Neutrophil infiltration combined with necrosis in the primary tumor is a useful prognostic indicator for three-year disease-free survival time in patients with colorectal cancer

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Abstract. Histopathological evaluation plays a key role in the diagnosis of colorectal cancer (CRC). Tumor-related local inflammation is regarded as a novel prognostic parameter. Neutrophils constitute one of the main types of inflammatory cells. The aim of the present study was to evaluate the prognostic value of intratumoral tumor-associated neutrophils (intraTANs), stromal TANs (stromaTANs) and necrosis, as well as their combined parametric value in formalin-fixed paraffin-embedded tissue sections from patients with CRC. For this purpose, a retrospective study of 160 patients with CRC who underwent surgery was conducted. The association of intraTANs, stromaTANs, necrosis and their combined parametric value with the clinicopathological features of patients with CRC was examined. The Kaplan-Meier method and the log-rank test were used to compare survival curves. To identify independent prognostic factors, uni- and multivariate Cox proportional hazards regression models were used. StromaTANs were associated with lymph node metastasis (P=0.049) and tumor deposits (P=0.041). In addition, necrosis was found to be associated with venous (P=0.003), lymphatic (P=0.007) and perineural (P=0.015) invasion, as well as with lymph node metastasis (P=0.033), the number of invaded lymph nodes (P=0.012), and lymph node pouch invasion (P=0.043). Furthermore, necrosis was found to be associated with the white blood cell count (P=0.030), neutrophil count (P=0.011), the combined neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (NLR-PLR) (P=0.038), and the combined platelet and NLR (PLT-NLR status) (P=0.030), as well as with the serum carcinoembryonic antigen (CEA) levels following surgery (P=0.011) and the monocyte-to-lymphocyte ratio (P=0.023). The combined parametric value was found to be associated with pT stage (P=0.049), venous (P=0.034) and lymphatic (P=0.026) invasion, and with serum CEA levels prior to surgery (P=0.029). The analysis of the 3-year disease-free survival (DFS) time revealed that tumor growth [hazard ratio (HR), 2.070; 95% CI, 1.837-3.808; P=0.003] and the combined parametric value (intraTANs, stromaTANs and necrosis, HR, 1.577; 95% CI, 1.372-3.032; P=0.028) were independent factors for patients with CRC. Taken together, the findings of the present study demonstrated that the combined value of neutrophils and necrosis examined in the cancerous tissue may be used as a prognostic factor for the 3-year DFS time in patients with CRC.

Introduction

Colorectal cancer (CRC) is one of the most common causes of cancer-related mortality and remains a significant challenge for oncological therapy. CRC was the 3rd most common type of cancer worldwide following lung and breast cancer in 2020 (1). Analysis of epidemiological trends has indicated that the incidence rate of colon cancer is constantly increasing. According to the World Health Organization (WHO) data for 2018, the estimated number of cases of CRC worldwide was 1,849,518 (1-3). Despite medical developments, the number of cases of CRC is estimated to increase by 679,280 in the world by the year 2030 (1). The 5-year survival rate largely depends on the stage of CRC. It is estimated to be 90% in patients with stage I cancer and 15% in patients with metastatic CRC (4). The increasing trend in the number of patients with CRC motivates continued research on prognostic factors that may be evaluated during routine histopathological examinations. There are several types of CRC. Adenocarcinoma accounts for 90% of all CRC cases. Other less common histological types include squamous cell, spindle cell, neuroendocrine and undifferentiated carcinoma (5).

Histopathological evaluation plays a key role in the diagnosis of CRC. In addition to primary diagnosis, a histopathological examination provides information on the stage

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of the disease, vascular-lymphatic invasion and/or prognostic parameters in CRC (5). Consequently, pathological analysis provides the information necessary to select a therapy and assess patient prognosis. The tumor microenvironment, which can be assessed in a histopathological specimen, is another important element. It is mainly created by inflammatory cells. Tumor-related local inflammation is regarded as a novel prognostic parameter (6,7) Neutrophils represent one of the main types of inflammatory cells, and these cells have multifaceted functions by releasing various effector molecules, cytotoxic mediators and cytokines (8). The histopathological assessment of inflammatory cells may have predictive value in CRC. The role of inflammation in colon carcinogenesis has been the subject of numerous studies (9,10). Researchers have demonstrated the effects of proinflammatory agents (factors produced by inflammatory cells) on the development of CRC or on the regulation of genes associated with carcinogenesis (9). The presence of intratumoral neutrophils is considered to indicate a poorer prognosis (10).

Necrosis is a common phenomenon in CRC. It is observed both during tumor development and in patients following treatment. Tumor necrosis may be the result of rapid tumor growth and may reflect the level of hypoxia (11,12). Its presence is relatively simple to assess in histopathological slides (13) Thus far, the incidence of necrosis has been associated with a high tumor grade and with a serrated histology (11). Recent studies have indicated the association between the type of molecular CRC and necrosis (14,15). The aim of the present study was to define the role of necrosis and neutrophil infiltration in tumor tissue, and to assess its clinical significance.

Patients and methods

Study population. Specimens from 160 patients, who had been surgically treated for CRC, were examined. Patients with CRC received surgery at the Department of Oncological Surgery, in the Comprehensive Cancer Center of Bialystok (Poland) between April 2014 and December 2016. The study was designed with no restrictive inclusion and exclusion criteria to obtain a sample reflecting a wide representation of patients with CRC. The 160 patients consisted of 96 males and 64 females, with a mean age of 67.5 years (range, 32-86 years). The study protocol was reviewed and approved by the Local Ethics Committee at the Medical University of Bialystok (approval no. APK.002.164.2020; Poland).

Clinical and laboratory characteristics of the study group. The majority of the patients presented with similar symptoms, including abdominal pain, anemia, rectal bleeding, constipation, diarrhea, vomiting and anorexia. In addition, patients received treatment for hypertension (n=45), type II diabetes (n=12), osteoarthritis (n=3) and coronary heart disease (n=7). However, none of the patients received any anti-inflammatory therapy. All patients underwent routine diagnostic tests, including basic diagnostic laboratory tests (morphological tests and lipid profiles), electrocardiography, spirometry, arterial blood gasometry test, X-rays and computerized chest tomography. The clinical stage of CRC was evaluated according to the tumor-node-metastasis (TNM) classification (16). Prior to surgery, patients with tumors identified in other sites had not received any anti-inflammatory or immunosuppressive therapy.

Patients diagnosed with neoplasms in the rectum received pre-operative therapy (n=53). They received radiotherapy (n=39), chemotherapy (n=7) or radio-chemotherapy (n=7) and were treated with a dose of 25 Gy in fractions of 5 Gy for 1 week in the pelvic area. The response to pre-operative therapy was determined according to the Response Evaluation Criteria in Solid Tumors (17). Stable disease (SD) was observed in 26 patients, while partial response (PR) was observed in 27 patients.

Blood samples were obtained within 3 days prior to and following surgery. The differential white blood cell count (WBC) was determined using an XN-1000 automated hematology analyzer (Sysmex Co.). The combined neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (NLR-PLR status) was calculated as previously described by Hirahara *et al* (18) and Jakubowska *et al* (19). Themonocyte-to-lymphocyte ratio (MLR) and platelet (PLT)-NLR status were calculated as previously described (19). Cancer biomarkers [carcinoembryonic antigen (CEA) and CA19-9] were analyzed using a Cobas 6000 analyzer (Roche).

The inclusion criteria were as follows: i) Pathologically confirmed CRC; ii) treatment with radical resection; iii) patients had not received any anti-inflammatory therapy. The exclusion criteria were as follows: i) Incomplete clinicopathological and follow-up data; ii) the presence of hematological disorders, such as anemia; and iii) evidence of an autoimmune disease.

Tissue samples. Tissues obtained from surgery were fixed in 4% buffered formalin for 24-72 h at room temperature. Small sections of tissue were embedded in paraffin. Sections (4-μm-thick) were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E; cat. no. 468802128; POCH S.A.; Avantor Performance Materials Poland) at room temperature for 4 min according to the manufacturer's protocol. The slides were deparaffinized in an oven at 60˚C for 5 min. Subsequently, the slides were washed with xylene (three washes, 10 min each) and rehydrated in a graded ethanol series (100, 95, 85 and 75%, 1 min at each concentration). A routine histopathological evaluation of the slides was performed in accordance with the recommendation from the WHO (20). The type of tumor growth, tumor size, histological type, the percentage of mucinous components, the grade of malignancy and the TNM stage were determined by pathologists (MK and K.L.) who were blinded to the clinical information. Venous, lymphatic and perineural invasions of cancer cells were also analyzed. The characteristic features of lymph node invasion were examined, including the number of resected and invaded lymph nodes, the presence of micro- and macro-metastases, the invasion of the pouch lymph node, the presence of distant metastases, and the size of metastases. The presence, number and size of the deposits of cancer cells were also assessed (21).

