Diagnostic value of inflammatory cell infiltrates, tumor stroma percentage and disease-free survival in patients with colorectal cancer

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Abstract. The anticancer immune defense mechanism involves humoral and cellular responses. The main effector mechanisms of antitumor responses involve the following: the activity of cytotoxic T cells; the activation of macrophages and neutrophils; the activity of cytokines secreted by T cells; and natural killer cell activity. Selected cell populations are responsible for the stimulation or suppression of the immune system against tumor cells. Therefore, the aim of the present study was to evaluate the location, extent and composition of the cellular inflammatory infiltration of tumors in patients with colorectal cancer (CRC). In addition, the correlation between cellular inflammatory infiltration, and anatomoclinical and histopathological features of patients was evaluated. The study involved 160 patients diagnosed with primary operable CRC. The local inflammatory infiltrate was assessed in the invasive front and center of the tumor using light microscopy with hematoxylin and eosin (H&E) staining, according to the Klintrup-Makinen criteria, tumor stroma percentage, and Glasgow microenvironment score. The inflammatory infiltrate in the invasive front of the tumor was correlated with gender (P=0.018), the invasion of blood vessels (P=0.020) and lymph vessels (P=0.038), the presence of tumor-infiltrating lymphocytes in the invasive front (P=0.033) and center (P<0.001) of the tumor, fibrosis (P<0.001), and the degree of desmoplasmic stroma (P=0.004). In contrast, inflammatory infiltration in the center of the tumor was associated with the tumor node metastasis stage (P=0.012), Dukes' stage (P=0.009), primary tumor stage (P=0.036), lymph node status (P=0.005), number of lymph nodes (P=0.006), invasion of lymph node pouches (P=0.021), size of lymph node metastasis (P=0.025) and the degree of desmoplasmic stroma (P=0.002). The low-group, who demonstrated an absent or weak inflammatory cell infiltrate in the invasive front of the tumor, had a statistically significant shorter disease-free survival (DFS) time (P=0.004). Inflammatory cell infiltrate in the invasive front was identified as an independent predictive factor in CRC (P=0.041). In conclusion, the degree of inflammatory cell infiltration in the invasive front of the primary tumor significantly affects various variables that determine disease progression and DFS rates of patients with CRC. Furthermore, the routine histopathological assessment of this parameter in tissue stained with H&E may have potential prognostic value.

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Introduction

Colorectal cancer (CRC) is the second-leading death-related malignant tumor in Poland in both sexes (1). The prognosis of patients is performed by staging of primary tumor and the involvement of metastases in the lymph nodes and distant organs by standard classification of the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) classification based upon the tumor-node-metastasis (TNM) (2). However, recent reports in the literature indicated that a considerable heterogeneity of the primary tumor of colon cancer requires a more detailed qualitative analysis, all of its components such as inflammatory response and connective tissue stroma (3,4).

Vascular connective tissue is an important part of the tumor which forms its framework. It transfers the nutrients into proliferating, neoplastic cells. Due to certain elements, the stromal tumor retains its integrity and has the ability to increase as a destructive parasite on the host organism. The production of biologically active compounds can be done during the interaction between the structural elements of the stroma such as inflammatory cell infiltration and cancer cells (5). It has been shown that the tumor stroma including fibroblasts, endothelial cells, and inflammatory

cells play an important role in promoting the progression of the disease (6,7). Desmoplasic stromal reaction (DR) is a poor prognostic factor for patients with CRC. Moreover, the appearance of liver metastases is accompanied by a large DR with SMA-positive myofibroblasts (8,9). Furthermore, Conti *et al* (10) found that DR stimulates the growth of primary tumor and decreases the chemosensitivity of CRC metastasis in the liver.

