

Tumor-infiltrating lymphocytes in tissue material combined with systemic lymphocyte inflammation in patients with colorectal cancer

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Abstract. The efficacy of cancer immunotherapy has been actively explored in the treatment of various malignant neoplasms of the gastrointestinal tract. In light of recent reports, the present study aimed to investigate the combination of the absolute lymphocyte count (ALC), percentage of tumor-infiltrating lymphocyte (TILs) and tumor progression status in patients with colorectal cancer (CRC) who underwent surgery. The medical records of 160 patients diagnosed with CRC were retrospectively reviewed. TILs were determined as a percentage of mononuclear inflammatory cells in the total intratumoral or stromal area as determined in five high power fields (magnification, x200-400), at the invasive front and in the centre of the tumour. Blood samples were obtained within 3 days prior to and 7 days following the surgical treatment. The assessment of the TIL percentage was performed in the tissue at the invasive front and in the centre of the primary tumour mass in combination with the determination of ALC in whole blood samples. The samples were obtained prior to and after surgery from patients with CRC, and the tumour progression status was also assessed (TILs/ALC/tumour progression status). A significant association was observed between the percentage of TILs in the main mass of tumour and the tumour size ($P=0.031$), the pT stage ($P=0.049$) and the incidence of necrosis ($P=0.037$) following surgery. The histological type was associated with the evaluated combined parameters prior to surgery ($P=0.046$). Lymph node pouch invasion was associated with TILs at the invasive front of tumour and with ALC prior to and after surgery ($P=0.006$ and

$P=0.037$). Furthermore, the data indicated that the percentage of TILs located on the invasive front and centre of the tumour, and the ALC prior to and following surgery correlated with the treatment status ($P=0.032$, $P=0.018$, $P\leq 0.001$ and $P\leq 0.001$). A significant association was noted between eight features and evaluated combined parameters following surgery. These included the tumour size ($P=0.021$), TNM stage ($P<0.001$), tumour deposits ($P=0.001$), incidence of necrosis ($P=0.042$) and lymph node metastasis ($P<0.001$). Furthermore, the degree of invasion of venous ($P<0.001$), lymphatic ($P<0.001$) and perineural ($P<0.001$) sites was also significantly associated with TILs, ALC obtained after surgical treatment and tumor progression status. The data demonstrated that local and systemic chronic inflammation was associated with tumour progression in patients with CRC.

Introduction

Cancer immunotherapy has been actively explored in the treatment of various malignant neoplasms of the gastrointestinal tract (1,2). Recently, nanotechnology-mediated delivery approaches have attracted considerable attention in colorectal cancer (CRC) immunotherapy. Nanoparticle-based immunotherapy exhibits higher specificity and efficacy in comparison with conventional immunotherapy (3). Those immune-related transmitters can locally deliver immune components to the antigen presenting cells with high efficiency and modulate the tumor immune microenvironment. In addition, they act as an adjuvant that can boost immune reactions to antigens (4). CRC is a heterogeneous group of malignant lesions with different types of immune responses (5). Previous studies have focused on the morphological assessment and molecular changes that occur during CRC, which includes mutational status and chromosomal instability, somatic copy number variation, immune infiltration and metabolic regulation. Based on this evidence CRC can be divided into five groups of tumors as follows: Microsatellite instability-associated, immune-associated, canonical, metabolic and mesenchymal (6). The proposed division of cancerous tumors is closely associated with tumor stage and survival time. Recent studies have shown that patients

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with a high status of microsatellite instability respond better to immunotherapy (7). Therefore, systemic immune response is associated with infiltration of immune cells in CRC tumors, which appear to be separated from the tumor microenvironment (8). The immune cells infiltrating CRC tumors are macrophages, dendritic cells, neutrophils and lymphocytes. Immunotherapy is focused on the ability of lymphocytes to infiltrate the cancerous tissue. It has been shown that lymphocytes infiltrates exhibit considerable variation with regard to their biological characteristics in the different parts of the tumor (9). Laghi *et al* (9) demonstrated that the infiltrate of T cells in the invasive margin of CRC tumors could improve disease prognosis compared with cells examined at the center of the tumor. In addition, Bindea *et al* (10) indicated that the amount of T cells present in the tumor was decreased over time, while the number of B cells and innate immune cells was increased. Moreover, local immune response in the tumor tissue was closely associated with systemic exposure to circulating cancer cells in the bloodstream. Therefore, the analysis of hematological parameters appears to be an important indicator of immune response to cancer. Currently, several studies using hematological factors, such as absolute lymphocyte count (ALC), absolute neutrophil count, neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been conducted (11-13). Tanio *et al* (11) indicated that the combined absolute number of lymphocytes, monocytes and neutrophils in the preoperative whole blood can be a useful prognostic factor in CRC patients (11). Kozak *et al* (12) proposed that the elevated NLR independently predicts worse overall survival in patients with CRC. In addition, Yang *et al* (13) demonstrated that an increased PLR value was associated with poor overall survival. In light of these reports, the present study aimed to investigate the combination of the ALC, tumor-infiltrating lymphocyte (TIL) percentage and tumor progression status in patients with CRC who underwent surgery.

