Systemic inflammation markers in blood samples of colorectal cancer patients

Grudzińska M.[1,A,C,D], Jakubowska K.*[2,B,C,E], Koda M.[1,A,B,F], Koda L.[2,A,B,F], Kisielewski W.[3,B], Smereczański N.[4,C,F], Rogoz-Jezierska N.[5,C], Famulski W.[3,F],

1. Department of General Pathomorphology, Medical University of Bialystok, Bialystok, Poland
2. Department of Pathology, Maria Sklodowska-Curie Memorial Bialystok Oncology Centre, Bialystok, Poland
3. Department of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland
4. Department of Toxicology, Medical University of Bialystok, Bialystok, Poland
5. Central Laboratory of Specialistic Hospital in Brzozow, Podkarpacki Oncology Centre, Brzozow, Poland

A- Conception and study design; **B -** Collection of data; **C -** Data analysis; **D -** Writing the paper;
E- Review article; **F** - Approval of the final version of the article; **G** - Other (please specify)

ABSTRACT

Introduction: Colorectal cancer (CRC) is one of the most common cancers in Poland. The aim of this study was to investigate the clinical significance of absolute monocyte count, neutrophil-to-monocyte ratio (NMR) and monocyte-to-lymphocyte ratio (MLR) in pre- and postoperative blood samples of patients with CRC.

Materials and Methods: We retrospectively reviewed medical records of 160 patients diagnosed with CRC who underwent surgery. Blood samples were obtained within 3 days before and after the surgical treatment. Venous blood samples were also obtained from 42 healthy controls.

Results: Pre- and postoperative NMR were significantly higher than healthy controls ($p<0.0001$; $p<0.0001$). Moreover, MLR in pre-and postoperative blood samples were higher than voluntaries ($p<0.001$; $p<0.001$). The area under the ROC curve for pre and postNMR showed that the parameter exhibits strong diagnostic power (1.000). Pre- and postMLR had moderate diagnostic power amount 0.751 and 0.746. There is also correlation between monocyte count in samples obtained before and after surgery and, lymph node metastasis and size of lymph node metastasis in both cases. PreNMR value was significantly associated with venous and lymphatic invasion and the presence of cancer deposits. PostNMR was found to correlate with presence of distant metastasis and cancer cell deposits ($R=0.633$, $p<0.001$; $R=0.158$, $p=0.040$). Moreover, preMLR value was correlated with only perineural invasion.

Conclusions: Analyzed hematologic markers may be useful as simply obtained parameters, next to histopathological examination, that determine a systemic immune response.

Keywords: Colorectal cancer, neutrophil-to-monocyte ratio, monocyte-to-lymphocyte ratio

DOI

***Corresponding author:**
Katarzyna Jakubowska, Department of Pathology, Maria Sklodowska-Curie Memorial Bialystok Oncology Centre, Bialystok, Poland; e-mail: kathian@wp.pl

Received: 25.03.20120
Accepted: 05.06.2020
Progress in Health Sciences
Vol. 10(1) 2020 pp 92-101

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancer in Poland. In the near future, number of deaths due to colorectalcancer probably will increase two-fold for the male population and about one third for the female population [1]. Diagnosis of histological type of colorectal cancer and chosen prognostic and predictive markers were performed in the basis of conventional the tumor-node-metastases (TNM) classification recommended by The American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) society [2]. Nowadays, it is well known that diagnosis of CRC patients is performed also in the basis of the molecular biomarkers such as mutations in the KRAS, NRAS, BRAF, and PIK3CA genes that were used in daily practice. Patients with mutations in KRAS and NRAS – codons 12 and 13 of exon 2; codons 59 and 61 of exon 3; codons 117 and 146 of exon 4 should not receive anti-EGFR therapy [3]. Approximately 5%–10% of CRC have BRAF mutation in codon V600 that results in constitutive activation of BRAF kinase [4]. Moreover, Exon 20 mutations of PIK3CA are linked with poor clinical response to cetuximab [5]. Literature data also noted prognostic and predictive value of the loss of MMR proteins as result of epigenetic silencing of the four most common MMR genes including MLH1, MSH2, MSH6, and PMS2 [6].

The next direction of biomarkers investigation includes immune cell response in primary tumor mass. The first effort to find such immunological markers was made by Galon and his colleagues. Scientists showed that tumors with elevated levels of $CD3^+$ T-cells in the main mass as well as at the invasive margin were associated with the best clinical outcome, beyond the conventional TNM system [7]. Since 2012, it has been defined the Immunoscore that quantified the immune infiltrate, including the density of CD8+ cytotoxic T-cells and CD45RO+ memory cells inside the tumor [8]. The validated, large group examination demonstrated that patients with “low” immunoscore have shorter time-to-tumor recurrence compared to those with “high” immunoscore [9]. The inadequate response to the treatment of CRC patients forces us to search new simple biomarkers that can be validated, with optimal efficacy [10]. It is now well established that examination of local immune response tumor mass of patients with CRC is not sufficient. Therefore, hematologic biomarkers, containing simple whole blood parameters, have been recently explored [11,12]. The investigators confirmed that CRC patients with elevated neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte count, and Glasgow prognostic score (GPS) have worse prognosis in overall survival (OS), cancer-specific survival (CSS), and disease-free survival (DFS) [12]. The aim of this study was to investigate the clinical significance of the absolute monocyte count, neutrophil-to-monocyte ratio (NMR) and monocyte-to-lymphocyte ratio (LMR) in pre- and postoperative blood samples of patients with CRC.

