (54.8%). In terms of tumor type, the majority were pancreatic neuroendocrine neoplasms (P-NENs) (N = 10, 32.2%). The Ki-67 proliferation index was quantified for 25 patients, of which 17 (68%) had a value between 3 and 20%. The present study included 3 patients with poorly differentiated tumors. Non-functioning NENs (N = 19, 61.2%) represented the majority in our group of patients. We report a death rate of 19.3%. The demographic characteristics of our cohort for the first analysis (case-control) are illustrated in Table 1.

The pre-treatment comparative analysis of inflammatory markers between patients with NENs and the control group

When comparing the group of patients with NENs (N = 31) with 21 age and gender matched healthy individuals, the analysis of inflammatory markers did not reveal a statistically significant difference in terms

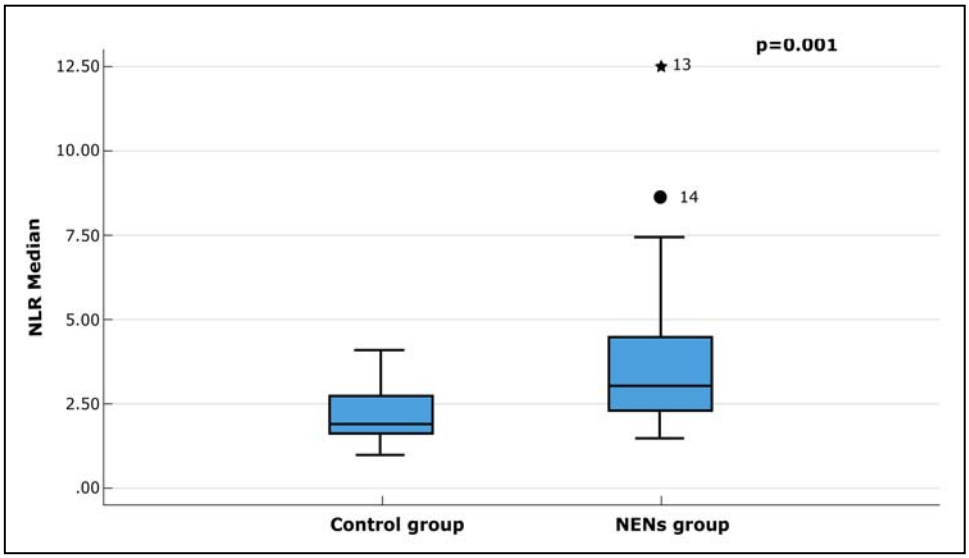

Fig. 1. Difference in median NLR between control group and NENs group
NENs – neuroendocrine neoplasms, NLR – neutrophil-to-lymphocyte ratio

Tab. 3. Comparison of inflammatory markers between GEP-NENs and other tumor sites

PARAMETER	GEP-NENs (N = 17)	NENs≠GEP-NENs (N = 14)	*p* value
Platelets, median (IQR) (*10^3/mm^3)	252 (122)	276.5 (129.25)	0.138
Neutrophils, mean ± SD (*10^3/mm^3)	5.31 ± 1.99	5.3 ± 1.84	0.986
Lymphocytes, mean ± SD (*10^3/mm^3)	1.66 ± 0.64	1.53 ± 0.49	0.516
WBC, mean ± SD (*10^3/mm^3)	7.7 ± 2.1	7.4 ± 2.1	0.753
NLR, median (IQR)	2.87 (2.9)	3.24 (1.92)	0.570
PLR, median (IQR)	162.96 (79.17)	171.33 (143.68)	0.297
SII, median (IQR)	721.83 (438.06)	801.25 (1143.74)	0.421
LWR, mean ± SD	0.22 ± 0.08	0.2 ± 0.06	0.632
MPV, median (IQR) [fl]	10.4 (1.1)	10.3 (1.22)	0.493

NENs – neuroendocrine neoplasms, GEP-NENs – gastroenteropancreatic neuroendocrine neoplasms, WBC – white blood cells, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – inflammatory systemic index, LWR – lymphocyte-to-white blood cell ratio, MPV – mean platelet volume.

of the absolute value of platelets and white blood cells. However, the total lymphocyte count was significantly lower in patients with NENs compared with our control group [1.57 (IQR = 0.75) versus 1.91 (IQR = 1.05), $p = 0.009$] (Table 2). Additionally, we observed significantly higher values of calculated ratios such as NLR, PLR and SII in the oncological group and a statistically significant difference of the mean LWR between our two groups (0.21 ± 0.07 compared to 0.3 ± 0.07, $p < 0.001$) (Table 2 and Figure 1).