Histopathological analysis of intratumoral and stromal tumor-associated neutrophils (TANs) in the primary tumor mass. In the present study, the intratumoral TANs (intraTANs) and stromal TANs (stromaTANs) in CRC tissues were assessed. The analysis was performed by two independent pathologists,

blinded to patient clinical information, treatment regimen and outcomes. Morphologically, neutrophils are recognized as polymorphonuclear cells with segmented nuclei that possess clumped chromatin, eosinophilic cytoplasm and pink granules (22). IntraTANs were determined according to the modified classification described in the study by Harbaum *et al* (23). Neutrophils were assessed in four H&E-stained slides under a light microscope (magnification, x400; Leica DM6 B, KAWA. SKA, Sp. z o.o.; Leica Microsystems, Inc.). They were counted and scored as the 'low' group [absent or <10 cells/high power field (HPF)], 'moderate' group (10-50 cells/HPF) and 'high' group (>50 cells/HPF).

The analysis of stromaTANs was performed as previously described (24). Briefly, TANs were assessed at the invasive front and in the tumor center under a light microscope at a high-power magnification (x400). The cells were counted and quantified as a percentage of all the cells examined. Neutrophils were divided into two groups as follows: 'Low' (0-20% neutrophils) and 'high' (>21% neutrophils).

Histopathological examination of necrosis. The degree of tissue necrosis in the center of the tumor (in the core region of the tumor) was classified using a modified version of the criteria described by Väyrynen *et al* (11) and Gao *et al* (25). Tumor necrosis was defined as an area with increased eosinophilia, neutrophilia and nuclear shrinkage, and the fragmentation and disappearance of cells in the tumor stroma. Intraluminal neutrophilic inflammatory infiltrate was excluded from the evolution of the necrosis tumor and classified as intraTANs. The areas of tumor necrosis were assessed semi-quantitatively and graded as follows: 1, 'Absent' (none); 2, 'focal' (<10% of tumor area); 3, 'moderate' (10-30%); or 4, 'extensive' (>30%). For analysis, the study group was divided into two subgroups as follows: i) The 'low' group (absent or focal necrosis); and ii) the 'high' group (moderate or extensive necrosis).

Combined parametric value. In the present study, the combination of intratumoral neutrophils, stromaTANs in the primary tumor mass and necrosis was also examined. The study group was divided into four groups as follows: Group 1 (low intraTANs, low stromaTANs and low necrosis); group 2 (low intraTANs, high stromaTANs and high necrosis); group 3 (high intraTANs, low stromaTANs and low necrosis); and group 4 (high intraTANs, high stromaTANs and high necrosis).

Follow-up data. Patients were followed up annually for 2-5 years. They were monitored by measuring CEA and CA19-9 levels, a physical examination, colonoscopy or/and radiological imaging, including computerized tomography of the chest, abdomen and pelvis, bone scan, and positron emission tomography scans. Local and distant recurrences were defined as pathological evidence of the spread of tumors in the region of anastomosis (local recurrence) and/or present outside of the primary tumor at other sites, such as the liver, lungs, bones, brain (distant recurrence) and confirmed by the aforementioned techniques.

Statistical analysis. Statistical analysis was performed using the STATISTICA software version 13.0 (StatSoft). Numerical data were analyzed using a χ^2 test. To analysis small number of cases (≤5), we use the Fisher's exact test. Comparisons amongst multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. The disease-free survival (DFS) time was calculated as the duration between the date of diagnosis and the date of disease progression, including local or distant relapse. The DFS rate was calculated using the Kaplan-Meier method and survival curves were compared using log-rank tests. Prognostic factors were assessed using univariate and multivariate analyses (Cox proportional hazard regression model). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of the study group. Overall, 12.5% of the patients (20/160) had a primary tumor on the right side of the colon, 10% (16/160) in the transverse colon and 9.375% (15/160) on the left side of the colon; 18.125% (29/160) had CRC in the sigmoid colon and 51.25% (82/160) in the rectum. Among the 160 tumors, 81.25% (130/160) were classified as adenocarcinoma and 18.75% (30/160) were classified as adenocarcinoma with mucosal component. According to the TNM classification, 45.625% of the patients (73/160) had stage Ior II cancer, and 54.375% of the patients (87/160) had stage III or IV cancer. The majority of the tumors were moderately differentiated (G2) (N=148; 92.5%), and the remaining tumors were poorly differentiated (G3) (N=12; 7.5%). Lymph node metastases were present in 49.375% of the patients (79/160) and distal metastases were present in 10.625% of the patients (17/160). Pre-operative treatment was performed in 53 cases.

Analysis of intraTAN infiltration, stromaTAN infiltration and tumor necrosis in patients with CRC. IntraTANs extensively infiltrated the tumor tissue in the majority of cases (n=82), while weak and moderate inflammation were observed in 43 and 35 cases, respectively. StromaTANs in the core region of the tumor tissue mainly exhibited a low cell infiltration (n=104, Fig. 1). Similar to stromaTANs, the majority of the patients in the study group exhibited low levels of necrosis in tissue (n=105; Fig. 2). The analysis of the combined parametric value indicated the majority of CRC cases were in groups 3 (high intraTANs, low stromaTANs and low necrosis; n=53) and 4 (high intraTANs, high stromaTANs and high necrosis; n=51), where the tissue had a large amount of intraTANs (Fig. 3). Fig. 4 demonstrates the distribution of intraTANs and stromaTANs in the center of the tumor mass, necrosis and combined parametric value.

Association between intraTAN infiltration, stromaTAN infiltration or tumor necrosis and the clinicopathological features of patients with CRC. IntraTANs were associated with the number of resected lymph nodes (P=0.004), fibrosis (P=0.032) and pre-operative treatment (P=0.015) (Table I). StromaTANs were associated with lymph node metastasis (P=0.049) and tumor deposits (P=0.041) (Table II). Necrosis was associated with venous (P=0.003), lymphatic (P=0.007) and perineural (P=0.015) invasion, as well as with lymph node metastasis (P=0.033), the number of invaded lymph