MATERIALS AND METHODS

Patients’ characteristics

We retrospectively reviewed medical records of 160 patients diagnosed with CRC (96 men and 64 women) who underwent surgery in the Department of Oncological Surgery, Comprehensive Cancer Center (Bialystok, Poland) between April 2014 and December 2016. The mean age of the patients was 67.5 years, including 40 patients <60 years-old and 120 patients >60 years-old. The majority of patients presenting similar symptoms, including abdominal pain, anemia, rectal bleeding, constipation, diarrhea, vomiting and anorexia. In the majority of cases, patients additionally received treatment for hypertension, type II diabetes, osteoarthritis and coronary heart disease. However, none of the patients had received inflammatory therapy. All patients underwent routine diagnostic tests, including basic diagnostic laboratory tests (morphological tests and lipid profiles), electrocardiography, spirometry, arterial blood gasometric test, X-ray and computerized chest tomography. The clinical stage of CRC was evaluated according to the Tumor-Node-Metastasis (TNM) classification [13]. Prior to surgery, patients with tumors identified in other sites received no inflammatory or immunosuppressive therapy. The response to preoperative therapy was estimated according to the Response Evaluation Criteria in Solid Tumors [14]. Tissues obtained from surgery were fixed in 4% buffered formalin for 24 to 72 h at room temperature. Small sections of tissue were embedded in paraffin. Sections (4 μm-thick) were cut from paraffin blocks and stained with hematoxylin and eosin (H&E) at room temperature for 4 min (cat. no. 468802128; POCH S.A.; Avantor Performance Materials Poland, Gliwice, Poland) according to the manufacturers' protocol. The slides were deparaffinized in an oven at 60°C for 5 min. Subsequently, slides were rehydrated in xylene (three washes, 10 min each) and graded ethanol (100, 95, 85 and 75%, 1 min at each concentration). Routine histopathological assessment of the sections was performed by two pathologists blinded to the clinical information. The type of tumor growth, tumor size, histological type, percentage of mucinous components, grade of malignancy, TNM and Dukes stages were determined by the pathologists. Venous, lymphatic and perineural invasion of cancer cells were also analyzed. Characteristic features of lymph node invasion were examined, including the number of resected and invaded lymph nodes, the presence of micro- and macro-metastases, invasion of the pouch

lymph node, presence of distant metastases and the size of metastases. The presence, number and size of the deposits of cancer cells were also assessed [15]. Tumor budding was analyzed as previously described by Morodomi et al. [16]. Crohn's-like aggregates of lymphocytes (CLR) were evaluated based on the Väyrynen criteria [17]. CLR was defined as lymphoid structures surrounding the primary tumors, excluding mucosa-associated lymphoid tissue or pre-existing lymph nodes. Histological categorization of fibrotic cancer stroma was performed based on the criteria from Ueno et al. [17]. Fibrotic cancer stroma was classified as follows: mature, composure of mature collagen fibers and elongated fibers with fibrocytes stratified into multiple layers; intermediate, broad bands of collagen with brightly eosinophilic hyalinization and keloid-like structures; and immature, composition of randomly oriented keloid-like collagen bundles surrounded by myxoid stroma.

The present study was performed in accordance with the Declaration of Helsinki for Human Experimentation, and the protocol was approved by The Bioethics Committee of the Medical University of Bialystok (approval no R-I-002/353/2016). Written informed consent was obtained from all participants.

Blood samples examination

Blood samples were obtained within 3 days before and after the surgical treatment. Venous blood samples were also obtained from 42 healthy controls (female-21, male-21; mean age 45 years old; min-max 25-65 years old).

The differential white blood cell count was analyzed using Sysmex XN-1000 based on the manufacturers' protocol. Cancer biomarkers were analyzed performed using Cobas 6000.

We analyzed monocyte count, neutrophil monocyte ratio (NMR) and monocyte lymphocyte ratio (MLR) in whole blood before and after surgery of patients with CRC. The NMR was defined as the absolute neutrophil count divided by the absolute monocyte count. The MLR was performed by the absolute monocyte count divided by absolute lymphocyte count.

Receiver operating characteristic (ROC) curve analysis was used to the investigation of cutoff values of the pre- and postoperative monocyte count (preMONO/postMONO), NMR (preNMR /postNMR) and MLR (preMLR/postMLR).

According to the ROC curves and significant correlation with each other, we defined the scores of monocyte count, NMR and MLR as 1 or 2 when patients had low or high analyzed parameters in pre- and postoperative blood samples.

Statistical analysis

Statistical analysis was performed using the STATISTICA 13.3 program (Statsoft, Cracow, Poland). A Mann-Whitney U-test was used to compare the groups. Correlations between the parameters were calculated using the Spearman's correlation coefficient test. $P<0.05$ was considered to indicate a statistically significant difference. The missing data was removed in pairs. The analysis of ROC curve was performed using MedCalc statistical software (MedCalc Software, Belgium).