Patients and methods

Patients and tissue specimens. A total of 160 patients who were diagnosed with CRC were retrospectively reviewed (96 male, 64 female; median age, 72 years). In the present study, the paraffin-embedded tissues were obtained from the Comprehensive Cancer Centre between April 2014 and December 2016. The patients who were diagnosed with CRC underwent surgery in the Division of Surgical Oncology. All of the tissue samples were processed by an identical dissection protocol. The tissues obtained from surgery were fixed in 4% buffered formalin and subsequently small sections of tissues were embedded in paraffin. The sections (4 μ m-thick) were cut from paraffin blocks and stained with haematoxylin and eosin (H&E). The slides were deparaffinised in an oven at 60°C for 5 min. Subsequently, the slides were rehydrated in xylene (three washes) and graded ethanol (100, 95, 85 and 75%). Histological assessment was performed by two pathologists, which were blinded to the study protocol. The following parameters were recorded from each patient: Type of tumour growth, Tumour-Node-Metastasis (TNM) stage, tumour size, histological type, percentage of mucinous components and grade of malignancy. The clinical stage of CRC was evaluated according to the TNM classification (14). Venous, perineural and

lymphatic invasion (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) were also assessed. The deposits of cancer (their number and size) were also included (15).

The patients enrolled in the present study presented similar symptoms. The most common symptoms were abdominal pain, vomiting, rectal bleeding, constipation or diarrhoea. The medical history demonstrated that some patients suffered from hypertension, diabetes (type II), coronary heart disease or osteoarthritis. However, none of the patients had received anti-inflammatory or immunosuppressive therapy. All of the patients underwent routine diagnostic tests prior to surgery (blood tests, electrocardiography and spirometry). The diagnostic examinations, such as a chest computerized tomography and chest X-ray were utilized to identify CRC distal metastasis. The patients with neoplasm in the rectum received preoperative therapy (n=53): Radiotherapy (n=39), chemotherapy (n=7) and radio-chemotherapy (n=7). The radiation dose used was 25 Gy, in fractions of 5 Gy during one week in the pelvic area. According to the Response Evaluation Criteria in Tumours (16), the response to preoperative therapy was evaluated. A total of 26 patients were recruited with a stable disease and 27 patients exhibited partial response.

The inclusion criteria were as follows: i) Pathologically confirmed CRC; ii) treatment with radical resection; and iii) lack of anti-inflammatory therapy. The exclusion criteria were the following: i) Incomplete clinicopathological and follow-up data; and ii) presence of haematological disorders, such as anaemia.

The present study was performed in conformity with the Declaration of Helsinki for Human Experimentation and the protocol was approved by the Bioethics Committee of the Medical University of Bialystok (no. R-I-002/353/2016).

Assessment of the percentage of TILs in CRC tissues. The analysis of TILs was described in our previous study (17). Briefly, tissue material obtained from routine histopathological diagnosis was stained with H&E and used to assess the TIL populations located in the tumor stroma at the invasive front and centre of the tumour by light microscopy (magnification, x200-400; Leica DM6 B; Leica Microsystems, Inc.). The analysis was evaluated by two independent pathologists who were blinded to the clinical information of the study. TILs in the stroma were identified according to recommendations by the International TILs Working Group, 2014 (18). They were determined as a percentage of mononuclear inflammatory cells in the total intratumoral or stromal area as counted in 5 high power fields (HPF; magnification, x200-400), at the invasive front and in the centre of the tumour, with the exception of tumour areas with crush artefacts, necrosis or regressive hyalinization. The invasive front of the tumour was defined as the percentage of most progressed cancer cells on the advanced edge of the tumour. For statistical analysis, the percentage of stromal TILs was scored as follows: 1, weak (0-10% stromal TILs); 2, moderate (20-40% stromal TILs); and 3, strong (50-90% stromal TILs). The study population was divided into two groups based on the stromal TIL score as follows: 1, score 1; and 2, score 2 or 3. Representative images of TILs are shown in Fig. 1.

Blood sample examination. Blood samples were obtained within 3 days prior to and 7 days following surgical treatment.