Tumor stroma, as myofibroblasts, may affect the organization of the inflammatory response. Dysregulation of the immune response is already visible in the early stages of precancerous adenoma-colorectal carcinoma sequence in which decrease in the activity of Th1 cells is observed (11). By contrast, the development of CRC on the basis of inflammatory bowel disease is characterized by the stimulation of immune responses by $CD3^+$ T-cells (12). Recent reports showed that both the inflammatory cell infiltration and tumor stroma affect the development and progression of CRC (13). Therefore, the aim of our study was to assess inflammatory cell infiltrate in the invasive front and in the center of primary tumor mass, and tumor stroma percentage (TSP) in correlation with anatomo-clinical features of CRC patients.

Materials and methods

Patients. The study group consisted of 160 patients diagnosed with colorectal carcinoma (female, 56, male, 88) and operated on at the Department of Oncological Surgery, in the Comprehensive Cancer Center of Bialystok, during years 2014-2016. The data collection procedures and statistical analysis were designed before the collection of study material had started. The mean age was 67.5 years, including 40 patients under 60 years of age and 120 patients over 6 decades of life. Mostly, patients were complained about abdominal ache, anemia and bleeding from rectum. Family's medical history of malignant neoplasms was noted in 16 out of 160 cases. Patients were taking medicine against hypertension, type II diabetes, osteoarthritis and coronary heart disease in most of cases. We excluded patients with clinical evidence of active infection and/or chronic inflammatory condition. Colonoscopy examination was performed in 62 cases that confirmed the presence of cancerous infiltrate in the intestinal wall. Macroscopically, cancerous infiltrate was limited to the gut wall in 69 cases, exceeded the wall focally in 17 cases and continuously in 74 cases.

All patients, during routine diagnostics, underwent a basic diagnostic laboratory examination, ECG, spirometry, arterial blood gasometric study and X-ray and computerized tomography of the chest. The clinical efficiency was performed by 5-point scale of Zubroda (WHO) (14). The clinical staging of CRC was evaluated according to TNM classification (2). Patients diagnosed with neoplasms in rectum received preoperative therapy (N=53). Patients received radiotherapy (N=39), chemotherapy (N=7) and radio-chemotherapy (N=7). They took a dose of 25 Gy in fractions of 5 Gy during one week in the pelvic area. Patients with tumors situated on other localization had received neither inflammatory nor immunosuppressive therapy. The response to preoperative therapy was estimated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (15).

The 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). Written informed consent was obtained from all participants.

Histopathological examination of CRC tumor. Sections, 4 μm-thick, were cut from paraffin blocks and stained with hematoxylin and eosin (H&E) (cat no. 468802128; POCH S.A., Poland). The routine histopathological assessment of the sections referred to type of tumor growth, tumor size, histological type and percentage of the mucinous component, grade of malignancy, pTNM and Duke stages. We also analyzed venous, lymphatic and perineural invasions, characteristic features of lymph node invasion such as number of resected and invaded lymph nodes, the presence of micro- and macrometastases, invasion of the pouch lymph node; presence of the distant metastases and their size in millimetre. We also assessed the presence of deposits, their number and size in millimetre (16). We analyzed tumor budding according to Morodomi *et al* (17). The extent of necrosis and fibrosis in the central tumor was evaluated according to Richards *et al* (18) and graded as 'absent' (none), 'focal' (<10% of tumor area), 'moderate' (10-30%) or 'extensive' (>30%). Crohn's-like aggregates of lymphocyte (CRL) were performed in the basis of Väyrynen's *et al* criteria (19). Histological categorization of fibrotic cancer stroma was performed based on classification described by the Ueno *et al* (20).

Examination of inflammatory cell infiltration, TSP and Glasgow microenvironment score (GMS). The inflammatory cell infiltrates were assessed according to Klintrup-Makinen (K-M) (21) criteria and performed by two independent pathologists who have been blinded to the clinical information. Briefly, inflammatory reaction in the invasive margin and centre of tumor were scored on 4-point scale where score '0' defined no increase in inflammatory cell infiltrate; score '1' defined a mild or patchy increase; score '2' denoted a prominent inflammatory reaction with some evidence of cancer cell destruction and score '3' denoted florid 'cup-like' inflammatory infiltrate. The inflammatory cell infiltrate were classified into low-group (score 0-1) and high-group (score 2-3). Invasive front of tumor was defined as the most progressed few cancer cells localized on the advanced edge of tumor. We assessed the TSP according to criteria described by Huijbers *et al* (22). TSP ratio was dived into two groups: 'high TSP group' (>50%) and 'low TSP group' (≤50%). We also analyzed the GMS (4) based on the K-M grade and TSP. Patients were characterized as having, i) good prognosis (high score of K-M and any TSP score); ii) intermediate prognosis (low K-M score and low TSP score) and iii) poor prognosis (low K-M score and high TSP score).