RESULTS

Estimation of cut-off values of monocyte count, NMR and MLR

Pre- and postoperative NMR were significantly higher than in healthy controls ($p<0.0001$; $p<0.0001$). Moreover, MLR in pre-and postoperative blood samples were higher than in voluntaries ($p<0.001$; $p<0.001$). Only monocyte count did not differ between the groups. The cut-off of pre- and postoperative monocyte count were 0.39 and 0.68 with sensitivity (preMONO-75.8%; postMONO- 27.89%) and specificity (preMONO-16.67%; postMONO-85.71%) The area under the ROC curve for pre- and post monocyte count had weak prognostic value of CRC patients (0.505 and 0.532). The cut-off of pre- and postoperative NMR was 0.48 and 0.48 with sensitivity and specificity (preNMR- 100% and 100%; postNMR- 89,16 and 100). The area under the ROC curve for pre- and postNMR showed that the parameter exhibits strong diagnostic power (1.000). Moreover, the cut-off of pre- and postoperative MLR was 1.46 in both cases. The sensitivity and specificity of analysis in pretreatment blood samples were 94.27% and 73.81% and were similar to those in postopreative samples (90.41% and 73.81%). The area under the ROC curve for pre- and postNMR showed that the parameter exhibits strong diagnostic power (1.000). Pre- and postMLR had moderate diagnostic power amount of 0.751 and 0.746.

Monocyte count, NMR, MLR in CRC and its correlation with clinicopatholgical parameters

PreMONO and postMONO counts correlated with pT ($R=0.296$, $p<0.001$; $R=0.295$; $p<0.001$). There is also correlation between monocyte count in samples obtained before and after surgery and lymph node metastasis and size of lymph node metastasis in both cases ($R=0.256$, $p=0.039$; $R=0.184$, $p=0.022$; $R=0.262$, $p=0.041$; $R=0.185$, $p=0.025$). PreNMR value was significantly associated with venous and lymphatic invasion and the presence of cancer deposits ($R=0.190$, $p=0.018$; $R= 0.191$, $p=0.017$; $R=0.230$, $p=0.004$).

PostNMR was found to correlate with the presence of distant metastasis and cancer cell deposits ($R=0.633$, $p<0.001$; $R=0.158$, $p=0.040$). Moreover, preMLR value correlated with only perineural invasion ($R=0.178$, $p=0.027$). We did not

find any connection between postMLR and clinicopathological parameters (Table 1).

Monocyte count, NMR, MLR in CRC and its correlation with morphological blood parameters

We also noted many various correlations between analyzed factors and selected morphological and cancer biomarkers that are shown in Table 2. Association between well-known markers, such CA-19-9 and monocyte count before and after surgery of CRC patients seems to be the most important in clinical usage (R=0.987, p<0.001; R=0.945, p<0.001).

Table 1. Correlations between monocyte count, NMR and MLR in pre and postoperative whole blood samples and clincopathological features of patients with CRC

Parameter		**N** 160	**preMONO** R, p-value	**postMONO** R, p-value	**preNMR** R, p-value	**postNMR** R, p-value	**preMLR** R, p-value	**postMLR** R, p-value
Age	<60 >60	40 120	-0.059 0.454	-0.060 0.460	0.074 0.357	0.083 0.286	0.043 0.592	-0.013 0.871
Gender	Female Male	64 96	-0.132 0.095	-0.137 0.093	0.010 0.895	-0.001 0.987	-0.053 0.506	-0.101 0.222
Localization	Right-side Transverse Left-side Sigmoid Rectum	20 14 15 29 82	0.113 0.170	0.115 0.173	-0.017 0.835	0.093 0.245	-0.034 0.679	-0.038 0.655
Tumor growth	Expanding Infiltrate	133 27	0.099 0.212	0.109 0.184	0.005 0.946	-0.100 0.198	0.019 0.805	-0.043 0.602
Tumor size	<2.5cm 2.5-5.0cm >5.0cm	27 106 27	0.031 0.969	0.003 0.967	-0.037 0.639	-0.041 0.599	0.092 0.249	0.097 0.241
TNM stage	1 2 3 4	42 31 69 18	0.137 0.086	0.138 0.092	0.090 0.262	0.072 0.352	0.123 0.124	-0.058 0.482
Grade of malignancies	2 3	148 12	0.153 0.054	0.158 0.053	-0.024 0.765	0.118 0.128	-0.030 0.708	-0.011 0.893
pT stage	1 2 3 4	3 62 91 4	**0.296** **<0.001**	**0.295** **<0.001**	0.045 0.576	-0.005 0.948	-0.015 0.844	-0.015 0.851
Venous invasion	Absent Present	113 46	0.050 0.529	0.055 0.503	**0.190** **0.018**	-0.058 0.455	-0.041 0.606	-0.012 0.879
Lymphatic invasion	Absent Present	121 38	-0.064 0.421	-0.065 0.430	**0.191** **0.017**	-0.018 0.817	-0.029 0.716	-0.033 0.689
Perineural invasion	Absent Present	143 17	-0.028 0.725	-0.037 0.652	0.054 0.503	0.147 0.060	**0.178** **0.027**	-0.054 0.521
Lymph node metastasis	Absent Present	81 79	**0.256** **0.039**	**0.262** **0.041**	-0.010 0.898	-0.048 0.532	0.089 0.264	-0.019 0.819
Size of lymph node metastasis	Micro Macro	30 49	**0.184** **0.022**	**0.185** **0.025**	-0.008 0.913	-0.098 0.212	0.093 0.250	-0.037 0.655
Distant metastasis	Absent Present	143 17	-0.011 0.884	-0.011 0.885	-0.018 0.817	**0.633** **<0.001**	-0.054 0.497	-0.038 0.504