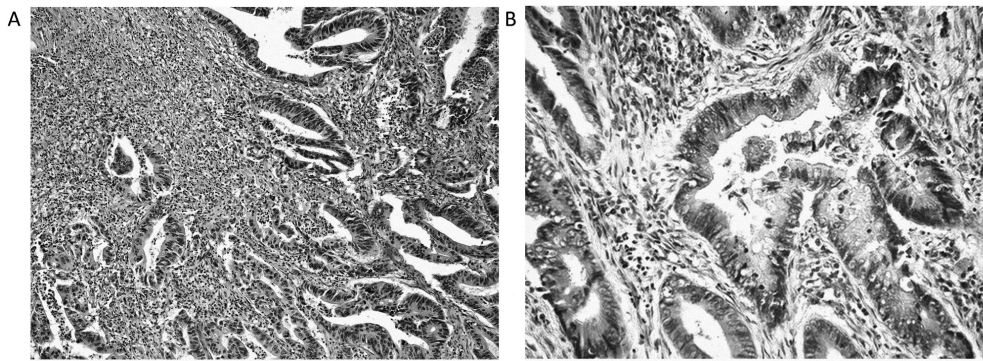


Figure 1. Representative images of complex of tumor-infiltrating lymphocytes (A) at the invasive front and (B) in the center of resected colorectal cancer tissue (H&E staining; original magnification, x100).

Venous blood samples were also obtained from 42 healthy control subjects (female-21, male-21; mean age 45 years old; min-max 25-65 years old). The differential white blood cell count was counted using an XN-1000 automated haematology analyzer (Sysmex America, Inc.). Normal total lymphocyte count ranges were between 0.90 and 4.00×10^3 cells/ μ l (mean 2.25×10^3 cells/ μ l). The total lymphocyte count in CRC patients ranged from 0.47 to 4.06×10^3 cells/ μ l (mean 1.05×10^3 cells/ μ l).

Combination of TIL percentage and ALC. The percentage of TILs was examined in the CRC tissue at the invasive front and centre of the primary tumour mass in combination with the ALC and in whole blood samples obtained prior to and following surgery from patients with CRC. The classification of the immune response system included: i) TILs at the invasive front and ALC prior to surgery; ii) TILs in the centre of the tumour mass and ALC prior to surgery; iii) TILs at the invasive front and ALC following surgery, iv) TILs in the centre of the tumour mass and ALC following surgery. All groups included 4 subgroups: i) High TILs (moderate or strong stromal TILs) and high ALC ($\geq 4.0 \times 10^3$ cells/ μ l); ii) high TILs (moderate or strong stromal TILs) and low ALC ($\leq 0.9 \times 10^3$ cells/ μ l); iii) low TILs (weak stromal TILs) and high ALC ($\geq 4.0 \times 10^3$ cells/ μ l); iv) low TILs (weak stromal TILs) and low ALC ($\leq 0.9 \times 10^3$ cells/ μ l).

In the present study, the combination of TIL percentage and ALC was also examined with regard to the tumor progression status (TILs/ALC/tumour progression status). The tumour progression status was determined on the basis of 3 parameters as follows: i) Lymphatic vessel invasion; ii) lymph node involvement and iii) distant metastasis. Based on the tumour progression status the study population was divided into 3 groups as follows: i) patients with local disease; ii) patients with lymphatic vessel invasion and positive lymph node status without distant metastasis; iii) patients that exhibited cancer cell invasion to all the aforementioned structures. The local and systemic response of lymphocytes prior to and following surgical treatment was investigated. Taken together, the patients were divided into 4 groups as follows: i) Negative status of tumour progression with high percentage of TILs at the invasive front and in the centre of the primary tumour mass and high ALC, ii) negative status of tumour progression with high percentage of TILs as the invasive front and in the centre of the primary tumour mass and low ALC, iii) positive status of tumour progression

(group 2 or 3) with high percentage of TILs at the invasive front and in the centre of the primary tumour mass and high ALC and iv) positive status of tumour progression (group 2 or 3) with high percentage of TILs at the invasive front and in the centre of the primary tumour mass and high ALC.

Statistical analysis. All calculations were performed using the statistical software STATISTICA 13.0 (StatSoft, Inc.). The enumeration data of examined parameters were analysed using the χ^2 test. The comparisons among multiple groups (examined parameters and clinicopathological features) were analysed using one-way ANOVA. Fisher's Least Significant Difference post hoc test was used to analyze multiple comparisons of TILs, ALC, progression status and histopathological factors following the ANOVA test (data not shown). Tukey's test was used to compare more than three groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics and distribution of the combination index including TIL percentage and ALC in CRC patients. The median age was 67.5 years (range, 32 to 88 years) and 60% of the patients were male. The rectum was the most common tumour location (51.25%). The pathological staging was the following: Stage I in 1.88% of patients, stage II in 38.75% of patients, stage III in 56.88% of patients and stage IV in 2.5% of patients. The patients developed adenocarcinoma (81.25%) more frequently than mucinous CRC (18.75%). The distribution of TILs was evaluated based on several factors, such as invasion of cancer cells, distant metastasis or status of tumour progression. The data indicated that the distribution of the combination index including TIL percentage and ALC differed significantly among the groups (Table I). However, statistical significance was not obtained for the distribution of the TILs/ALC/progression tumour status (Table II).