Follow-up. Patients were followed-up during last 2-2.5 years. They were monitored by the measurement of carcinoembryonic antigen (CEA) and CA19-9 levels, 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 pathologic evident of the spread

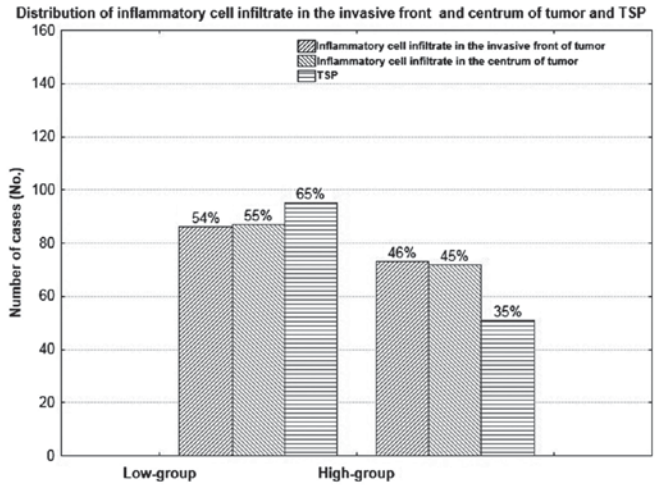

Figure 1. Distribution of inflammatory cell infiltration in the invasive front, in centre of primary tumor mass and TSP in low and high group of patients with colorectal cancer. TSP, tumor stroma percentage.

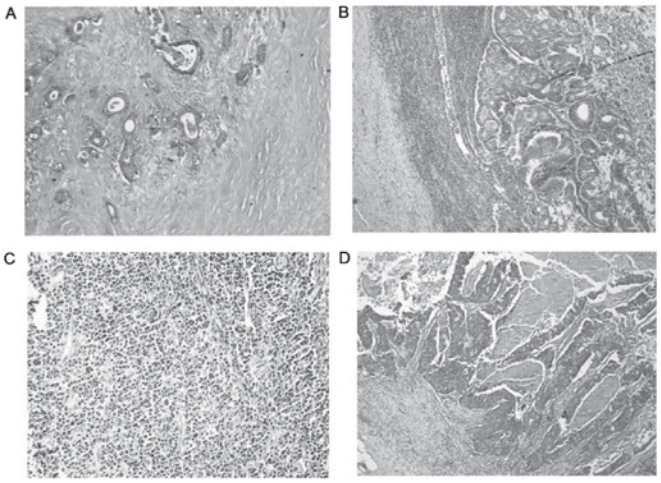

Figure 2. Examination of inflammatory cell infiltrate in colorectal cancer tissue. (A) Low-group: Lack of inflammatory response in the invasive front of tumor, (B and C) High-group: Strong infiltration of mononuclear cells in the tumor margin, (D) low-group: Weak inflammatory infiltrate in the centrum of the main mass associated with necrosis (H&E stain). H&E, hematoxylin and eosin.

of tumors in the region of the anastomosis (local recurrence) or/and present outside of the primary tumor at other sites such as liver, lungs, bones, brain (distant recurrence) and confirmed by mentioned above techniques.

Statistical analysis. Statistical analysis was conducted using the STATISTICA 10.0 program (StatSoft, Kracow, Poland). Mann-Whitney U-test was use to compare the groups. Correlations between the parameters were calculated by the Spearman's correlation coefficient tests. Disease-free survival (DFS) was calculated from the date of diagnosis to the date of disease progression (local or distant relapse). DFS were estimated using Kaplan Meier method and the survival curves were compared using log-rank tests. Multivariate Cox proportional hazards models were used to estimate hazard ratios. A P-value of <0.05 was considered statistically significant.