The analysis of pre-treatment inflammatory markers depending on tumor location

As presented in the previous section, we studied a heterogeneous group of patients in terms of tumor location. In order to analyze the variability of inflammatory markers according to tumor site, we initially divided the group of patients into two categories, namely those with gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) and those with other localizations of the primary lesion. We did not find any statistically significant differences in terms of inflammatory markers collected prior to any line of treatment between the two categories (Table 3).

In contrast, when we clustered the patients according to the pancreatic location of the primary tumor, we observed a significant difference of the absolute count of lymphocytes and also of NLR, PLR and LWR (Table 4 and Figure 2).

The correlation between baseline inflammatory markers and neuroendocrine specific biomarkers

Chromogranin A (CgA) and 5-hydroxyindolacetic acid (5-HIAA) are valuable biomarkers that have a significant contribution to the workup plan of patients diagnosed with NENs. In our group of 31 patients, we managed to obtain the results of the baseline level of CgA and 5-HIAA in 30 and 21 cases, respectively. We observed a positive correlation between PLR and the initial value of 5-HIAA ($p = 0.006$, $\rho = 0.582$), whereas the CgA concentration was negatively correlated with the absolute count of lymphocytes (Table 5).

The comparative analysis of inflammatory markers before and after treatment initiation

In the comparative study of inflammatory markers prior to and post-treatment we had to exclude 5 patients due to: lack of data regarding postoperative blood count evaluation, chemotherapy as the first line of therapy and "wait and see" treatment policy. Furthermore, of the 26 patients included in this analysis, 22 underwent surgical resection of the primary tumor and 4 received systemic therapy with SSA. Table 6 details the demographic and clinical characteristics of our patients.

When we compared the values of inflammatory markers and the ratios resulting from this analysis, we observed significantly higher levels of neutrophils, NLR and SII in the pre-treatment group. Additionally, the LWR was significantly higher in patients that underwent surgery or systemic therapy with SSA (Table 7).

The comparative analysis in terms of tumor location (GEP-NENs versus non-GEP-NENs) showed no significant differences in the absolute value of post-treatment inflammatory markers (and related ratios) in the two subgroups. However, in the P-NENs subgroup, statistically significant differences were observed only for the absolute number of post-treatment platelets (Table 8).

Of the 26 patients, the value of the Ki-67 proliferation index was determined in 21 cases. Segregation of patients according to a Ki-67 value greater than or equal to 10 (N = 6/21) revealed that the MPV value is

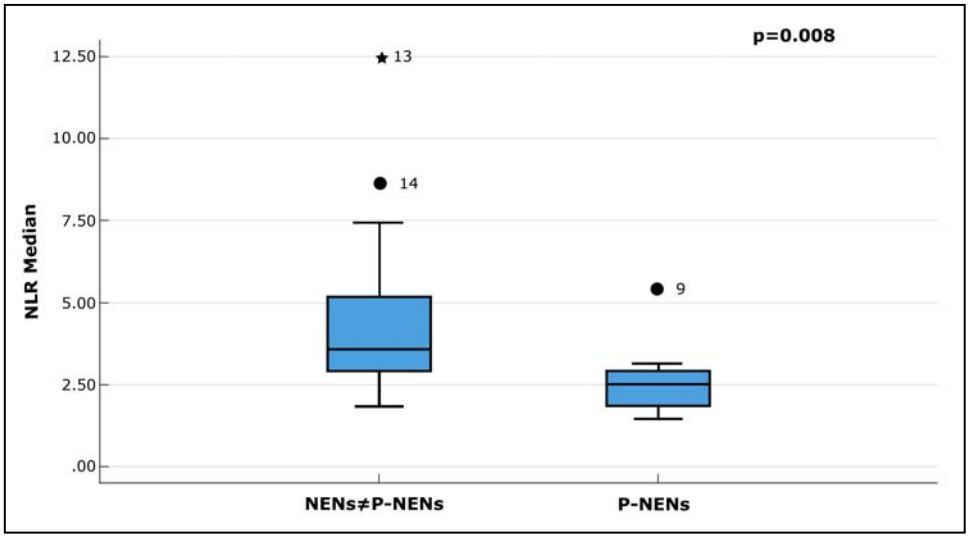

Fig. 2. Difference in median NLR according to the pancreatic location of the primary tumor
P-NENs – pancreatic neuroendocrine neoplasms, NENs – neuroendocrine neoplasms, NLR – neutrophil-to-lymphocyte ratio

not similar between groups ($p = 0.021$). Thus, we report a higher MPV value in patients with a Ki-67 above 10%.