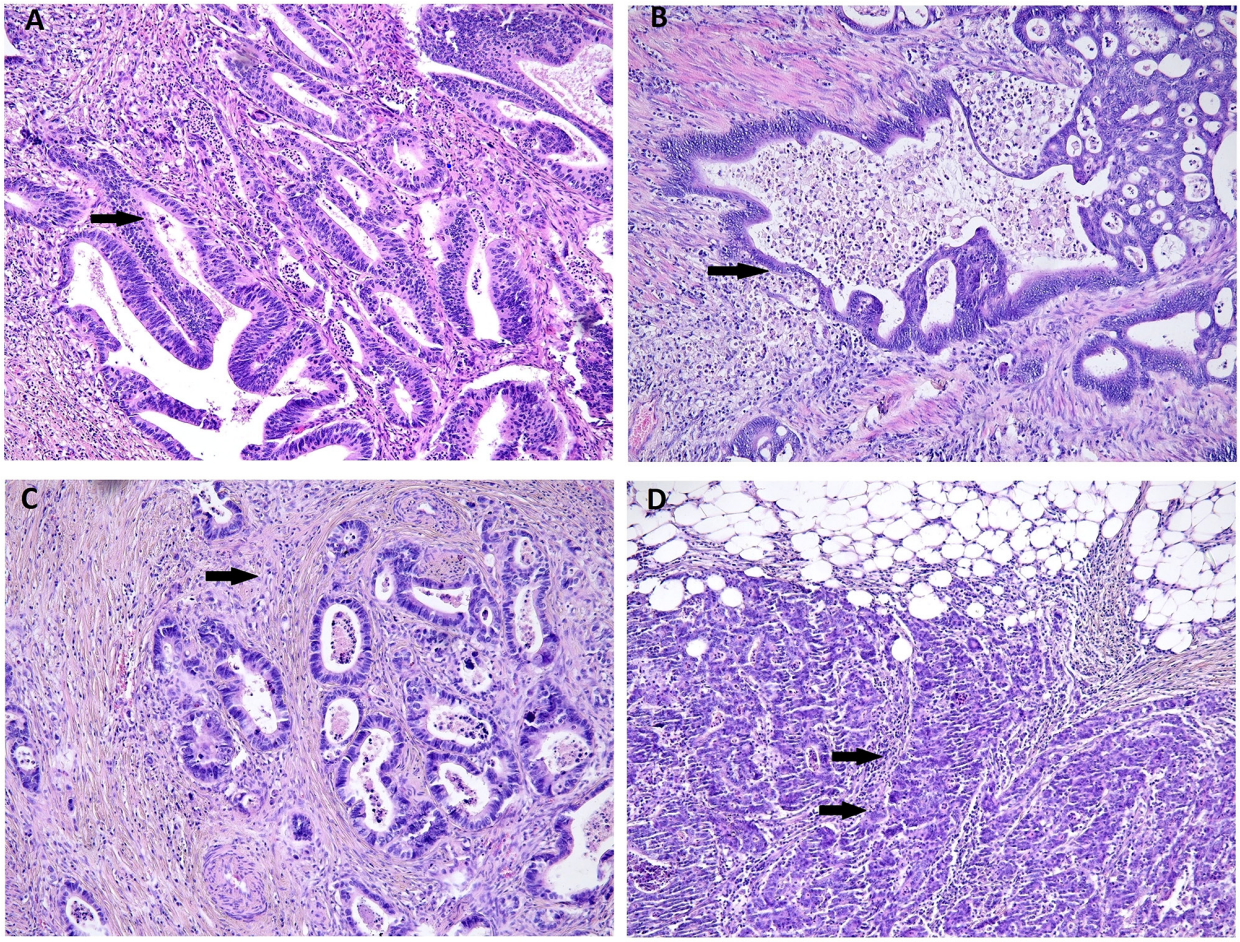

Figure 1. Microscopic analysis of the presence of intratumoral TANs and stromal TANs in the centre of the tumor mass in hematoxylin and eosin-stained CRC tissues. Representative examples of (A) low and (B) high levels of intratumoral TANs in CRC tissue (black arrows). Representative images of samples with (C) low and (D) highlevels of stromal TANs (black arrows). Magnification, x100. CRC, colorectal cancer; TANs, tumor-associated neutrophils.

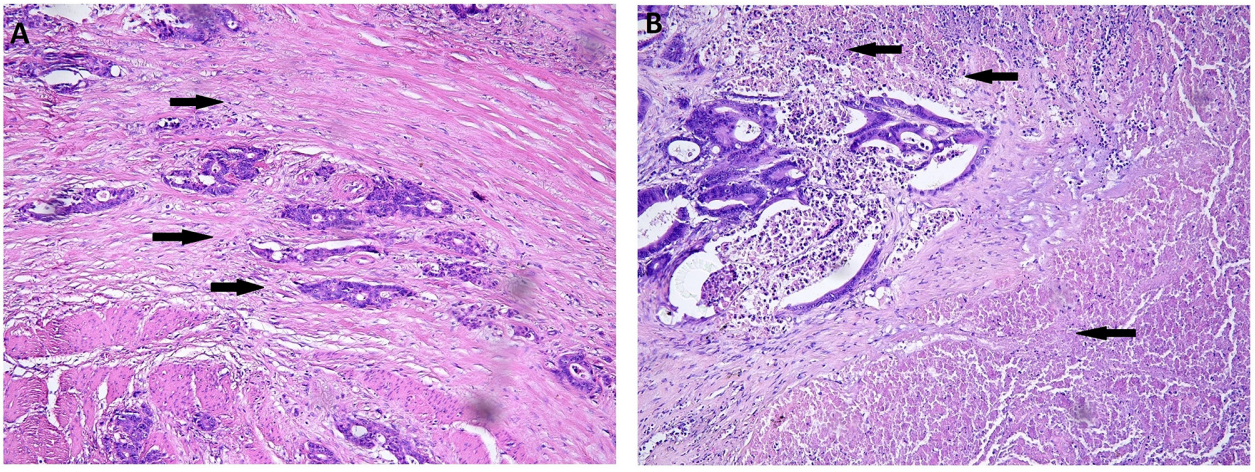

Figure 2. Histological examination of necrosis in hematoxylin and eosin-stained CRC tissue (A) The specimens from the majority of patients with CRC exhibited a lack of necrotic mass (low group) in the centre of the tumour tissue (B) Representative image of massively extensive necrosis together with the disintegration of cancerous tissue (high group). Magnification, x100. Arrows indicate necrosis. CRC, colorectal cancer.

nodes (P=0.012) and lymph node pouch invasion (P=0.043) (Table III). The combined parametric value was associated with pT stage (P=0.049), and with venous (P=0.034) and lymphatic (P=0.026) invasion (Table IV).

Association between intraTAN infiltration, stromaTAN infiltration or tumor necrosis and the hematological parameters of patients with CRC. IntraTANs were found to be associated with lymphocyte count following surgery

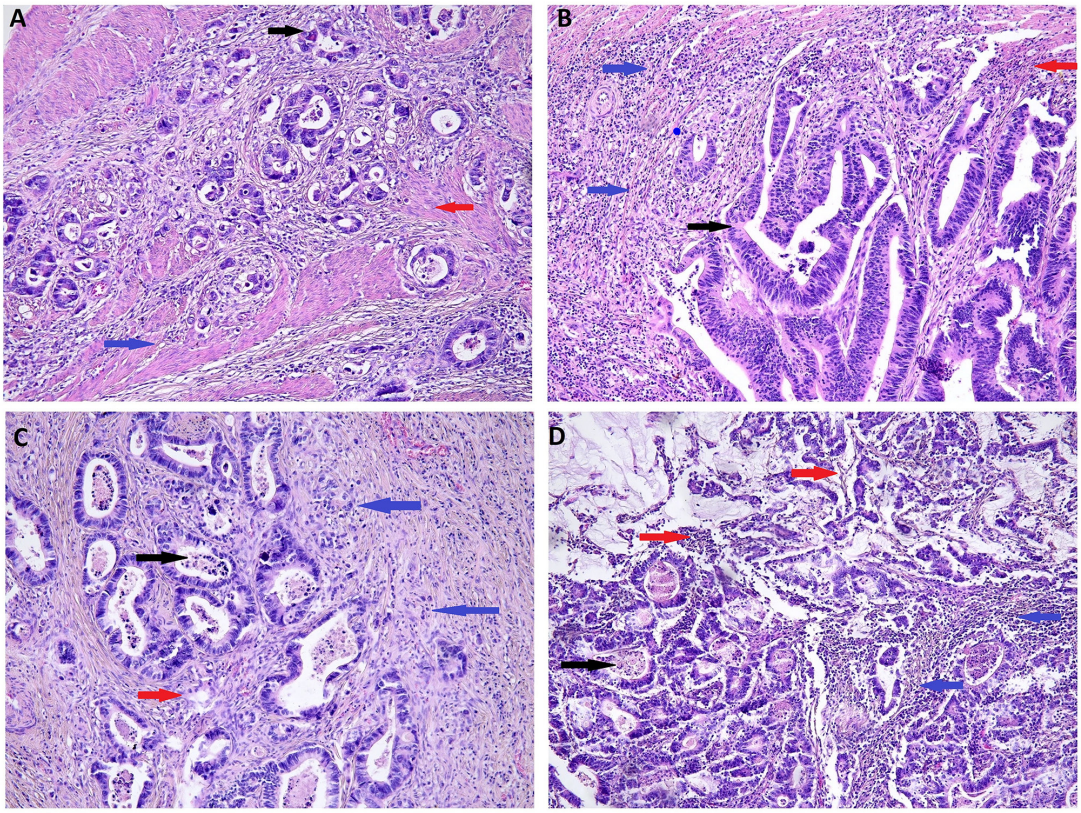

Figure 3. Representative examples of the combined parametric value in hematoxylin and eosin-stained CRC tissue. Histological findings in resected CRC tissues from patients with (A) low levels of intraTANs, low levels of stromaTANs and a low degree of necrosis (group 1) (B) low levels of intraTANs, high levels of stromaTANs and a high degree of necrosis (group 2) (C) high levels of intraTANs, low levels of stromaTANs and a low degree of necrosis (group 3), and (D) high levels of intraTANs, high levels of stromaTANs and a high degree of necrosis (group 4). magnification, x100. IntraTANS (black arrow), stromaTANs (blue arrow), necrosis (red arrow). CRC, colorectal cancer; intraTANs, intratumoral tumor-associated neutrophils; stromaTANs, stomal tumor-associated neutrophils.