Tumour Deposits	Absent Present	133 27	-0.042 0.589	-0.044 0.593	**0.230** **0.004**	**0.158** **0.040**	0.037 0.644	-0.036 0.663
Tumor budding	Absent Present	94 66	0.018 0.824	0.022 0.788	0.102 0.530	0.029 0.708	-0.059 0.467	0.096 0.250
Crohn's-like aggregates of lymphocyte	Absent Present	113 42	0.107 0.181	0.110 0.184	-0.061 0.449	0.001 0.982	-0.050 0.530	-0.033 0.693
Necrosis	Absent Focal Moderate Extensive	45 61 36 18	0.043 0.594	0.038 0.646	0.008 0.913	0.095 0.223	0.155 0.055	-0.008 0.920
Fibrosis	Absent Focal Moderate Extensive	11 72 43 34	0.117 0.143	0.115 0.162	0.009 0.909	0.025 0.751	-0.120 0.138	0.111 0.183
Maturation of fibrotic stroma	Immature Intermediate Mature	12 91 57	-0.048 0.547	-0.052 0.528	0.062 0.441	0.031 0.690	0.031 0.702	-0.025 0.761

Spearman's correlation coefficient test

Table 2. Correlations between monocyte count, NMR and MLR in pre and postoperative samples and chosen morphological and serum cancer markers of patients with CRC

Parameter	**N 160**	**preMONO** R, p-value	**postMONO** R, p-value	**preNMR** R, p-value	**postNMR** R, p-value	**preMLR** R, p-value	**postMLR** R, p-value
EOSINOPHILS (K/uL)	Before	**0.709** **<0.001**	**0.710** **<0.001**	-0.110 0.170	-0.025 0.753	-0.109 0.173	0.061 0.460
	After	**0.709** **<0.001**	**0.698** **<0.001**	0.064 0.440	0.149 0.071	0.061 0.457	**-0.207** **0.012**
BASOPHILS (K/uL)	Before	**0.999** **<0.001**	**0.998** **<0.001**	**-0.255** **0.010**	**-0.304** **<0.001**	-0.086 0.281	-0.042 0.611
	After	**0.999** **<0.001**	**0.998** **<0.001**	-0.061 0.456	-0.003 0.970	-0.054 0.510	**-0.246** **0.003**
RBC (M/ul)	Before	**0.998** **<0.001**	**0.993** **<0.001**	-0.128 0.109	**-0.156** **0.050**	**-0.168** **0.035**	0.149 0.072
	After	**0.707** **<0.001**	**0.708** **<0.001**	0.134 0.105	0.022 0.791	0.080 0.330	0.012 0.884
HGB (g/dl)	Before	**0.980** **<0.001**	**0.986** **<0.001**	-0.091 0.256	-0.087 0.277	**-0.222** **0.005**	0.022 0.787
	After	**0.963** **<0.001**	**0.969** **<0.001**	0.153 0.067	0.059 0.478	-0.046 0.580	-0.080 0.332
PLT (x103/ uL)	Before	**-0.997** **<0.001**	**-0.967** **<0.001**	-0.074 0.351	-0.053 0.503	-0.079 0.322	-0.032 0.694
	After	**-0.791** **<0001**	**-0.781** **<0.001**	0.030 0.716	0.064 0.435	0.053 0.521	0.037 0.655
CEA (j/ml)	Before	-0.018 0.881	-0.018 0.882	0.016 0.893	0.185 0.130	0.202 0.099	-0.053 0.682
	After	0.002 0.986	0.028 0.848	-0.081 0.482	-0.048 0.680	-0.044 0.702	-0.009 0.934
CA19-9 (j/ml)	Before	**0.987** **<0.001**	**0.945** **<0.001**	-0.047 0.788	0.064 0.719	0.178 0.312	-0.100 0.583
	After	-0.020 0.906	-0.157 0.366	**0.492** **0.002**	0.209 0.213	-0.041 0.796	-0.203 0.242