DISCUSSION

The complete blood count (CBC) is a reliable low cost and reproducible test that can help physicians monitor different oncological treatments in terms of side effects. In addition, the study of blood parameters represents a relatively new research direction with significant prognostic implication in oncology. NENs are an extremely heterogeneous group of tumors, with an apparent indolent evolution and symptoms that are often nonspecific. Due to these characteristics, patient follow-up requires the definition of new prognostic and predictive markers. The analysis of hematological indices and the resulted ratios (NLR, PLR, LWR, SII) reflect the degree of systemic inflammation. In recent years, increasing evidence points to the causal link between carcinogenesis and chronic inflammation (Grivennikov *et al.* 2010; Sethi *et al.* 2012).

The first aim of our study was to compare the pre-treatment hematological indices between patients diagnosed with NENs and a group of gender matched healthy individuals. Thus, we observed a significantly higher median value of neutrophils in the NENs patients compared to the control group [4.8 (IQR = 2.68) compared to 3.78 (IQR = 2.11), $p = 0.035$]. In addition, our analysis demonstrated a higher lymphocyte value in healthy adults without an oncological disease compared with our NENs group [1.91 (IQR = 1.05) versus 1.57 (IQR = 0.75), $p = 0.009$].

The relationship between the absolute lymphocyte count and the survival of patients with different types of cancer has been extensively explored in the literature. In this regard, patients with ovarian, breast, colorectal and renal cancer with a low lymphocyte count had a poorer outcome (Liang *et al.* 2016; Mehrazin *et al.* 2015; Milne *et al.* 2012, Tredan *et al.* 2013). Furthermore, Zhao and colleagues published in 2017 an interesting study that evaluated the relationship of peripheral lymphocyte count, LWR and the overall survival in patients with advanced cancer (Zhao *et al.* 2017). The authors concluded that changes in LWR rather than the lymphocyte count (due to its day-to-day variability) prior to treatment and also during follow-up is a valuable prognostic tool in oncological patients. Moreover, this study showed that patients with a lower LWR had a significantly shorter survival, thus demonstrating the clinical value of this parameter (Zhao *et al.* 2017).

In our study, the comparative analysis of pre-treatment inflammatory markers showed statistically significant differences in NLR, PLR and SII between our NENs patients and the control group. Thus, higher median values of NLR, PLR and SII were found in patients with NENs [3.01 (IQR = 2.32), 162.96 (IQR = 93.71), 747.5 (IQR = 669.3)] compared to healthy adults [1.84 (IQR = 1.13), 137.5 (IQR = 37.98), 540.58 (IQR = 272.45)] (Table 2). We also demonstrated a higher mean value of LWR in the control group in comparison with the values obtained prior to treatment in our NENs group (0.3 ± 0.07 versus 0.21 ± 0.07, $p = <0.001$).

Malignant tumors can be perceived as unhealed wounds (Byun & Gardner 2013). The tumor microenvironment is modulated by activated platelets that have the capacity of releasing pro-inflammatory cytokines (Olsson & Cedervall 2018). MPV is a marker of platelet activity that proved to be useful in monitoring the prognosis of various inflammatory conditions, including cancer (Gong *et al.* 2016; Korniluk *et al.* 2019). Moreover, according to Karaman's publication the analysis of MPV was used in the differential