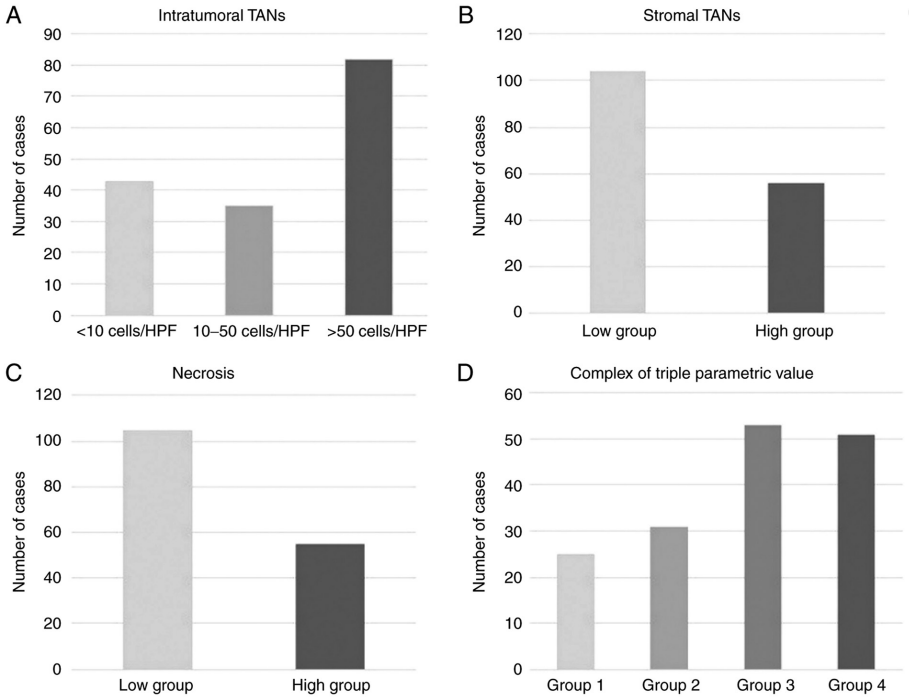

Figure 4. Distribution of (A) intraTANs and (B) stromaTANs in the center of the tumor mass, (C) necrosis, and (D) their combined parametric value. Stromal TANs scored as low group (0-20% neutrophils) and high group (more than 21% neutrophils). Necrosis scored as low group (absent or focal necrosis) and high group (moderate or extensive necrosis). Group 1, low levels of intraTANs, low levels of stroma TANs and a low degree of necrosis; group 2, low levels of intraTANs, high levels of stromaTANs and a high degree of necrosis; group 3, high levels of intraTANs, low levels of stromaTANs and a low degree of necrosis; group 4, high levels of intraTANs, high levels of stromaTANs and a high degree of necrosis.

Table I. Association between intraTAN infiltration and clinicopathological features in patients with colorectal cancer (n=160).

	IntraTAN infiltration			
Clinicopathological variable	Low, <10 cells/HPF	Moderate, 10-50 cells/HPF	High, >50 cells/HPF	P-value
Age, years				
<60	11	13	16	0.756
>60	29	44	47	
Sex				
Female	13	18	33	0.132
Male	27	39	30	
Location				
Rightside	3	8	9	0.899
Transverse	3	2	11	
Leftside	2	2	11	
Sigmoid	1	13	15	
Rectum	29	26	27	
Tumor growth				
Expanding	34	48	51	0.670
Infiltrate	6	9	12	
Tumor size, cm				
<2.5	7	10	10	0.672
2.5-5.0	25	40	41	
>5.0	8	7	12	
Histological type				
Mucinous	8	9	13	0.825
Adenocarcinoma	32	48	50	
Percentage of mucinous component, %				
10-30	2	4	9	0.971
30-50	5	5	5	
TNM stage				
I+II	16	26	31	0.702
III+IV	24	29	34	
Grade ofmalignancies				
2	37	54	57	0.763
3	3	3	6	
pT stage				
1+2	18	22	25	0.122
3+4	22	35	38	
Venous invasion				
Absent	31	36	46	0.712
Present	9	21	16	
Lymphatic invasion				
Absent	32	41	48	0.805
Present	8	16	14	
Perineural invasion				
Absent	37	52	54	0.780
Present	3	5	9	
Lymph nodemetastasis				
Absent	21	34	26	0.752
Present	19	23	37	
Number of resected lymph nodes				
<5	7	4	2	0.035
5-10	12	10	7	
≥10	21	43	38	

Table I. Continued.

Clinicopathological variable	IntraTAN infiltration			P-value
	Low, <10 cells/HPF	Moderate, 10-50 cells/HPF	High, >50 cells/HPF	
Number of invaded lymph nodes				
<5	13	17	21	0.922
≥5	6	6	16	
Lymph node pouch invasion				
Absent	25	9	8	0.957
Present	15	18	2	
Distant metastasis				
Absent	37	49	57	0.949
Present	3	8	6	
Tumor Deposits				
Absent	29	48	56	0.160
Present	10	9	8	
Tumor budding				
Absent	24	34	36	0.572
Present	16	23	27	
Necrosis				
Absent	10	17	18	0.942
Focal	7	27	27	
Moderate	14	8	14	
Extensive	9	5	4	
Fibrosis				
Absent	1	7	3	0.034
Focal	19	28	25	
Moderate	11	14	18	
Extensive	9	8	17	
Crohn's-like aggregates				
Absent	34	37	42	0.271
Present	6	20	16	
Response to neoadjuvant treatment				
SD	10	5	1	0.517
PR	12	9	17	
Pre-operative treatment				
Yes	12	11	31	0.016
No	28	46	30	

SD, stable disease; PR, partial response; HPF, high-power field; intraTANs, intratumoral tumor-associated neutrophils.

(P=0.046) and serum CEA level prior to surgery (P=0.042) (Table SI). The analysis of stromaTANs and hematological parameters did not reveal a significant association (Table SII). The degree of necrosis was associated with hematological parameters measured following surgery, such as WBC (P=0.030), neutrophil count (P=0.011), the NLR-PLR status (P=0.038), the PLT-NLR status (P=0.030) and serum CEA level following surgery (P=0.011). Furthermore, necrosis was associated with MLR, measured in pre-operative whole blood samples (P=0.023; Table SIII). The combined parametric value was significantly associated with serum CEA levels prior to surgery (P=0.029) (Table SIV).

Prognostic value of intraTANs, stromaTANs and necrosisin patients with CRC, and their combined parametric value. To investigate the association between intraTANs, stromaTANs and necrosis and prognosis, and their combined parametric value in patients with CRC, the survival curves of 3- and 5-year DFS time in all the patients were determined usingthe Kaplan-Meier method. The median of the 3- and 5-year DFS was 11.6 and 27.6 months, respectively. Patients with low levels of intraTANs survived ~10.9 months (3-year DFS time) and 28.2 months (5-year DFS time) compared with that in patients with moderate and strong levels ofintraTANs [(10.9 and 12.4 months for 3-year DFS time, respectively

Table II. Association between stromaTANs and clinicopathological features in patients with colorectal cancer (n=160).[a]

Clinicopathological feature	StromaTANs in the center of tumor		
	Low	High	P-value
Age, years			
<60	35	5	0.602
>60	109	11	
Sex			
Female	58	6	0.862
Male	86	10	
Location			
Rightside	15	5	0.320
Transverse	11	3	
Leftside	14	1	
Sigmoid	28	1	
Rectum	78	4	
Tumor growth			
Expanding	121	12	0.494
Infiltrate	23	4	
Tumor size, cm			
<2.5	23	4	0.892
2.5-5.0	100	6	
>5.0	21	6	
Histological type			
Mucinous	18	12	0.982
Adenocarcinoma	126	4	
Percentage of mucinous component, %			
10-30	14	1	0.302
30-50	11	4	
TNM stage			
I+II	65	8	0.383
III+IV	79	8	
Grade ofmalignancies			
2	133	15	0.852
3	11	1	
pT stage			
I+II	61	4	0.622
III+IV	83	12	
Venous invasion			
Absent	105	8	0.193
Present	38	8	
Lymphatic invasion			
Absent	110	11	0.415
Present	33	5	
Perineural invasion			
Absent	127	16	0.833
Present	17	0	
Lymph nodemetastasis			
Absent	72	9	0.047
Present	72	7	
Number of resected lymph nodes			
<5	11	2	0.142
5-10	27	2	
≥10	90	12	
Number of invaded lymph nodes			
<5	44	7	0.736
≥5	28	0	
Lymph node pouch invasion			
Absent	31	10	0.887
Present	33	6	
Distant metastasis			
Absent	128	15	0.252
Present	16	1	
Tumor deposits			
Absent	118	15	0.052
Present	26	1	
Tumor budding			
Absent	85	9	0.130
Present	59	7	
Necrosis			
Absent	44	1	0.319
Focal	56	5	
Moderate	30	6	
Extensive	14	4	
Fibrosis			
Absent	10	1	0.742
Focal	64	8	
Moderate	39	4	
Extensive	31	3	
Crohn's-like aggregates			
Absent	100	13	0.083
Present	39	3	
Response to neoadjuvant treatment			
SD	15	1	0.973
PR	35	3	
Pre-operative treatment			
Yes	45	9	0.252
No	101	5	