Correlation of the combination index including TIL percentage and ALC with the clinicopathological parameters of CRC patients. The percentage of TILs and the ALC prior to and following surgery were significantly associated with the tumour growth in the main mass (Tables III and IV, $P = 0.001$, $P = 0.007$). A significant correlation was observed in the percentage of TILs with the tumour size ($P = 0.031$), pT

Table I. Distribution of the combined parameters of TILs and ALC.

Parameter	Groups				P-value
	High TILs/high ALC, n	High TILs/low ALC, n	Low TILs/high ALC, n	Low TILs/low ALC, n	
TILs at the invasive front and ALC before surgery	39	116	2	9	<0.001
TILs at the invasive front and ALC after surgery	75	72	4	1	
TILs in the main mass of primary tumor and ALC before surgery	39	121	2	7	<0.001
TILs in the main mass of primary tumor and ALC after surgery	74	69	3	4	

TILs, tumor-infiltrating lymphocytes; ALC, absolute lymphocyte count.

Table II. Distribution of the TILs/ALC/tumor progression status.

Parameter	Groups				P-value
	1, n	2, n	3, n	4, n	
TILs at the invasive front, ALC and tumor progression status	12	48	28	72	0.164
TILs in the main mass of the primary tumor, ALC and tumor progression status	29	30	47	54	

TILs, tumor-infiltrating lymphocytes; ALC, absolute lymphocyte count.

stage ($P=0.049$) and necrosis percentage ($P=0.037$) following surgery. The histological type correlated with the combination index prior to surgery ($P=0.046$). Lymph node pouch invasion was associated with the percentage of TILs at the invasive front of the tumour and with ALC prior to and following surgery ($P=0.006$, $P=0.037$). Moreover, the percentage of TILs examined at the invasive front and centre of the tumour, and the ALC prior to and following surgery correlated with the treatment status ($P=0.032$, $P=0.018$, $P\leq 0.001$, $P\leq 0.001$). The patients with neoadjuvant therapy (status treatment group 1 and 2) exhibited better local and systemic chronic inflammatory response. Furthermore, no significant difference was observed in the remaining evaluated parameters (Tables III and IV).

Subsequent correlation analysis was performed between different clinical parameters. Specifically, the percentage of TILs, the ALC, the tumour progression status and the clinicopathological features were compared (Table V). The histopathological type, percentage of mucinous component and lymph node pouch invasion correlated significantly with the evaluated combined parameters prior to and following surgery. The growth of the tumour was associated with the percentage of TILs, the ALC and the tumour progression status prior to surgery ($P=0.037$). A significant correlation was observed between eight features with evaluated combined parameters following surgery. These were the tumour size ($P=0.021$), TNM stage ($P<0.001$), tumour deposits ($P=0.001$), necrosis ($P=0.042$) and lymph node metastasis ($P<0.001$). Moreover, the percentage of invasion of venous ($P<0.001$), lymphatic ($P<0.001$) and perineural ($P<0.001$) sites exhibited a significant correlation with the combination index including the percentage of TILs and the ALC. Moreover, no additional correlations were noted between the percentage

of TILs and the ALC, the tumour progression status and the remaining clinicopathological parameters. Furthermore, multi-parametric analysis was associated with the treatment status for patients who had received preoperative treatment. Moreover, TILs/ALC/tumour progression status and recurrence/prognosis were not statistically significant (data not shown).

Discussion

Multiple studies have emphasized on the importance of the host immunity during the development of CRC. The local inflammation has been previously measured in the tumour mass of CRC patients following surgical resection (19,20). More specifically, these studies were based on the measurement of CD3⁺ T cells, CD8⁺ T cells, and/or CD45RO⁺ memory T cells that were localized in the invasive front and in the centre of the primary tumour mass. The main finding was that the immune score could be used as a superior predictor of CRC recurrence in everyday practice (19,20). Furthermore, in response to the predictive value of the intensive local inflammation caused by high density TILs, the systemic inflammation is also frequently investigated. It has been shown that systemic inflammatory response is associated with a pro-tumour response and poor prognosis for CRC patients (21).