Tab. 4. Comparison of inflammatory markers between P-NENs and other tumor sites

PARAMETER	P-NENs (N = 10)	NENs≠P-NENs (N = 21)	*p* value
Platelets, median (IQR) (*10^3/mm^3)	267.5 (114.5)	257 (148)	0.983
Neutrophils, mean ± SD (*10^3/mm^3)	4.53 ± 1.04	5.68 ± 2.1	0.115
Lymphocytes, mean ± SD (*10^3/mm^3)	1.92 ± 0.62	1.45 ± 0.49	**0.029**
WBC, mean ± SD (*10^3/mm^3)	7.14 ± 1.43	7.8 ± 2.36	0.423
NLR, median (IQR)	2.48 (1.13)	3.54 (2.41)	**0.008**
PLR, mean ± SD	143.8 ± 38.91	206.88 ± 87.7	**0.039**
SII, median (IQR)	716.98 (393.68)	855 (1201.98)	0.096
LWR, mean ± SD	0.26 ± 0.09	0.19 ± 0.06	**0.008**
MPV, median (IQR), [fl]	10.5 (1.96)	10.3 (0.8)	0.787

NENs – neuroendocrine neoplasms, P-NENs – pancreatic neuroendocrine neoplasms, WBC – white blood cells, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – inflammatory systemic index, LWR – lymphocyte-to-white blood cell ratio, MPV – mean platelet volume.

diagnosis of P-NENs from pancreatic adenocarcinomas (Karaman *et al.* 2011). Despite these results, we did not observe any significant differences in the pre-treatment MPV values between patients with NENs and healthy individuals, nor between tumor types (P-NENs versus other sites).

When analyzing the inflammatory markers prior to any line of treatment according to tumor location (pancreatic versus other primary sites), we obtained significantly lower values of NLR and PLR in the P-NENs group (Table 4). We also report significantly higher mean values of LWR in the P-NENs group compared to other tumor sites (0.26 ± 0.09 versus 0.19 ± 0.06, $p = 0.008$). These findings are partially in contradiction with an interesting research published by Salman and colleagues in 2016. This study evaluated 132 patients with GEP-NENs in which NLR and PLR had higher mean values in the P-NENs group compared to patients with gastroenteric NENs (3.3 ± 0.9 compared to 1.9 ± 0.7, $p = 0.0001$, and 303.1 ± 91.2 compared to 161.1 ± 67.5, $p = 0.0001$, respectively) (Salman *et al.* 2016). A possible explanation for these differences is the small number of patients included in our study, and also the fact that the population with which the P-NENs group was compared included both gastroenteric NENs and other tumor types, such as MTC, PHEO / PGL, Up-NENs.

Patients stratified according to a Ki-67 index ≥10% did not show any statistically significant differences in terms of inflammatory markers prior to any line of treatment. However, Yucel and colleagues demonstrated an association between NLR, SII and tumor grade in a heterogeneous population of NENs, the majority being gastroenteropancreatic tumors (Yucel

Tab. 5. Correlations between inflammatory markers, CgA and 5-HIAA

PARAMETER	CgA (N = 30)	5-HIAA (N = 21)
Neutrophils (*10^3/mm^3)	$p = 0.122$, $\rho = 0.288$	$p = 0.563$, $\rho = 0.134$
Platelets (*10^3/mm^3)	$p = 0.442$, $\rho = 0.146$	$p = 0.416$, $\rho = 0.187$
WBC (*10^3/mm^3)	$p = 0.315$, $\rho = 0.190$	$p = 0.996$, $\rho = 0.001$
Lymphocytes (*10^3/mm^3)	$p = 0.021$, $\rho = -0.419$	$p = 0.028$, $\rho = -0.480$
NLR	$p = 0.008$, $\rho = 0.472$	$p = 0.042$, $\rho = 0.447$
PLR	$p = 0.017$, $\rho = 0.432$	$p = 0.006$, $\rho = 0.582$
SII	$p = 0.014$, $\rho = 0.446$	$p = 0.049$, $\rho = 0.434$
LWR	$p = 0.007$, $\rho = -0.481$	$p = 0.043$, $\rho = -0.446$
MPV, [fl]	$p = 0.268$, $\rho = 0.209$	$p = 0.997$, $\rho = -0.001$

CgA – chromogranin A, 5-HIAA – 5-hydroxyindolacetic acid, WBC – white blood cells, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – inflammatory systemic index, LWR – lymphocyte-to-white blood cell ratio, MPV – mean platelet volume.