[a]StromaTANS were divided into two groups: Low (0-20% neutrophils) and 'high' (more than 21% neutrophils). SD, stable disease; PR, partial response; stromaTANs, stromaltumor-associated neutrophils.

and 26.0 and 29.1 months for5-year DFS time, respectively). The results revealed that patients in the low stromaTANs level group exhibited significantly longer 3- and 5-year DFS rates compared with that in patients in the high stromaTANs level group (P=0.061 and 0.162, respectively). The mean

Table III. Association between necrosis and clinicopathological features in patients with colorectal cancer (n=160).[a]

Clinicopathological feature	Necrosis		
	Low	High	P-value
Age, years			
<60	25	15	0.623
>60	67	53	
Sex			
Female	40	24	0.212
Male	53	43	
Location			
Right-side	9	11	0.552
Transverse	4	10	
Left-side	6	11	
Sigmoid	6	23	
Rectum	46	36	
Tumor growth			
Expanding	75	58	0.422
Infiltrate	18	9	
Tumor size, cm			
<2.5	17	10	0.499
2.5-5.0	57	49	
>5.0	19	8	
Histological type			
Mucinous	19	11	0.946
Adenocarcinoma	74	56	
Percentage of mucinous component, %			
10-30	5	10	0.715
30-50	14	1	
TNM stage			
I+II	50	23	0.069
III+IV	43	44	
Grade ofmalignancies			
2	88	60	0.532
3	5	7	
pT stage			
1+2	38	27	0.822
3+4	55	40	
Venous invasion			
Absent	71	42	0.002
Present	22	24	
Lymphatic invasion			
Absent	75	46	0.006
Present	18	20	
Perineural invasion			
Absent	88	55	0.011
Present	5	12	
Lymph node metastasis			
Absent	60	21	0.031
Present	33	46	
Number of resected lymph nodes			
<5	8	5	0.682
5-10	17	12	
≥10	68	34	
Number of invaded lymph nodes			
<5	23	28	0.011
≥5	10	18	
Lymph node pouch invasion			
Absent	14	27	0.040
Present	27	12	
Distant metastasis			
Absent	83	60	0.622
Present	10	7	
Tumor deposits			
Absent	79	54	
Present	13	14	0.087
Tumor budding			
Absent	56	38	0.432
Present	37	29	
Fibrosis			
Absent	7	4	0.822
Focal	43	29	
Moderate	25	18	
Extensive	18	16	
Crohn's-like aggregates			
Absent	64	49	0.815
Present	29	13	
Response to neoadjuvant treatment			
SD	8	8	0.521
PR	9	29	
Pre-operative treatment			
Yes	16	38	0.682
No	76	30	

[a]Necrosis was scored as low (absent or focal necrosis) or high group (moderate or extensive necrosis). SD, stable disease; PR, partial response.

3- and 5-year DFS rates were 11.6 and 29.0 months in the high necrosis group, and 9.85 and 23.8 months, in the low necrosis group, respectively. The analysis of the combined parametric value indicated that patients in groups 1 (low intraTANs, low stromaTANs and low necrosis), 3 (high intraTANs, low stromaTANs and low necrosis) and 4 (high intraTANs, high stromaTANs and high necrosis) had significantly longer 3-year DFS rates compared with that inpatients in group 2 (low intraTANs, high stromaTANs and high necrosis) (P=0.027). However, the analysis of 5-DFS rate did not confirm this trend (P=0.895) (Fig. 5).

To compare the prognostic value of intraTANs, stromaTANs, necrosis and their combined parametric value with other histopathological parameters, univariate and multivariate analyses were performed (Tables V and VI). Thefactors

Table IV. Association between intraTAN infiltration, stromaTAN infiltration and tumor necrosis and clinicopathological features in patients with colorectal cancer (n=160).[a]

Clinicopathological variable	Group number 1	2	3	4	P-value
Age, years					
<60	7	9	14	10	0.584
>60	18	18	41	43	
Sex					
Female	12	8	27	17	0.257
Male	13	19	28	36	
Location					
Rightside	3	2	5	10	0.500
Transverse	1	5	1	7	
Leftside	2	1	10	2	
Sigmoid	2	2	23	2	
Rectum	17	15	23	27	
Tumor growth					
Expanding	21	22	47	43	0.796
Infiltrate	4	5	8	10	
Tumor size, cm					
<2.5	5	5	7	10	0.437
2.5-5.0	13	18	43	36	
>5.0	7	4	6	7	
Histological type					
Mucinous	5	7	12	8	0.433
Adenocarcinoma	20	20	45	45	
Percentage of mucinous component, %					
10-30	0	3	8	4	0.094
30-50	5	4	2	4	
TNM stage					
I+II	12	13	29	19	0.462
III+IV	13	14	26	34	
Grade ofmalignancies					
2	23	26	49	50	0.566
3	2	1	6	3	
pT stage					
1+2	9	14	19	23	0.049
3+4	16	13	36	30	
Venous invasion					
Absent	20	16	47	30	0.034
Present	9	11	3	23	
Lymphatic invasion					
Absent	21	18	48	34	0.026
Present	4	9	6	19	
Perineural invasion					
Absent	24	24	50	45	0.362
Present	1	3	5	8	
Lymph nodemetastasis					
Absent	15	16	24	26	0.511
Present	10	11	31	27	
Number of resected lymph nodes					
<5	3	5	0	4	0.254
5-10	3	9	8	9	
≥10	19	13	31	39	
Number of invaded lymph nodes					
<5	7	6	16	22	0.256
≥5	3	5	15	5	
Lymph node pouch invasion					
Absent	16	19	1	5	0.511
Present	9	8	0	22	
Distant metastasis					
Absent	23	24	49	47	0.678
Present	2	3	6	6	
Tumor deposits					
Absent	19	21	48	45	0.328
Present	5	6	8	8	
Tumor budding					
Absent	15	15	33	31	0.998
Present	10	12	22	22	
Necrosis					
Absent	7	7	17	14	0.023
Focal	6	5	24	26	
Moderate	5	13	7	11	
Extensive	7	2	7	2	
Fibrosis					
Absent	1	1	4	5	0.459
Focal	10	6	35	21	
Moderate	7	5	15	16	
Extensive	7	0	16	11	
Crohn's-like aggregates					
Absent	20	20	37	36	0.156
Present	5	6	14	17	
Response to neoadjuvant treatment					
SD	3	1	7	5	0.852
PR	2	5	26	5	
Pre-operative treatment					
Yes	4	10	31	9	0.442
No	21	17	25	43	

[a]The study group was divided into 4 groups: group 1 (low intraTANs, low stromaTANs, low necrosis), group 2 (low intraTANs, high stromaTANs, high necrosis), group 3 (high intraTANs, low stromaTANs, low necrosis), group 4 (high intraTANs, high stromaTANs, high necrosis). SD, stable disease; PR, partial response.

found to be predictive of 3-year DFS times in univariate Cox regression analysis included tumor growth [hazard ratio (HR),