Results

Distribution of inflammatory cell infiltrates and characteristics of TSP and GMS in CRC. The inflammatory cell infiltrate in the invasive front of tumor was low in 86 (54%) and high in 73 (46%) of cases, and was similar to those observed in the centrum of tumor mass. Low-group of TSP was present in 95 (65%) cases in comparison with 51 (35%) cases observed in high TSP group (Figs. 1-3). Examined group of parameters did not differ significantly (P=0.059; P=0.065; P=0.910). Patient prognosis, based on GMS was: good in 55, intermediate in 49 and poor in 56 cases.

Inflammatory cell infiltrates in the invasive front of CRC and its correlation with anatomoclinical variables. Inflammatory cell infiltrate in the invasive front was found to correlate negatively with female (P=0.018, R=-0.197), venous and lymphatic invasion (P=0.020, R=-0.193; P=0.038, R=-0.173, respectively), invasion of lymph node pouch (P=0.020, R=-0.212), TSP (P=0.015, R=-0.212) and the stage of fibrosis (P<0.000, R=-0.293). The increase of the inflammatory cell infiltrate in the invasive front of tumor was

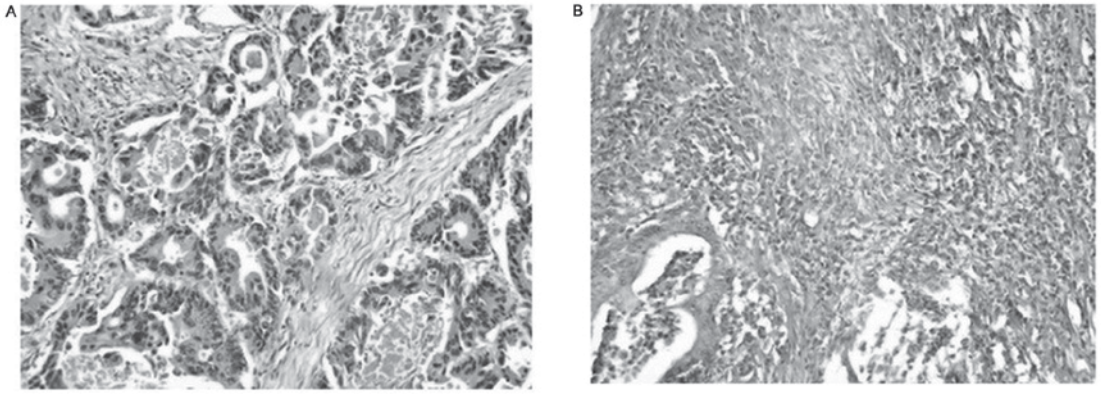

Figure 3. Assessment of TSP based on H&E staining. (A) Low-TSP group presents less than 50% tumor stroma with strong inflammatory cell infiltrate in the centrum of primary tumor mass. (B) Sections with high-TSP group are characterized by undifferentiated fibrotic stroma and lack of inflammatory cells in most of cases. TSP, tumor stroma percentage; H&E, hematoxylin and eosin.

Table I. Correlation between inflammatory cell infiltration in the invasive front and main mass of primary tumor and anatomo-clinical variables of colorectal cancer.

Variables	N	Inflammatory cell infiltration in the invasive front of tumor		Inflammatory cell infiltration in the center of tumor mass	
	160	R	P-value	R	P-value
Age					
<60	40	NS	NS	NS	NS
>60	120				
Gender					
Female	64	**-0.197**	**0.018**	NS	NS
Male	96				
Localization					
Right-side	20	NS	NS	NS	NS
Transverse	14				
Left-side	15				
Sigmoid	29				
Rectum	82				
Tumor growth					
Expanding	133	NS	NS	NS	NS
Infiltrate	27				
Tumor size, cm					
<2.5	27	NS	NS	NS	NS
2.5-5.0	106				
>5.0	27				
TNM stage					
1	42	NS	NS	**-0.200**	**0.012**
2	31				
3	69				
4	18				
Duke stage					
A	39	NS	NS	**-0.218**	**0.009**
B	35				
C	69				
D	17				
Adenocarcinoma type					
Partim muc	30	NS	NS	NS	NS
Nonmuc	130				
Percentage of mucinous component					
10-30%	15	NS	NS	NS	NS
30-50%	15				
Grade of malignancies					
2	148	NS	NS	NS	NS
3	12				
Preoperative treatment					
Yes	53	NS	NS	NS	NS
No	107				
Treatment response					
SD	26	NS	NS	NS	NS
PR	27				