Tab. 6. Demographic and clinical characteristics of 26 patients with NENs for which we analyzed the inflammatory markers prior to and post-treatment

PARAMETER	NUMBER OF PATIENTS (%), N = 26
Gender	
Male	14 (53.9%)
Female	12 (46.1%)
Age at diagnosis, median (IQR)	60 (30.25)
Tumor loction	
Lu-NENs	2 (7.6%)
P-NENs	8 (30.7%)
Si-NENs	2 (7.6%)
Du-NENs	1 (3.8%)
G-NENs	2 (7.6%)
MTC	5 (19.3%)
PHEO/PGL	4 (15.4%)
Pr-NENs	1 (3.8%)
Up-NENs	1 (3.8%)
Metastatic disease at diagnosis	10 (38.4%)
Liver metastases	5 (19.2%)
Lymph node metastases	11 (42.3%)
Ki-67	
≤2	4 (19%)
3-20	15 (71.5%)
>20	2 (9.5%)
Undetermined	5
Type of treatment	
Surgery	22 (84.6%)
SSA	4 (15.4%)

Lu-NENs – lung neuroendocrine neoplasms, P-NENs – pancreatic neuroendocrine neoplasms, Si-NENs – small intestine neuroendocrine neoplasms, Du-NENs – duodenal neuroendocrine neoplasms, G-NENs – gastric neuroendocrine neoplasms, MTC – medullary thyroid carcinoma, PHEO/PGL – pheocromocytoma/paraganglioma, Pr-NENs – prostatic neuroendocrine neoplasms, Up-NENs – unknown primary neuroendocrine neoplasms, F-NENs – functional neuroendocrine neoplasms, NF-NENs – non-functioning neuroendocrine neoplasms, SSTR – somatostatin receptors, SSA – somatostatin analogs.

et al. 2018). Regarding our analysis, we did not compare the values of the preoperative inflammatory markers according to the WHO 2010 classification, as our study included other types of tumors besides those of the gastroenteropancreatic tract.

The European Neuroendocrine Tumor Society (ENETS) Consensus Guidelines convey that CgA is a valuable biomarker for diagnosis and treatment follow-up for patients with NENs, with a sensitivity and specificity between 60 and 90%. In addition, urinary 5-HIAA is a very useful test in the clinical setting of patients with carcinoid syndrome (Oberg *et al.* 2017). Taking into account the importance of these biomarkers and also the fact that, to the best of our knowledge, this analysis has not been assessed in the literature, we evaluated the relationship between CgA, 5-HIAA at diagnosis and the pre-treatment hematological indices.

We observed a negative correlation between the absolute count of lymphocytes and also LWR with both CgA and 5-HIAA. Furthermore, NLR, PLR and SII were positively correlated with both markers (Table 5). Thus, we believe that the corroboration of circulating biomarkers with the results of the CBC could help clinicians to better predict patients' prognosis and response to certain treatments. Furthermore calculating the ratios between the absolute count of neutrophils, platelets, lymphocytes and white blood cells and including the results in future prognostic models together with CgA, 5-HIAA and many other specific NENs markers, represent a future line of research.

In the comparative analysis of inflammatory markers before and after initiating a line of treatment, five patients were excluded as explained in the material and methods section. For the remaining 26 patients, a significantly lower median neutrophil value was observed after treatment [4.01 (IQR 1.05) compared to 4.74 (IQR 2.3), $p = 0.012$]. Furthermore, the analysis revealed lower values of NLR and SII and a higher mean LWR value (Table 7) in the post-treatment group. A possible explanation for these results is the reduction of tumor burden and the subsequent chronic inflammation once treatment was initiated (in our case resection of the primary tumor or the antitumoral effect of SSA).

When patients were stratified according to tumor location, we observed a significantly higher mean value of platelets in the pancreatic group in comparison to the rest of tumor sites included in our study (333.16 ± 96 versus 234.5 ± 50.07, $p = 0.007$). Moreover, a significantly higher mean MPV value (post-treatment) was found in patients with a Ki-67 proliferation index ≥10% (11.4 ± 0.7 versus 10.3 ± 0.9, $p = 0.021$). The literature provides a single publication that suggests the diagnostic value of MPV in differentiating pancreatic adenocarcinomas from P-NENs. The latter showed lower pre-treatment values of MPV compared to adenocarcinomas with the same localization (Karaman *et al.* 2011). Extrapolating this information, supported by the idea that a more aggressive tumor (adenocarcinoma) has a higher MPV value in comparison with a tumor exhibiting an indolent evolution such as P-NENs, we can speculate that there could be a causal relationship between the histopathological aggressiveness of a tumor and MPV.