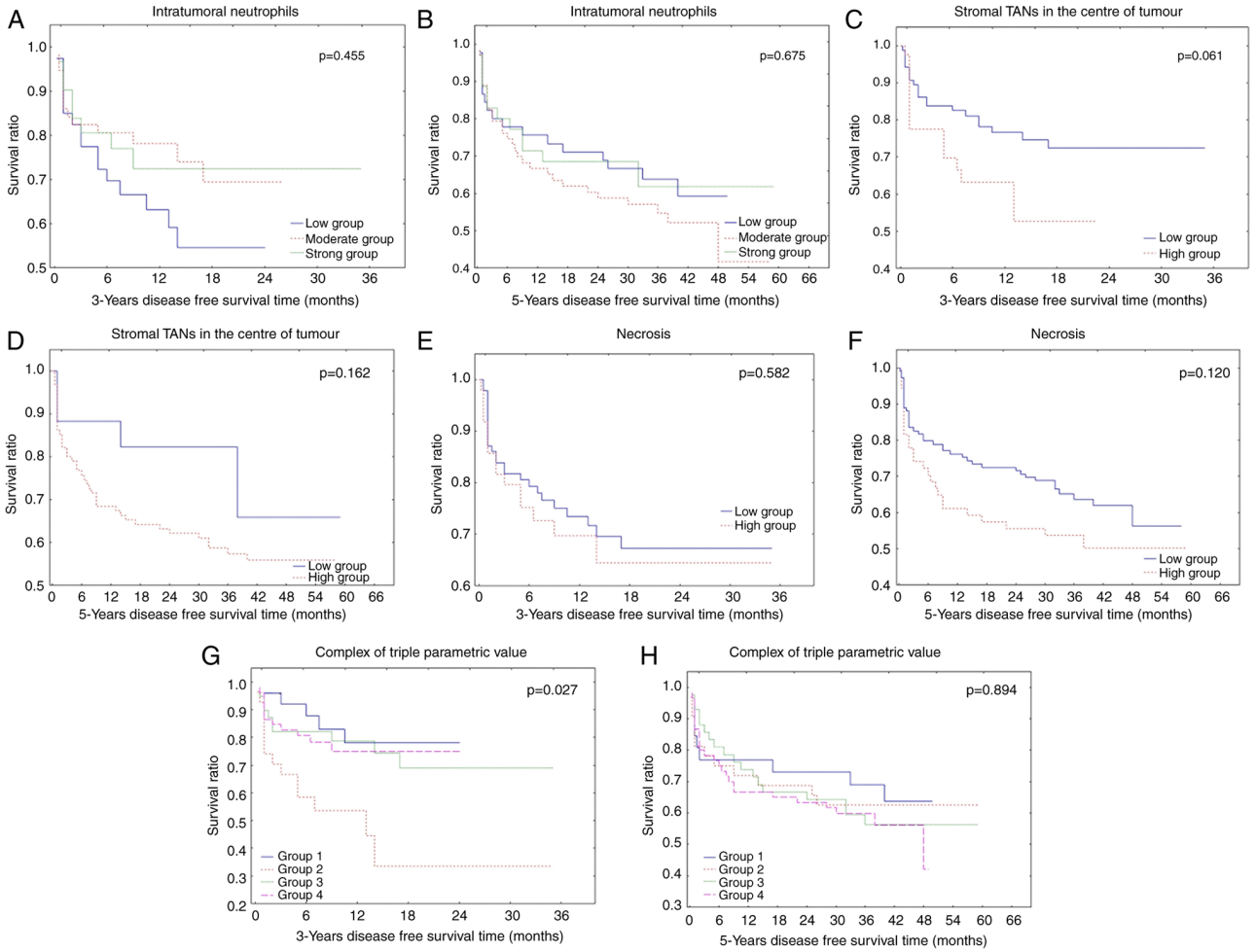

Figure 5. Effect of intraTANs, stromaTANs, necrosis and their combined parametric value on the DFS time in patients with CRC. Kaplan-Meier plots of the 3- and 5-year DFS times based on (A and B) intraTANs, (C and D) stromal TANs, (E and F) necrosis, and (G and H) their combined triple parametric value, respectively. Group 1, low levels of intraTANs, low levels of stroma TANs and a low degree of necrosis; group 2, low levels of intraTANs, high levels of stromaTANs and a high degree of necrosis; group 3, high levels of intraTANs, low levels of stromaTANs and a low degree of necrosis; group 4, high levels of intraTANs, high levels of stromaTANs and a high degree of necrosis. IntraTANs, intratumoral tumor-associated neutrophils; stromaTANs, stomal tumor-associated neutrophils. IntraTANs scored as low group (absent or <10 cells/HPF), moderate group (10-50 cells/HPF) and high group (>50 cells/HPF). Stromal TANs scored as low group (0-20% neutrophils) and high group (more than 21% neutrophils). Necrosis scored as low group (absent or focal necrosis) and high group (moderate or extensive necrosis).

2.070; 95% confidence interval (CI), 1.837-3.808; P=0.040], tumor budding (HR, 1.932; 95% CI,-1.036-0.613; P=0.049) and intraTANs/stromaTANs/necrosis (HR, 1.577; 95% CI, 1.372-3.032; P=0.014). According to multivariate Cox proportional model, tumor growth (HR, 1.925; 95% CI, 1.145-3.420; P=0.003) and intraTANs/stromaTANs/necrosis (HR, 1.344; 95% CI, 1.235-3.015; P=0.028) were independent factors of 3-year DFS time in patients with CRC. However, other factors such as age, sex, tumor size, TNM stage, histopathological type, grade of malignancies, venous invasion, metastasis to the lymphatic vessels and perineural spaces, lymph node involvement, distant metastasis, tumor deposits, tumor budding, intraTANs, stromaTANs and their combined parametric value were not significant in univariate and multivariate analyses of the 5-year DFS times in patients with CRC.

Discussion

Neutrophilic cell infiltration and tumor necrosis often occur in solid tumors (26,27). Physiologically, immune cells, particularly neutrophils, are able to protect the host using immune surveillance, eliminating both microbial pathogens and cancerous cells. Notwithstanding, the classical function of these cells can be modified, whereby they are recruited into the tumor mass. They can then be activated via alternative pathways, and thus exhibit immunosuppressive activities, including tumor growth and metastasis (28). In the present study, tissues from patients with CRC exhibited numerous neutrophils localized inside the tumor tissue in the majority of cases, while a low neutrophilic inflammation was observed in the center of the tumor stroma. Both types of neutrophils were associated with pro-tumor factors, such as the CEA level, the number of resected lymph nodes, lymph node metastasis and the presence of tumor deposits. Ye *et al* (29) indicated that a higher TAN abundance was found in tissues from patients with CRC with well-to-moderate tumor differentiation, and fewer numbers of lymph nodes or metastases, TNM stage I-II disease or rectal cancer. Subsequent studies have also demonstrated that an increasing TAN density was associated with a higher stage disease in patients with CRC (10,30). Furthermore, some

Table V. Prognostic factors for 3-year disease-free survival time in patients with colorectal cancer.

	Univariate		Multivariate	
Variable	HR (95%CI)	P-value	HR (95%CI)	P-value
Age, years (≤60 vs. ≥60)	0.649 (1.048-1.597)	0.512	-	-
Sex (female vs. male)	0.822 (1.198-1.465)	0.414	-	-
Tumor growth (expanding vs. infiltrate)	2.070 (1.837-3.808)	0.040	1.925 (1.145-3.420)	0.003[a]
Tumor size, cm <2.5 vs. 2.5-5 vs. >5	0.19.1 (0.264-1.225)	0.829	-	-
TNM stage (I-II vs. III-IV)	-1.491 (-0.801-0.514)	0.121	-	-
Adenocarcinoma type (non-muc vs. partim mucin)	-0.062 (-0.120-1.738)	0.944	-	-
Grade of malignancy (2 vs. 3)	1.639 (2.715-4.442)	0.104	-	-
Pre-operative treatment (yes vs. no)	-2.044 (-4.677-2.286)	0.443	-	-
pT stage (1-2 vs. 3-4)	1.331 (1.490-2.125)	0.156	-	-
Venous invasion (yes vs. no)	0.046 (-0.885-3.135)	0.777	-	-
Lymphatic invasion (yes vs. no)	0.596 (-0.288-3.434)	0.933	-	-
Perineural invasion (yes vs. no)	0.578 (1.266-2.728)	0.643	-	-
Number of removed lymph nodes (<5 vs. 5-10 vs. <10)	1.458 (1.017-1.762)	0.566	-	-
Lymph node metastasis (yes vs. no)	1.623 (1.250-1.964)	0.848	-	-
Type of lymph node metastasis (micro vs. macro)	1.244 (0.442-1.144)	0.700	-	-
Number of metastatic lymph nodes (<5 vs. >5)	1.556 (2.653-3.778)	0.160	-	-
Lymph node pouch invasion (yes vs. no)	-0.249 (-0.963-2.496)	0.701	-	-
Distant metastasis (yes vs. no)	0.079 (-0.395-2.083)	0.937	-	-
Distant metastasis size, mm (<10 vs. >10)	0.140 (0.052-0.307)	0.850	-	-
Tumor deposits (yes vs. no)	-1.367 (-1.183-1.506)	0.435	-	-
Tumor budding (yes vs. no)	0.932 (-1.036-0.613)	0.049[a]	0.895 (-0.926-1.201)	0.093
IntraTANs (yes vs. no)	0.560 (1.212-1.587)	0.446	-	-
StromaTANs (low vs. high)	-0.946 (-1.013-1.706)	0.382	-	-
Necrosis (low vs. high)	-0.869 (-1.013-0.097)	0.553	-	-
IntraTANs/stromaTANs/necrosis (group 1-2 vs. 3-4)	1.577 (1.372-3.032)	0.014[a]	1.344 (1.235-3.015)	0.028[a]

[a]P<0.05. HR, hazard ratio; CI, confidence interval; TANs, tumor-associated neutrophils; non-muc, conventional carcinoma/adenocarcinoma; partim mucin, partim mucinous adenocarcinoma

studies found an association between a higher TAN count and the survival time of patients with cancers of the gastrointestinal tract (30,31). In the present study, only a slight tendency for a longer DFStime was observed in patients with a low stromaTAN infiltration. This is in contrast to the findings of Berry *et al* (32) who reported that patients with stage II disease and high TAN scores had a longer overall survival time as compared with that in patients with stage II disease and a low TAN score. In addition, Wikberg *et al* (33) demonstrated that poor infiltration of CD66b-expressing neutrophils at the invasive front of surgically resected CRC tumors was associated with a worse prognosis. All the aforementioned data prove that TANs may have a significant effect on tumor progression and patient prognosis.