Table I. Continued.

Variables	N 160	Inflammatory cell infiltration in the invasive front of tumor		Inflammatory cell infiltration in the center of tumor mass	
		R	P-value	R	P-value
pT stage					
1	3	NS	NS	**-0.175**	**0.036**
2	62				
3	91				
4	4				

Bold numbers indicate values considered to be statistically significant. Spearman's correlation coefficient test; NS, not statistically significant.

Table II. Correlation between inflammatory cell infiltration in the invasive front and main mass of primary tumor and morphological variables of colorectal cancer.

Variables	N 160	Inflammatory cell infiltration in the invasive front of tumor		Inflammatory cell infiltration in the center of tumor mass	
		R	P-value	R	P-value
Venous invasion					
Absent	113	**-0.193**	**0.020**	NS	NS
Present	46				
Lymphatic invasion					
Absent	121	**-0.173**	**0.038**	NS	NS
Present	38				
Perineural invasion					
Absent	143	NS	NS	**-0.191**	**0.022**
Present	17				
No. of removed lymph nodes					
<5	13	NS	NS	NS	NS
5-10	29				
>10	116				
Lymph node metastasis					
Absent	81	NS	NS	**-0.230**	**0.005**
Present	79				
Type of lymph node metastasis					
Micro	27	NS	NS	**-0.198**	**0.017**
Macro	52				
Number of metastatic lymph nodes					
<5	49	NS	NS	**-0.323**	**0.012**
>5	26				
Lymph node pouch invasion					
Absent	11	**-0.212**	**0.010**	**-0.191**	**0.021**
Present	68				
Distant metastasis					
Absent	143	NS	NS	NS	NS
Present	17				

Table II. Continued.

Variables	N 160	Inflammatory cell infiltration in the invasive front of tumor		Inflammatory cell infiltration in the center of tumor mass	
		R	P-value	R	P-value
Distant metastasis size (mm)					
<10	11	NS	NS	NS	NS
>10	6				
Tumor deposits					
Absent	133	NS	NS	NS	NS
Present	27				
Size of tumor deposits (mm)					
<2.5	10	NS	NS	NS	NS
>2.5	17				
TSP (%)					
<50	94	**-0.212**	**0.015**	NS	NS
>50	66				
Tumor budding					
Absent	94	NS	NS	NS	NS
Present	66				
Crohn's-like aggregates of lymphocyte					
Absent	113	NS	NS	**0.195**	**0.019**
Present	42				
Necrosis					
Absent	45	NS	NS	NS	NS
Focal	61				
Moderate	36				
Extensive	18				
Fibrosis					
Absent	11	**-0.293**	**0.000**	NS	NS
Focal	72				
Moderate	43				
Extensive	34				
Maturation of fibrotic stroma					
Immature	12	**0.238**	**0.004**	**0.256**	**0.002**
Intermediate	91				
Mature	57				

Bold numbers indicate values considered to be statistically significant. Spearman's correlation coefficient test; NS, not statistically significant; TSP, tumor stroma percentage.

associated with increase of stromal maturation (P=0.004, R=0.238) (Tables I and II).