Recent data have suggested that local and systemic inflammatory response in CRC should be assessed in combination. This type of assessment may affect patient prognosis. Park *et al* (22) demonstrated a significant role of the density of T lymphocytes within the cancer cell areas. Moreover, an increased level of modified Glasgow Prognostic Score (mGPS) was noted in patients with mismatch repair-deficient (dMMR) CRC (22).

Table III. Associations between combined parameters of TILs and absolute lymphocyte count obtained before surgery, and clinicopathological features of patients with CRC (n=160).

Parameter	No.	TILs and absolute lymphocyte count before surgery									
		Invasive front					Main mass				
		1, n	2, n	3, n	4, n	P-value	1, n	2, n	3, n	4, n	P-value
Age, years											
<60	40	5	30	0	5	0.420	5	32	0	3	0.420
≥60	120	29	69	2	20		29	70	2	19	
Sex											
Female	64	12	41	0	11	0.696	12	41	0	11	0.696
Male	96	22	58	2	14		22	61	2	11	
Localization											
Right-side	20	8	11	1	0	0.407	4	12	1	3	0.561
Transverse	14	4	4	0	6		1	4	0	9	
Left-side	15	1	5	0	9		1	5	0	10	
Sigmoid	29	1	11	0	7		8	8	0	13	
Rectum	82	17	54	1	10		16	54	0	12	
Tumor growth											
Expanding	133	28	82	2	21	0.109	28	82	2	21	0.001
Infiltrate	27	6	17	0	3		6	20	0	1	
Tumor size, cm											
<2.5	27	6	15	1	5	0.412	6	17	1	3	0.181
2.5-5.0	106	26	63	0	17		25	64	1	16	
>5.0	27	2	21	1	3		3	21	0	3	
Histological type											
Muc	30	4	25	0	1	0.322	8	22	0	0	0.046
Adca	130	30	74	2	24		30	79	2	19	
Mucinous component, %											
10-30	15	0	10	0	5	0.392	0	10	0	5	0.148
31-50	15	4	11	0	0		4	11	0	0	
TNM stage											
1+2	73	10	49	1	13	0.518	11	53	0	9	0.417
3+4	87	24	50	1	12		23	49	2	13	
Grade of malignancies											
2	148	32	93	2	21	0.812	32	96	2	18	0.482
3	12	2	6	0	4		2	6	0	4	
pT stage											
1+2	65	13	39	1	12	0.159	13	41	1	10	0.154
3+4	95	21	60	1	13		21	61	1	12	
Venous invasion											
Absent	113	22	70	1	20	0.642	23	73	0	17	0.083
Present	46	12	29	1	4		11	29	2	4	
Lymphatic invasion											
Absent	121	24	75	1	21	0.952	24	78	0	19	0.961
Present	38	10	24	1	3		10	24	2	2	
Perineural invasion											
Absent	143	30	89	2	22	0.415	30	92	2	19	0.478
Present	17	4	10	0	3		4	10	0	3	
Lymph node metastasis											
Absent	81	16	60	1	4	0.350	16	62	1	2	0.405
Present	79	18	39	1	21		18	40	1	20	

Table III. Continued.

Parameter	No.	TILs and absolute lymphocyte count before surgery									
		Invasive front					Main mass				
		1, n	2, n	3, n	4, n	P-value	1, n	2, n	3, n	4, n	P-value
Lymph node pouch invasion											
Absent	41	17	9	2	13	0.006	10	5	13	13	0.775
Present	39	17	3	0	19		14	2	6	17	
Distant metastasis											
Absent	143	28	91	2	22	0.916	29	95	1	18	0.873
Present	17	6	8	0	3		5	7	1	4	
Tumor deposits											
Absent	133	24	84	2	23	0.565	25	87	1	20	0.474
Present	27	10	14	0	3		9	14	1	2	
Tumor budding											
Absent	94	20	57	1	16	0.436	20	59	1	14	0.945
Present	66	14	42	1	9		14	43	1	8	
Necrosis											
Absent	45	6	29	0	10	0.632	5	31	1	8	0.538
Focal	61	13	41	0	7		13	39	0	9	
Moderate	36	9	20	2	5		11	24	1	0	
Extensive	18	6	9	0	3		5	8	0	5	
Fibrosis											
Absent	11	6	5	0	0	0.603	6	4	1	0	0.917
Focal	72	14	48	1	9		13	49	0	10	
Moderate	43	7	26	1	9		8	28	1	6	
Extensive	34	7	20	0	7		7	20	0	7	
Treatment status ^a											
1	36	14	11	5	6	0.032	14	20	1	1	0.018
2	22	5	14	1	2		11	11	0	0	
3	71	36	22	12	1		7	59	1	5	
4	31	4	12	2	13		7	21	0	3	

^a1, RCHT-resection-no treatment; 2, RCHT-resection-RT/RCHT; 3, no treatment-resection-no treatment; 4, no treatment-resection-RT/RCHT. Adca, adenocarcinoma; Muc, mucinous; RCHT, radiochemotherapy; RT, radiotherapy.