The present study also determined tumor necrosis in the primary tumor tissues of patients with CRC. It was demonstrated that the majority of cases had tumors with <30% necrosis across the entire tumor area. Furthermore, it was revealed that the level of necrosis was associated with venous, lymphatic and perineural invasion, lymph node involvement, the number of invaded lymph nodes and the infiltrate of cancerous cells beyond the lymph node pouch, as well as with post-operative level of CEA in serum. These observations are reflected by the hypothesis whereby tumor necrosis in CRC results from rapid tumor cell invasion, the insufficient vascular supply of the tumor and the development of intratumoral hypoxia (34). Schneider *et al* (35) indicated that the extent of necrosis was found to be significantly associated with histopathological features of disease progression, such as lymph node metastases, TNM stage, poor tumor differentiation, a large tumor size and venous invasion. Moreover, Väyrynen *et al* (11) demonstrated that more extensive tumor necrosis in CRC was associated with higher stage tumors and more frequently with conventional carcinoma (adenocarcinoma). They also proved the lack of a connection between tumor necrosis and pre-operative treatment. This finding is in accordance with the findings of the present study, where no significant difference was found between the presence of tumor necrosis and patients who received pre-operative therapy. It has been demonstrated that short-term neoadjuvant chemo- and radiotherapies do not markedly modify the morphological appearance of the tumor tissue (36). The presence of tumor necrosis revealed in

Table VI. Univariate analysis of prognostic factors for 5-year disease-free survival time in patients with colorectal cancer.

Variable	HR (95%CI)	P-value
Age, years (≤60 vs. ≥60)	-1.023 (-0.095-0.259)	0.714
Sex (female vs. male)	0.274 (0.629-3.306)	0.849
Tumor growth (expanding vs. infiltrate)	-1.113 (-4.291-4.125)	0.300
Tumor size, cm <2.5 vs. 2.5-5 vs. >5	1.437 (2.758-3.976)	0.151
TNM stage (I-II vs. III-IV)	-0.883 (-0.705-1.215)	0.562
Adenocarcinoma type (non-muc vs. partim mucin)	0.798 (3.094-3.901)	0.428
Grade of malignancy (2 vs. 3)	-0.198 (-1.180-6.094)	0.846
Pre-operative treatment (yes vs. no)	-0.274 (0.125-0.356)	0.326
pT stage (1-2 vs. 3-4)	0.122 (2.669-3.590)	0.458
Venous invasion (yes vs. no)	-0.165 (3.718-7.466)	0.619
Lymphatic invasion (yes vs. no)	0.031 (-1.057-8.059)	0.895
Perineural invasion (yes vs. no)	0.807 (-8.567-6.800)	0.210
Number of removed lymph nodes (<5 vs. 5-10 vs. <10)	0.750 (3.401-4.463)	0.450
Lymph node metastasis (yes vs. no)	-0.318 (-2.875-4.565)	0.530
Type of lymph node metastasis (micro vs. macro)	-1.810 (-1.600-1.817)	0.383
Number of metastatic lymph nodes (<5 vs. >5)	0.204 (2.297-9.057)	0.801
Lymph node pouch invasion (yes vs. no)	-0.255 (1.427-6.800)	0.834
Distant metastasis (yes vs. no)	-1.399 (5.922-6.379)	0.355
Distant metastasis size, mm (<10 vs. >10)	1.056 (-1.314-1.361)	0.336
Tumor deposits (yes vs. no)	-0.652 (-2.044-2.210)	0.356
IntraTANs (yes vs. no)	0.674 (3.282-3.846)	0.395
StromaTANs (low vs. high)	-1.061 (-1.558-2.281)	0.495
Necrosis (low vs. high)	-0.796 (-2.833-4.140)	0.489
IntraTANs/stromaTANs/necrosis (group 1-2 vs. 3-4)	-0.886 (-3.041-3.040)	0.319

HR, hazard ratio; CI, confidence interval; TANs, tumor-associated neutrophils; non-muc, conventional carcinoma/adenocarcinoma; partim mucin, partim mucinous adenocarcinoma.

the histological examination may be reflected in the morphological parameters of whole blood. With respect to necrosis, the increased recruitment of inflammatory cells, such as neutrophils was observed. The accumulation of inflammatory cells in the tissue activates the production of these erythropoietic cells in the peripheral blood (37). In support of this hypothesis, in the present study, high levels of necrosis were found to be associated with morphological parameters measured following surgical treatment, including WBC, neutrophil count, and NLR-PLR and PLT-NLR status. It was also demonstrated that tumor necrosis was associated with MLR calculated from pre-operative whole blood parameters. Notably, previous studies have highlighted the potential prognostic value of tumor necrosis in the survival time of patients with CRC (13,38,39). In the present study, however, there was a trend for a shorter 5-year DFS time in patients with extensive tumor necrosis.

Previous studies have highlighted the role of immune cell infiltrates in CRC (23,40). To determine the role of neutrophils and necrosis, the present study examined the significance of these parameters in combination. Patients in groups 3 (high intraTANs, low stromaTANs and low necrosis) and 4 (high intraTANs, high stromaTANs and high necrosis) exhibited increased deep primary tumor invasion, and metastases to venous and lymphatic vessels. The results of the present study also indicated that the combined parametric value was significantly associated with serum CEA level prior to surgery. Furthermore, one of the most notable observations was the prognostic significance of this novel combined parameter. In the present study, it was established that patients in groups 1 (low intraTANs, low stromaTANs and low necrosis), 3 (high intraTANs, low stromaTANs and low necrosis) and 4 (high intraTANs, high stromaTANs and high necrosis) had significantly longer 3-year DFS rates compared with that in patientsin group 2 (low intraTANs, high stromaTANs and high necrosis). In addition, the analysis of the 3-year DFS rate revealed that the combined parametric value was an independent factor for patients with CRC.

The present study has certain limitations, which should be taken into consideration when interpretating the results. In the present study, neutrophils were only examined on the basis of morphological features and counted; however, it is strongly postulated that the performance of immunohistochemical staining is also required to reveal the phenotypical characterization of TANs.

In conclusion, the present study demonstrated that the examination of neutrophils and necrosis in the tumor tissue could improve the understanding of CRC pathogenesis.