The potential role of MPV as a predictive marker of pathologic complete response after neoadjuvant chemotherapy in patients with breast cancer was investigated by Multu and colleagues in 2016. The authors concluded that a lower MPV predicts a higher pathologic complete response rate (Multu *et al.* 2016). Gathering all this information, we believe that the study of pre- and post-treatment MPV can provide important information on inflammatory status, especially in patients with cancer.

Tab. 7. Pre/post-treatment analyses of inflammatory markers

PARAMETER	PRE-TREATMENT	POST-TREATMENT	*p* value
Neutrophils, median (IQR) (*10^3/mm^3)	4.74 (2.3)	4.01 (1.05)	**0.012**
Platelets, median (IQR) (*10^3/mm^3)	262 (126)	255 (73)	0.647
Lymphocytes, mean ± SD (*10^3/mm^3)	1.6 ± 0.57	1.8 ± 0.56	0.216
WBC, median (IQR) (*10^3/mm^3)	7.25 (3.25)	6.33 (1.88)	0.191
NLR, median (IQR)	3 (2.38)	2.39 (1.03)	**0.003**
PLR, median (IQR)	160.52 (59.87)	155.62 (61.04)	0.323
SII, median (IQR)	734.66 (480.37)	617.69 (266.26)	**0.006**
LWR, mean ± SD	0.21 ± 0.06	0.26 ± 0.06	**0.004**
MPV, median (IQR), [fl]	10.4 (1.1)	10.3 (1.58)	0.883

WBC – white blood cells, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – inflammatory systemic index, LWR – lymphocyte-to-white blood cell ratio, MPV – mean platelet volume.

The limitations of our study consist in the small number of patients and also the heterogeneous population of tumors included in the analysis. Prospective and larger studies should evaluate the prognostic and predictive value of inflammatory markers in order to avoid potential selection bias.

CONCLUSIONS

The CBC is a routine test that can be easily performed at diagnosis, but also in the course of follow-up in cancer patients. Despite diagnostic and therapeutic advances, NENs represent a challenge, requiring a multidisciplinary approach and new biomarkers useful for stratifying patients in terms of prognostic and treatment decision-making. The degree of systemic inflammation is reflected by the measurement of hematological indices and the resulting ratios (NLR, PLR, LWR, SII). Our study confirms the practicality of hematological parameters in the NENs setting. Thus, in comparison to the control group, the NENs patients had a considerably higher median neutrophil value. Additionally, in the post-treatment group the reduction of tumor burden and the subsequent chronic inflammation once treatment was initiated revealed lower values of NLR and SII and a higher mean LWR value. In conclusion we believe that the assessment of peripheral blood cells parameters is a low-cost and reliable marker of ongoing cancer-related inflammation which can be extrapolated in tumors of neuroendocrine origin. In addition, the

Tab. 8. Comparison of inflammatory markers between P-NENs and other tumor sites after treatment initiation

PARAMETER	P-NENs (N = 8)	NENs≠P-NENs (N = 18)	*p* value
Neutrophils, median (IQR) (*10^3/mm^3)	4.3 (1.09)	4.11 (1.34)	0.397
Platelets, mean ± SD (*10^3/mm^3)	333.16 ± 96	234.5 ± 50.07	0.007
Lymphocytes, mean ± SD (*10^3/mm^3)	2.23 ± 0.57	1.61 ± 0.19	0.059
WBC, median (IQR)(*10^3/mm^3)	7.29 (2.06)	6.5 (1.55)	0.216
NLR, median (IQR)	1.83 (1.51)	2.45 (1.33)	0.531
PLR, mean ± SD	155.84 ± 51.47	147.01 ± 33.43	0.636
SII, median (IQR)	545.89 (515.06)	607.49 (354.17)	0.724
LWR, mean ± SD	0.3 ± 0.06	0.24 ± 0.06	0.336
MPV, mean ± SD [fl]	10.8 ± 0.8	10.5 ± 0.89	0.560

NENs – neuroendocrine neoplasms, P-NENs – pancreatic neuroendocrine neoplasms, WBC – white blood cells, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – inflammatory systemic index, LWR – lymphocyte-to-white blood cell ratio, MPV – mean platelet volume.

evaluation of CBC can add value to prognostic models, which could better estimate the risk of future outcome in cancer patients.

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