Another study indicated that the combination of the number of intratumoral CD8-positive T cells with a high level of C-reactive protein (CRP) may possess predictive value for recurrence in CRC patients receiving chemotherapy treatment (23). The present study indicated that local and systemic chronic inflammation was simultaneously observed in 24% of cases. The majority of the cases (~73%) exhibited a predominantly local chronic response (high percentage of TILs and low ALC). Moreover, Turner *et al* (24) demonstrated the presence of the local chronic response consisting of lymphocytes, plasma cells, macrophages within tumour areas (24). In addition, systemic chronic inflammation was assessed by the expression of neutrophil-to-lymphocyte ratio (NLR) in preoperative blood samples in only 10% of stage II colon cancer cases (24). The authors of that study further indicated that patients with predominantly local response exhibited the best outcomes, while those with systemic inflammatory response exhibited reduced survival and poor prognosis (24).

The present study demonstrated the association between the combination index of TIL percentage and ALC with clinicopathological features. Patients with low local and systemic inflammatory response exhibited higher tumour growth and larger tumour mass associated with necrosis. Moreover, the patients from group 4 who presented with lymph node metastasis indicated higher percentage of cancer cell invasion beyond the lymph node pouch to the local fat tissue. Wu *et al* (25) demonstrated that the percentage of TILs and the ALC correlated with local recurrence. Moreover, Xiao *et al* (26) postulated that the density of CD8⁺ lymphocytes in the biopsy materials could be combined with the circulating lymphocyte ratio in order to predict complete response following neoadjuvant chemoradiotherapy in rectal cancer. Similar observations were found in studies conducted on breast cancer (27,28). The present study further indicated that patients with neoadjuvant therapy (status treatment group 1 and 2) exhibited

Table IV. Correlations between combined parameters of tumor-infiltrating lymphocytes and absolute lymphocyte count obtained after surgery, and clinicopathological features of patients with colorectal cancer (n=160).

Parameter	No.	TILs and absolute lymphocyte count									
		After surgery									
		Invasive front					Main mass				
		1, n	2, n	3, n	4, n	P-value	1, n	2, n	3, n	4, n	P-value
Age, years											
<60	40	22	12	0	6	0.245	21	13	1	5	0.545
≥60	120	47	47	1	25		47	44	2	27	
Sex											
Female	64	30	20	0	14	0.984	31	20	0	13	0.719
Male	96	39	39	1	17		37	37	3	19	
Localization											
Right-side	20	8	7	0	5	0.320	10	6	1	3	0.438
Transverse	14	2	2	1	9		2	2	0	10	
Left-side	15	3	3	0	9		3	2	0	10	
Sigmoid	29	12	7	0	10		2	7	0	20	
Rectum	82	37	30	0	20		34	28	0	20	
Tumor growth											
Expanding	133	54	51	1	27	0.489	53	49	3	28	0.007
Infiltrate	27	15	8	0	4		15	8	0	4	
Tumor size, cm											
<2.5	27	9	12	0	6	0.426	8	14	1	4	0.031
2.5-5.0	106	48	38	0	19		48	35	2	21	
>5.0	27	12	9	1	6		12	8	0	7	
Histological type											
Muc	30	16	12	1	1	0.079	16	7	0	7	0.056
Adca	130	53	47	0	30		52	50	3	25	
Mucinous component, %											
10-30	15	5	5	1	4	0.148	5	4	0	6	0.081
31-50	15	11	2	0	2		11	3	0	2	
TNM stage											
1+2	73	33	24	0	16	0.913	33	27	1	12	0.651
3+4	87	36	35	1	3		35	30	2	20	
Grade of malignancies											
2	148	66	54	1	27	0.618	65	52	3	28	0.806
3	12	3	5	0	4		3	5	0	4	
pT stage											
1+2	65	28	22	0	15	0.124	27	24	2	12	0.049
3+4	95	41	37	1	16		41	33	1	20	
Venous invasion											
Absent	113	48	40	0	25	0.861	48	40	3	22	0.300
Present	46	21	19	1	5		20	17	0	9	
Lymphatic invasion											
Absent	121	51	44	0	26	0.935	51	43	1	26	0.879
Present	38	18	15	1	4		17	14	2	5	
Perineural invasion											
Absent	143	61	53	1	28	0.803	60	52	3	28	0.903
Present	17	8	6	0	3		8	5	0	4	
Lymph node metastasis											
Absent	81	42	31	0	8	0.788	41	32	2	6	0.623
Present	79	27	28	1	23		27	25	1	26	

Table IV. Continued.