Spearman's correlation coefficient test

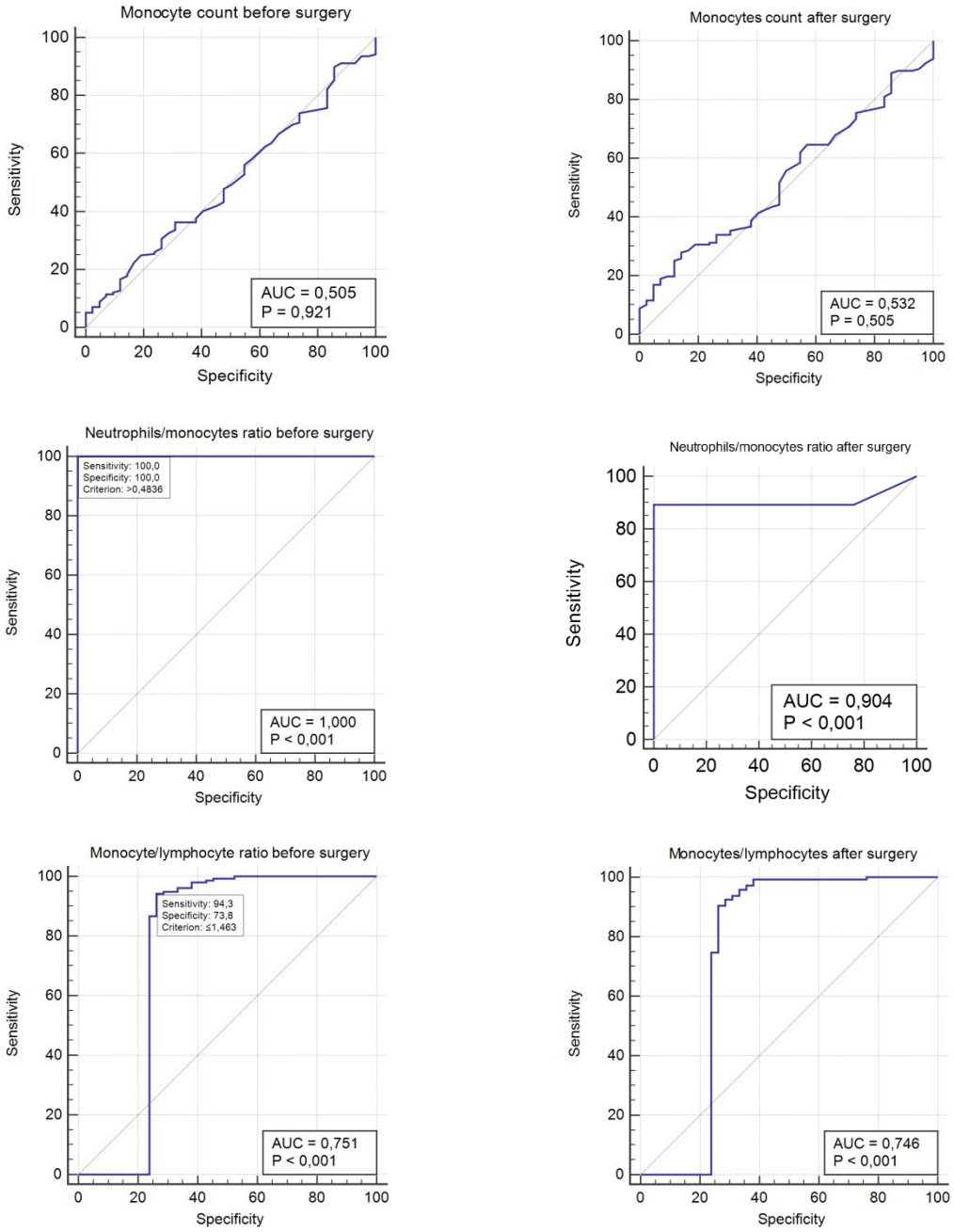

Figure 1. ROC curve of pre- and postoperative monocyte count, NMR and MLR

	preMONO	**postMONO**	**preNMR**	**postNMR**	**preMLR**	**postMLR**
Cut-off value	>0.39	>0.68	>0.48	>0.48	**≤1.46**	**≤1.46**
AUC	0.505	0.532	1.000	0.904	0.751	0.746
Sensitivity(%)	75.80	27.89	100.0	89.16	94.27	90.41
Specificity (%)	16.67	85.71	100.0	100.0	73.81	73.81
p-value	0.920	0.504	**<0.0001**	**<0.0001**	**0.0001**	**0.0002**

AUC- Area under the ROC curve

DISSCUSION

Activation of immune cell response is the one way to defend the organism against cancer cells expansion. Innate immune cells that circulate in the bloodstream and go to tissues during inflammation are mononuclear phagocyte called monocytes [18]. In physiological condition, monocytes take part in