Furthermore, it was found that the combined value of neutrophil infiltration and necrosis may be used as an independent factor for patients with CRC, and may be used in routine pathomorphological diagnostics in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KJ collected and analyzed the data, reviewed the literature and wrote, and revised the manuscript. MK and KL analyzed and interpreted the pathological results. WF and WK collected and analyzed data. MG wrote the paper, reviewed the literature and acquired the data. All authors have read and approved the final manuscript. KJ and MG confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of the Medical University of Bialystok, Poland.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. World Health Organization (WHO): Global Cancer Observatory. WHO, Geneva, 2020. https://gco.iarc.fr. Accessed November 10, 2020.
2. Høydahl Ø, Edna TH, Xanthoulis A, Lydersen S and Endreseth BH: Long-term trends in colorectal cancer: Incidence, localization, and presentation. BMC Cancer 20: 1077, 2020.
3. Mattiuzzi C, Sanchis-Gomar F and Lippi G: Concise update on colorectal cancer epidemiology. Ann Transl Med 7: 609, 2019.
4. American Cancer Society: Survival Rates for Colorectal Cancer. American Cancer Society, Atlanta, GA, 2020. https://www.cancer.org/camcer/colon-rectal-cancer/detection-diagnosis-staging/survival-rates.html. Accessed November 10, 2020.
5. Hamilton SR, Bosman FT and Boffetta P: Carcinoma of the colon and rectum. In: WHO Classification of Tumours of the Digestive System. Bosman FT, Carneiro F, Hruban RH and Theise ND (eds). IARC Press, Lyon, pp134-46, 2000.
6. Cammarota R, Bertolini V, Pennesi G, Bucci EO, Gottardi O, Garlanda C, Laghi L, Barberis MC, Sessa F, Noonan DM and Albini A: The tumor microenvironment of colorectal cancer: Stromal TLR-4 expression as a potential prognostic marker. J Transl Med 8: 112, 2010.
7. Park JH, McMillan DC, Powell AG, Richards CH, Horgan PG, Edwards J and Roxburgh CS: Evaluation of a tumor microenvironment-based prognostic score in primary operable colorectal cancer. Clin Cancer Res 21: 882-888, 2015.
8. Wang X, Qiu L, Li Z, Wang XY and Yi H: Understanding the multifaceted role of neutrophils in cancer and autoimmune diseases. Front Immunol 9: 2456, 2018.
9. Ullman TA and Itzkowitz SH: Intestinal inflammation and cancer. Gastroenterology 140: 1807-1816, 2011.
10. Rao HL, Chen JW, Li M, Xiao YB, Fu J, Zeng YX, Cai MY and Xie D: Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. PLoS One 7: e30806, 2012.
11. Väyrynen SA, Väyrynen JP, Klintrup K, Mäkelä J, Karttunen TJ, Tuomisto A and Mäkinen MJ: Clinical impact and network of determinants of tumour necrosis in colorectal cancer. Br J Cancer 114: 1334-1342, 2016.
12. Szabó C: Mechanisms of cell necrosis. Crit Care Med 33 (Suppl 12): S530-S534, 2005.
13. Pollheimer MJ, Kornprat P, Lindtner RA, Harbaum L, Schlemmer A, Rehak P and Langner C: Tumor necrosis is a new promising prognostic factor in colorectal cancer. Hum Pathol 41: 1749-1757, 2010.
14. Klarskov L, Holck S, Bernstein I, Okkels H, Rambech E, Baldetorp B and Nilbert M: Challenges in the identification of MSH6-associated colorectal cancer: Rectal location, less typical histology, and a subset with retained mismatch repair function. Am J Surg Pathol 35: 1391-1399, 2011.
15. Zhang X and Chen L: The recent progress of the mechanism and regulation of tumor necrosis in colorectal cancer. J Cancer Res Clin Oncol 142: 453-463, 2016.
16. Benson AB, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Engstrom PF, *et al*: NCCN guidelines insights: Colon cancer, version 2.2018. J Natl Compr Canc Netw 16: 359-369, 2018.
17. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
18. Hirahara T, Arigami T, Yanagita S, Matsushita D, Uchikado Y, Kita Y, Mori S, Sasaki K, Omoto I, Kurahara H, *et al*: Combined neutrophil-lymphocyte ratio and platelet-lymphocyte ratio predicts chemotherapy response and prognosis in patients with advanced gastric cancer. BMC Cancer 19: 672, 2019.
19. Jakubowska K, Koda M, Grudzińska M, Kańczuga-Koda K and Famulski W: Monocyte-to-lymphocyte ratio as a prognostic factor in peripheral whole blood samples of colorectal cancer patients. World J Gastroenterol 26: 4639-4655, 2020.
20. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F and Cree IA; WHO Classification of Tumours Editorial Board: The 2019 WHO classification of tumours of the digestive system. Histopathology 76: 182-188, 2020.
21. Lin Q, Wei Y, Ren L, Zhong Y, Qin C, Zheng P, Xu P, Zhu D, Ji M and Xu J: Tumor deposit is a poor prognostic indicator in patients who underwent simultaneous resection for synchronous colorectal liver metastases. Onco Targets Ther 8: 233-240, 2015.
22. Mayadas TN, Cullere X and Lowell CA: The multifaceted functions of neutrophils. Annu Rev Pathol 9: 181-218, 2014.
23. Harbaum L, Pollheimer MJ, Kornprat P, Lindtner RA, Bokemeyer C and Langner C: Peritumoral eosinophils predict recurrence in colorectal cancer. Mod Pathol 28: 403-413, 2015.
24. Jakubowska K, Koda M, Kisielewski W, Kańczuga-Koda L and Famulski W: Prognostic significance of inflammatory cell response in patients with colorectal cancer. Oncol Lett 18: 783-791, 2019.
25. Gao JF, Arbman G, Wadhra TI, Zhang H and Sun XF: Relationships of tumor inflammatory infiltration and necrosis with microsatellite instability in colorectal cancers. World J Gastroenterol 11: 2179-2183, 2005.
26. Ling YH, Chen JW, Wen SH, Huang CY, Li P, Lu LH, Mei J, Li SH, Wei W, Cai MY and Guo RP: Tumor necrosis as a poor prognostic predictor on postoperative survival of patients with solitary small hepatocellular carcinoma. BMC Cancer 20: 607, 2020.
27. Liu X, Jiang C, Zhang D, Gao M, Peng F, Huang D, Sun Z, Ni Y, Zhang J and Yin Z: Tumor necrosis targeted radiotherapy of non-small cell lung cancer using radioiodinated protohypericin in a mouse model. Oncotarget 6: 26400-26410, 2015.

28. Whalen GF: Solid tumours and wounds: Transformed cells misunderstood as injured tissue? Lancet 336: 1489-1492, 1990.
29. Ye L, Zhang T, Kang Z, Guo G, Sun Y, Lin K, Huang Q, Shi X, Ni Z, Ding N, *et al*: Tumor-infiltrating immune cells act as a marker for prognosis in colorectal cancer. Front Immunol 10: 2368, 2019.
30. Galdiero MR, Bianchi P, Grizzi F, Di Caro G, Basso G, Ponzetta A, Bonavita E, Barbagallo M, Tartari S, Polentarutti N, *et al*: Occurrence and significance of tumor-associated neutrophils in patients with colorectal cancer. Int J Cancer 139: 446-456, 2016.
31. Kuang DM, Zhao Q, Wu Y, Peng C, Wang J, Xu Z, Yin XY and Zheng L: Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. J Hepatol 54: 948-955, 2011.
32. Berry RS, Xiong MJ, Greenbaum A, Mortaji P, Nofchissey RA, Schultz F, Martinez C, Luo L, Morris KT and Hanson JA: High levels of tumor-associated neutrophils are associated with improved overall survival in patients with stage II colorectal cancer. PLoS One 12: e0188799, 2017.
33. Wikberg ML, Ling A, Li X, Oberg A, Edin S and Palmqvist R: Neutrophil infiltration is a favorable prognostic factor in early stages of colon cancer. Hum Pathol 68: 193-202, 2017.
34. Swinson DE, Jones JL, Richardson D, Cox G, Edwards JG and O'Byrne KJ: Tumour necrosis is an independent prognostic marker in non-small cell lung cancer: Correlation with biological variables. Lung Cancer 37: 235-240, 2002.
35. Schneider NI and Langner C: Prognostic stratification of colorectal cancer patients: Current perspectives. Cancer Manag Res 6: 291-300, 2014.
36. O'Neil M and Damjanov I: Histopathology of colorectal cancer after neoadjuvant chemoradiation therapy. Open Pathol J 3: 91-98, 2009.
37. Bredholt G, Mannelqvist M, Stefansson IM, Birkeland E, Bø TH, Øyan AM, Trovik J, Kalland KH, Jonassen I, Salvesen HB, *et al*: Tumor necrosis is an important hallmark of aggressive endometrial cancer and associates with hypoxia, angiogenesis and inflammation responses. Oncotarget 6: 39676-39691, 2015.
38. Richards CH, Roxburgh CS, Anderson JH, McKee RF, Foulis AK, Horgan PG and McMillan DC: Prognostic value of tumour necrosis and host inflammatory responses in colorectal cancer. Br J Surg 99: 287-294, 2012.
39. Komori K, Kanemitsu Y, Kimura K, Hattori N, Sano T, Ito S, Abe T, Senda Y, Misawa K, Ito Y, *et al*: Tumor necrosis in patients with TNM stage IV colorectal cancer without residual disease (R0 Status) is associated with a poor prognosis. Anticancer Res 33: 1099-1105, 2013.
40. Jakubowska K, Kisielewski W, Kańczuga-Koda L, Koda M and Famulski W: Diagnostic value of inflammatory cell infiltrates, tumor stroma percentage and disease-free survival in patients with colorectal cancer. Oncol Lett 14: 3869-3877, 2017.