Parameter	No.	TILs and absolute lymphocyte count									
		After surgery									
		Invasive front					Main mass				
		1, n	2, n	3, n	4, n	P-value	1, n	2, n	3, n	4, n	P-value
Lymph node pouch invasion											
Absent	41	12	10	1	18	0.037	23	15	0	3	0.888
Present	39	5	23	0	10		12	7	2	18	
Distant metastasis											
Absent	143	63	52	1	27	0.526	63	51	2	27	0.539
Present	17	6	7	0	3		5	6	1	5	
Tumor deposits											
Absent	133	52	51	1	29	0.953	52	50	2	29	0.546
Present	27	16	8	0	3		15	7	1	4	
Tumor budding											
Absent	94	40	33	1	20	0.230	39	34	2	19	0.090
Present	66	29	26	0	11		29	23	1	13	
Necrosis											
Absent	45	17	15	1	12	0.201	16	16	1	12	0.037
Focal	61	26	27	0	8		25	24	1	11	
Moderate	36	15	14	0	7		17	15	0	4	
Extensive	18	11	3	0	4		10	2	1	5	
Fibrosis											
Absent	11	7	4	0	0	0.687	7	3	1	0	0.455
Focal	72	32	27	1	12		32	24	0	16	
Moderate	43	15	16	0	12		16	17	2	8	
Extensive	34	15	12	0	7		13	12	0	19	
Treatment status ^a											
1	36	27	3	0	6	<0.001	26	3	2	5	<0.001
2	22	17	5	0	0		17	5	0	0	
3	71	19	41	1	11		19	37	1	14	
4	31	12	19	0	0		12	19	0	0	

^a1, RCHT-resection-no treatment; 2, RCHT-resection-RT/RCHT; 3, no treatment-resection-no treatment; 4, no treatment-resection-RT/RCHT. Adca, adenocarcinoma; Muc, mucinous; RCHT, radiochemotherapy; RT, radiotherapy.

optimal local and systemic chronic inflammatory response. Unfortunately, the present study contains certain limitations. Initially, the sample size that corresponded to the patients who responded to preoperative treatment was relatively small. In addition, our analysis did not provide complete data regarding the type of chemotherapeutic drugs used and the number of chemotherapy cycles. Despite these limitations, the present study indicated that the measured parameters were associated with tumour progression and neoadjuvant therapy.

The previous study conducted by our group demonstrated that the percentage of stromal TILs present at the invasive tumour front correlated with the histopathological features of tumour progression (17). In the present study, the data demonstrated that approximately half of all patients exhibited a locally advanced tumour with lymph node involvement, whereas the incidence of distant metastases was less common. Therefore,

the study group was divided into 3 subgroups including patients without invasion of cancer cells, patients with invasion of cancer cells to lymphatic vessels and local lymph nodes, patients with lack of distant metastasis and patients with invasion of cancer cells to all aforementioned structures. In addition, the current study attempted to select CRC patients whose TILs and lymphocyte count may have an optimal prognostic value. In the current study, the combination index of different parameters (TILs/lymphocyte count/status progression) exhibited the highest diagnostic value in patients of group 4 (high TILs/high lymphocyte count/presence of local or distant metastases). The aforementioned combination of parameters was significantly associated with numerous pathological and clinical variables, such as tumour growth, tumour size, histological type, percentage of mucinous components, TNM stage, venous, lymphatic and perineural invasions, lymph node metastasis,

Table V. Associations between combined parameters of tumor-infiltrating lymphocytes, absolute lymphocyte count and tumor progression status, and clinicopathological features of patients with colorectal cancer (n=160).