supporting tissue homeostasis and propagating host responses to pathogens [19]. Recently, the role of monocytes in the cancer development and progression is underlined [20]. Monocytes are divided into the main three groups in the basis of their chemokine and adhesion molecule profiles: classical, nonclassicaland intermediate monocytes. Classical monocytes have high level expression of the CD14 cell surface receptor while nonclassical subtype is characterized by the low level expression of CD14 and additional co-expression of the CD16 receptor. Intermediate monocytes show overexpression of CD14 and down-regulate expression of CD16 [21]. Data literature demonstrate that only 1% of classical monocytes convert into intermediate and subsequently non classical monocytes during homeostasis. Other cells get across in blood circulation or undergocell death [22]. Moreover, monocytes take part at different stages of tumor growth and progression, depending on the type of origin and subtle differences in tumor microenvironment [18].Monocytes appear to be recruited due to increased CCL2 expression in model of colitis-associated colorectal cancer[23]. In our study, we showed that monocytes count in both pre- and postoperative blood samples correlated with pT stage of CRC patients. Peripheral blood monocytes expressed IFN-γ can produce TRAIL protein that is able to stimulate cell death [24].Monocytes can also take part in phagocytosis. However, some literature data demonstrated that cancer cells are able to change immunophenotype of peripheral blood monocytes and activate their tumorogenic properties [25,26]. Cancer cells are able to activate by themselves phagocytosis in blood stream or in tumor mass sites. Cancer cells also express CD47 protein that connects with surface ligand CD172a localized on circulating monocytes and promote monocyte-mediated phagocytosis [27]. We proved that increased monocyte count was found to correlate with lymph nodes invasion and the size of lymph node metastasis. Hu et al. [28] showed that preoperative peripheral blood monocyte count is linked with liver metastasis in colorectal cancer. Zhao et al. [29] found that CD47 expression correlates with the presence of lymph node and distal metastases in non-small cell lung cancer. Abu-Shawer et al. [30] observed that high baseline absolute monocyte count had more distant metastases in comparison to patients with low value of patients diagnosed with gynecological cancers. Moreover, we noted the association between increased level of peripheral blood monocyte count of samples obtained before surgery and CA 19-9 levels. Our results suggest that monocytes in CRC patients are probably responsible for tumor progression via the inhibition of cancer cell death or monocyte-mediated phagocytosis.

Monocytes and monocyte-derived cells interact with adaptive immunity by directing the recruitment and function of lymphocytes, especially their ability to suppress T cell function [31]. Circulating monocytes negatively correlated with infiltration of cytotoxic CD8+T cells. Monocyte-derived cells can recruit immunosuppressive regulatory Tcells (Tregs) via CCL5 production [32]. We analyzed correlation between monocytes and lymphocytes as a monocyte-lymphocyte ratio in peripheral blood samples of CRC patients. We found that pre- and postoperative MLR value were significantly higher than in healthy controls. Moreover, ROC curves of those parameters had high sensitivity and specificity. We also noted that high LMR value was associated with perineural invasion of cancer cells. Also according to Abu-Shawer M et al. [33] MLR correlated with lung metastasis at the time of diagnosis. Probably, the high level of circulating monocytes in the blood streamreflects to the inhibition of immune response in the basis of T cells.

We analyzed connection between neutrophils and monocytes as a neutrophil-to-monocyte ratio. According to our knowledge, this is the first study which conducted such interaction. Monocytes are circulating in peripheral blood, subsequently settle down the tissue material where they became macrophages. It has been proven that neutrophils undergoing apoptosis are permanently accumulated in the mucous membrane of colorectal adenomas, and those which are not removed by macrophages may cause tissue damage and loss of control over their regeneration [34]. We found to NMR value was associated with the invasion of venous and lymphatic vessels, the presence of cancer cell deposits and distant metastasis. NMR value is appearing to be reflected an insufficient immune response that need further, more detailed investigations.

In conclusion, we found that hematologic markers such as absolute monocyte count, NMR and MLR values of blood samples in colorectal cancer are associated with tumor progression. Analyzed markers may be useful as simply obtained parameters, next to histopathological examination, that determine a systemic immune response.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Financial disclosure/funding

The authors received funding support from Medical University of Bialystok for this work.

ORCID

Grudzińska Małgorzata: 0000-0002-1809-2383
Jakubowska Katarzyna: 0000-0002-1960-7204
Koda Mariusz: 0000-0002-1678-567X
Kańczuga-Koda Luzia: 0000- 0001-6261-2596

Kisielewski Wojciech: 0000-0003-1357-2000
Smereczański Nazar Michał: 0000-0002-4565-4150
Famulski Waldemar:-0000-0002-6579-912X

REFERENCES

1. Didkowska J, Wojciechowska U, Zatoński W. Prediction of cancer incidence and mortality in Poland up to the year 2025. Krajowy Rejestr Nowotworów, Warszawa 2009.
2. Nagtegaal ID, Quirke P, Schmoll HJ. Has the new TNM classification for colorectal cancer improved care? Nat Rev Clin Oncol. 2011 Oct 18;9(2):119-23.
3. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, Kopetz SE, Lieu C, Lindor NM, Minsky BD, Monzon FA, Sargent DJ, Singh VM, Willis J, Clark J, Colasacco C, Rumble B, Temple-Smolkin R, Ventura CB, Nowak JA, Molecular biomarkers for the evaluation of colorectal cancer: Guideline from the American society for clinical pathology, college of American pathologists, association for molecular pathology, and American society of clinical oncology. Arch Pathol Lab Med. 2017; 141(5):625–57.
4. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras K, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Oden Gangloff A, Ciardiello F, Pfeiffer P, Qvortrup C, Plato Hansen T, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S, Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis. Lancet Oncol. 2010 Aug; 11(8):753–62.
5. Mao C, Yang ZY, Hu XF, Chen Q, Tang JL. PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic colorectal cancer: A systematic review and meta-analysis. Ann Oncol. 2012 Jun;23(6):1518–25.
6. Atlas TCGN, Muzny D, Bainbridge M, Chang K, Dinh H, Drummond J, Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012 Jul;487(7407):330–7.
7. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pages F, Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006 Sep 29;313(5795):1960-4.
8. Galon J, Franck P, Marincola FM, Angell HK, Thurin M, Lugli A, Zlobec I,Berger A, Bifulco C, Botti G, Tatangelo F, Britten CM, Kreiter S, Chouchane L, Delrio P, Hartmann A, Asslaber M, Maio M, Masucci GV, Mihm M, Vidal-Vanaclocha F, Allison JP, Gnjatic S, Hakansson L, Huber C, Singh-Jasuja H, Ottensmeier C, Zwierzina H, Laghi L, Grizzi F, Ohashi PS, Shaw PA, Clarke BA, Wouters BG, Kawakami Y, Hazama S, Okuno K, Wang E, O'Donnell-Tormey J, Lagorce C, Pawelec G, Nishimura MI, Hawkins R, Lapointe R, Lundqvist A, Khleif SN, Ogino S, Gibbs P, Waring P, Sato N, Torigoe T, Itoh K, Patel PS, Shukla SN, Palmqvist R, Nagtegaal ID, Wang Y, D'Arrigo C, Kopetz S, Sinicrope FA, Trinchieri G, Gajewski TF, Ascierto PA, Fox BA. Cancer classification using the Immunoscore: A worldwide task force. J Transl Med. 2012 Oct 3; 10:205.
9. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, Lugli A, Zlobec I, Rau TT, Berger MD, Nagtegaal ID, Vink-Börger E, Hartmann A, Geppert C, Kolwelter J, Merkel S, Grützmann R, Van den Eynde M, Jouret-Mourin A, Kartheuser A, Léonard D, Remue C, Wang JY, Bavi P, Roehrl MHA, Ohashi PS, Nguyen LT, Han S, MacGregor HL, Hafezi-Bakhtiari S, Wouters BG, Masucci GV, Andersson EK, Zavadova E, Vocka M, Spacek J, Petruzelka L, Konopasek B, Dundr P, Skalova H, Nemejcova K, Botti G, Tatangelo F, Delrio P, Ciliberto G, Maio M, Laghi L, Grizzi F, Fredriksen T, Buttard B, Angelova M, Vasaturo A, Maby P, Church SE, Angell HK, Lafontaine L, Bruni D, El Sissy C, Haicheur N, Kirilovsky A, Berger A, Lagorce C, Meyers JP, Paustian C, Feng Z, Ballesteros-Merino C, Dijkstra J, van de Water C, van Lent-van Vliet S, Knijn N, Mușină AM, Scripcariu DV, Popivanova B, Xu M, Fujita T, Hazama S, Suzuki N, Nagano H, Okuno K, Torigoe T, Sato N, Furuhata T, Takemasa I, Itoh K, Patel PS, Vora HH, Shah B, Patel JB, Rajvik KN, Pandya SJ, Shukla SN, Wang Y, Zhang G, Kawakami Y, Marincola FM, Ascierto PA, Sargent DJ, Fox BA, Galon J. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018 May;391(10135): 2128–39.
10. Goel G. Molecular characterization and biomarker identification in colorectal cancer: Toward realization of the precision medicine dream. Vol. 10, Cancer Manag Res. 2018 Nov 19;10:5895-08.
11. Li H, Zhao Y, Zheng F. Prognostic significance of elevated preoperative neutrophil-to-lymphocyte ratio for patients with colorectal cancer undergoing curative surgery: A meta-