Parameter	No.	TILs, absolute lymphocyte count and tumor progression status									
		Before surgery					After surgery				
		1, n	2, n	3, n	4, n	P-value	1, n	2, n	3, n	4, n	P-value
Age, years											
<60	40	2	12	3	21	0.112	9	6	13	10	0.688
≥60	120	11	34	22	37		25	21	28	32	
Sex											
Female	64	15	17	8	25	0.353	15	8	17	16	0.966
Male	96	3	29	17	33		19	19	24	26	
Localization											
Right-side	20	3	6	5	8	0.287	5	5	7	5	0.864
Transverse	14	1	1	4	4		5	14	5	3	
Left-side	15	1	9	1	3		2	4	2	2	
Sigmoid	29	0	3	1	4		0	1	4	6	
Rectum	82	7	26	11	30		20	3	20	22	
Tumor growth											
Expanding	133	10	36	21	49	0.037	25	23	33	37	0.605
Infiltrate	27	3	10	4	9		9	4	8	5	
Tumor size, cm											
<2.5	27	5	3	4	12	0.599	4	4	6	10	0.021
2.5-5.0	106	7	35	19	34		23	20	29	24	
>5.0	27	1	9	2	12		7	3	6	8	
Histological type											
Mucinous	30	1	11	3	14	0.001	8	4	9	8	0.002
Adenocarcinoma	130	12	35	22	44		26	23	32	34	
Mucinous component, %											
10-30	15	12	6	0	4	0.008	3	3	2	2	0.013
31-50	15	1	29	3	10		5	1	7	6	
TNM stage											
1	42	4	15	5	14	0.085	13	8	10	8	<0.001
2	31	2	11	1	14		7	5	7	9	
3	69	5	17	4	26		13	12	18	20	
4	18	2	3	15	4		1	2	6	5	
Grade of malignancies											
2	148	13	44	23	54	0.961	33	26	39	38	0.659
3	12	0	2	2	4		1	1	2	4	
pT stage											
1	3	0	2	0	1	0.770	0	2	1	0	0.096
2	62	6	16	10	22		15	9	16	15	
3	91	6	27	15	34		19	14	24	26	
4	4	1	1	0	1		0	2	0	1	
Venous invasion											
Absent	113	10	35	14	39	0.518	24	21	27	26	<0.001
Present	46	3	11	11	19		10	6	10	16	
Lymphatic invasion											
Absent	121	11	37	15	43	0.397	27	21	28	30	<0.001
Present	38	2	9	10	15		7	6	13	12	
Perineural invasion											
Absent	143	12	41	22	53	0.661	31	24	36	39	0.001
Present	17	1	5	3	5		3	3	5	3	

Table V. Continued.

Parameter	No.	TILs, absolute lymphocyte count and tumor progression status									
		Before surgery					After surgery				
		1, n	2, n	3, n	4, n	P-value	1, n	2, n	3, n	4, n	P-value
Lymph node metastasis											
Absent	81	8	31	10	34	0.196	23	17	23	21	<0.001
Present	79	5	46	15	24		11	10	18	21	
Lymph node pouch invasion											
Absent	41	10	33	11	40	<0.001	25	19	25	26	0.006
Present	39	3	13	14	18		9	8	16	16	
Distant metastasis											
Absent	143	11	41	20	56	0.851	34	19	35	41	0.218
Present	17	2	5	5	2		0	5	6	1	
Tumor deposits											
Absent	133	9	39	18	51	0.654	26	23	32	37	0.001
Present	27	4	6	7	7		7	4	9	4	
Tumor budding											
Absent	94	8	24	15	35	0.459	15	19	29	21	0.941
Present	66	5	22	10	23		19	8	12	21	
Necrosis											
Absent	45	3	13	5	16	0.401	11	7	10	11	0.042
Focal	61	7	18	6	24		12	13	14	16	
Moderate	36	1	11	10	13		6	6	11	12	
Extensive	18	2	4	4	5		5	1	6	3	
Fibrosis											
Absent	11	2	24	4	5	0.365	2	1	5	3	0.294
Focal	72	5	11	9	27		19	10	17	20	
Moderate	43	5	11	6	17		6	9	11	12	
Extensive	34	3	0	6	9		7	7	8	7	
Treatment status ^a											
1	36	12	6	16	2	0.396	2	12	5	17	0.007
2	22	5	12	5	0		14	2	2	4	
3	71	45	20	5	1		25	20	11	15	
4	31	23	2	2	4		4	12	3	12	

^a1, RCHT-resection-no treatment; 2, RCHT-resection-RT/RCHT; 3, no treatment-resection-no treatment; 4, no treatment-resection-RT/RCHT. Adca, adenocarcinoma; Muc, mucinous; RCHT, radiochemotherapy; RT, radiotherapy.

cancer cell invasion beyond the lymph node pouch, tumour deposits and incidence of necrosis. It is important to note that the findings regarding the examined parameters obtained following surgery exhibited the highest diagnostic value.

In light of these observations, the present study demonstrated that the investigation of local and systemic chronic inflammation was associated with tumour progression of CRC patients. Due to this fact, the diagnosis and prognosis of CRC patients should be undertaken on the basis of numerous local and systemic factors.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

KJ collected data, performed analysis, wrote the paper, reviewed the literature, acquired the data and contributed to manuscript drafting. MK analysed and interpreted the

pathological examination and approved the final version of the article. WF and KL collected and analysed data, and approved the final version of the article. MG wrote the paper, reviewed the literature, acquired the data and contributed to manuscript drafting. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was performed in conformity with the Declaration of Helsinki for Human Experimentation. Written informed consent was not required, and informed consent included an opt-out clause approved by the Medical Ethics Committee. The protocol was approved by the Bioethics Committee of the Medical University of Białystok (NoR-I-002/353/2016).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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