analysis. Medicine (Baltimore) 2019 Jan;98(3):e14126.
12. Inamoto S, Kawada K. Prognostic impact of the combination of neutrophil-to-lymphocyte ratio and Glasgow prognostic score in colorectal cancer : a retrospective cohort study. Int J Colorectal Dis. 2019;Jul;34(7):1303-15
13. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. [Internet, cited 2019 Jul 1] Available from: www.nccn.org.asp.
14. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom A, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst. 2000 Feb;92(3):205–16.
15. Lin Q, Wei Y, Ren L, Zhong Y, Qin C, Zheng P, Xu P, Zhu D, Ji M, Xu J. Tumor deposit is a poor prognostic indicator in patients who underwent simultaneous resection for synchronous colorectal liver metastases. Onco Targets Ther. 2015 Jan 22; 8:233-40
16. Morodomi T, Isomoto H, Shirouzu K, Kakegawa K, Irie K, Morimatsu M. An index for estimating the probability of lymph node metastasis in rectal cancers. Lymph node metastasis and the histopathology of actively invasive regions of cancer. Cancer 1989;63(3):539–43.
17. Väyrynen JP, Sajanti SA, Klintrup K, Mäkelä J, Herzig KH, Karttunen TJ, Tuomisto A, Mäkinen MJ. Characteristics and significance of colorectal cancer associated lymphoid reaction. Int J Cancer. 2014 May 1;134(9):2126-35.
18. Olingy CE, Dinh HQ, Hedrick CC. Monocyte heterogeneity and functions in cancer. J Leukoc Biol. 2019 Aug;106(2):309-322.
19. Shi C. Monocyte Recruitment During Infection and Inflammation. Nat Rev Immunol. 2011 Oct;11(11):762–74.
20. Hanna RN, Cekic C, Sag D, Tacke R, Thomas GD, Nowyhed H, Herrley E, Rasquinha N, McArdle S, Wu R, Peluso E, Metzger D, Ichinose H, Shaked I, Chodaczek G, Biswas SK, Hedrick CC. Patrolling monocytes control tumor metastasis to the lung. Science. 2015 Nov 20;350(6263):985-90.
21. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, Ivanov S, Duan Q, Bala S, Condon T, van Rooijen N, Grainger JR, Belkaid Y, Ma'ayan A, Riches DW, Yokoyama WM, Ginhoux F, Henson PM, Randolph GJ. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. Immunity 2013 Sep 19;39(3):599-610.
22. Sunderkötter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA, Leenen PJ. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol. 2004 Apr 1;172(7):4410-7.
23. Chun E, Lavoie S, Michaud M, Gallini CA, Kim J, Soucy G, Odze R, Glickman JN, Garrett WS. CCL2 Promotes Colorectal Carcinogenesis by Enhancing Polymorphonuclear Myeloid-Derived Suppressor Cell Population and Function. Cell Rep. 2015 Jan;12(2):244–57.
24. Griffith TS, Wiley SR, Kubin MZ, Sedger LM, Maliszewski CR, Fanger NA. Monocyte-mediated Tumoricidal Activity via the Tumor Necrosis Factor–related Cytokine, TRAIL. J Exp Med. 1999 Apr 19;189(8):1343-54.
25. Chittezhath M, Dhillon MK, Lim JY, Laoui D, Shalova IN, Teo YL, Chen J, Kamaraj R, Raman L, Lum J, Thamboo TP, Chiong E, Zolezzi F, Yang H, Van Ginderachter JA, Poidinger M, Wong AS, Biswas SK. Molecular Profiling Reveals a Tumor-Promoting Phenotype of Monocytes and Macrophages in Human Cancer Progression. Immunity. 2014;41(5):815–29.
26. Hamm A, Prenen H, Van Delm W, Di Matteo M, Wenes M, Delamarre E, Schmidt T, Weitz J, Sarmiento R, Dezi A, Gasparini G, Rothé F, Schmitz R, D'Hoore A, Iserentant H, Hendlisz A, Mazzone M. Tumour-educated circulating monocytes are powerful candidate biomarkers for diagnosis and disease follow-up of colorectal cancer. Gut 2016 Jun;65(6):990–1000.
27. Willingham SB, Volkmer JP, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, Wang J, Contreras-Trujillo H, Martin R, Cohen JD, Lovelace P, Scheeren FA, Chao MP, Weiskopf K, Tang C, Volkmer AK, Naik TJ, Storm TA, Mosley AR, Edris B, Schmid SM, Sun CK, Chua MS, Murillo O, Rajendran P, Cha AC, Chin RK, Kim D, Adorno M, Raveh T, Tseng D, Jaiswal S, Enger PØ, Steinberg GK, Li G, So SK, Majeti R, Harsh GR, van de Rijn M, Teng NN, Sunwoo JB, Alizadeh AA, Clarke MF, Weissman IL. The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci. 2012 Apr;109(17):6662–7.
28. Hu S, Zou Z, Li H, Zou G, Li Z, Xu J, Wang L, Du X. The preoperative peripheral blood monocyte count is associated with liver metastasis and overall survival in colorectal cancer patients. PLoS One. 2016 Jun 29;11(6):e0157486.
29. Zhao H, Wang J, Kong X, Li E, Liu Y, Du X, Kang Z, Tang Y, Kuang Y, Yang Z, Zhou Y, Wang Q. CD47 promotes tumor invasion and metastasis in non-small cell lung cancer. Sci Rep. 2016 Jul 14;6:29719.
30. Abu-Shawer O, Abu-Shawer M, Hirmas N, Alhouri A, Massad A, Alsibai B, Sultan H, Hammo H, Souleiman M, Shebli Y, Al-Hussaini M. Hematologic markers of distant metastases and poor prognosis in gynecological cancers. BMC Cancer 2019 Feb 12;19(1):141.
31. Veglia F, Perego M, Gabrilovich D. Myeloid-

derived suppressor cells coming of age review-article. Nat Immunol. 2018 Feb;19(2):108-19.
32. Schlecker E, Stojanovic A, Eisen C, Quack C, Falk CS, Umansky V, Cerwenka A. Tumor-Infiltrating Monocytic Myeloid-Derived Suppressor Cells Mediate CCR5-Dependent Recruitment of Regulatory T Cells Favoring Tumor Growth. J Immunol. 2012 Dec 15;189(12):5602-11
33. Abu-Shawer M, Abu-Shawer O, Souleiman M, Akkawi M, Alshakhatreh O, Altamimi T, Al-Omari A, Al-Hussaini M. Hematologic Markers of Lung Metastasis in Stage IV Colorectal Cancer. J Gastrointest Cancer 2019 Sep;50(3):428-33.
34. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 2004 Aug;21(2):137-48.