Inflammatory cell infiltrates in the centrum of the mass of CRC in correlation with anatomoclinical variables. Inflammatory cell infiltrate in the centrum of the tumor mass was associated with parameter response for disease progression. Inflammatory cell infiltrate in this localization in tumor was negatively correlated with TNM and Duke stage (P=0.012, R=-0.200; P=0.009, R=-0.218), pT stage (P=0.036, R=-0.175), invasion of perineural structures (P=0.022, R=-0.191), lymph node status (P=0.005, R=-0.230), type of lymph nodes (P=0.017, R=-0.198), number of metastatic lymph nodes (P=0.012, R=-0.323) and the invasion of lymph node pouches (P=0.021, R=-0.151). Cronh's-like aggregates of lymphocyte and maturation of fibrotic stroma were positively associated with the increase of inflammatory cell infiltrate in the centre of tumor mass (P=0.019, R=0195; P=0.002,

Table III. Prognostic factors in patients with CRC.

Variables	Univariate p-value	Multivariate p-value	HR (95% CI)
Age (≤60 vs. ≥60)	0.059	-	1.21 (0.36-1.53)
Gender (female vs. male)	0.597	-	2.19 (1.88-3.53)
Tumor growth (expanding vs. infiltrate)	0.288	-	1.68 (1.06-1.90)
Tumor size (<2.5 vs. 2.5-5 vs. >5 cm)	0.349	-	0.77 (0.27-.87)
TNM stage (I-IV)	0.258	-	0.85 (0.13-1.27)
Duke stage (A-D)	0.628	-	1.22 (0.42-1.45)
Adenocarcinoma type (nonmuc. vs. partim mucin)	0.359	-	0.62 (0.51-0.84)
Grade of malignancies (2 vs. 3)	0.220	-	0.39 (0.23-1.49)
Preoperative treatment (yes vs. no)	**0.048**	0.784	1.05 (0.75-1.52)
pT stage (1-4)	0.674	-	1.00 (0.17-.1.2)
Venous invasion (yes vs. no)	0.109	-	2.72 (1.62-2.93)
Lymphatic invasion (yes vs. no)	0.149	-	0.36 (0.23-2.08)
Perineural invasion (yes vs. no)	0.121	-	2.30 (1.54-2.40)
No. of removed lymph nodes (<5 vs. 5-10 vs. <10)	0.816	-	0.94 (0.05-1.42)
Lymph node metastasis (yes vs. no)	0.079	-	1.47 (0.92-3.07)
Type of lymph node metastasis (micro vs. macro)	**0.039**	0.103	1.21 (0.19-2.65)
Number of metastatic lymph nodes (<5 vs. >5)	0.951	-	0.96 (0.61-1.22)
Lymph node pouch invasion (yes vs. no)	0.374	-	0.55 (0.45-0.78)
Distant metastasis (yes vs. no)	0.702	-	0.96 (0.14-1.23)
Distant metastasis size <10 vs. >10 mm	0.637	-	1.05 (0.22-1.24)
Tumor deposits (yes vs. no)	0.099	-	0.53 (0.37-2.72)
Tumor budding (yes vs. no)	0.267	-	0.65 (0.47-0.77)
Number of tumor budding	**0.025**	0.059	1.05 (0.57-3.53)
Fibrosis (low vs. high)	0.524	-	1.25 (0.40-1.48)
Necrosis (low vs. high)	0.615	-	0.84 (0.25-1.12)
Maturation of tumor stroma (low vs. high)	0.471	-	0.83 (0.51-1.26)
Inflammatory cell infiltrate in the invasive front of tumor (present vs. absent)	**0.037**	**0.041**	0.50 (0.33-4.14)
Inflammatory cell infiltrate in center of tumor (present vs. absent)	0.733	-	0.92 (0.23-1.56)
TSP (low vs. high)	0.054	-	0.46 (0.35-4.66

Bold numbers indicate values considered to be statistically significant.

R=0.256, respectively). Results of correlation are showed in Tables I and II.

Inflammatory cell infiltrates TSP and GMS in CRC DFS. Low-group who showed an absent or weak inflammatory cell infiltrate in the invasive front of the tumor had statistically significant shorter DFS (P=0.004). The 1-year and 2-year DFS of the low group with inflammatory cell infiltrate in the invasive front of the tumor were 64 and 51%, whereas patients with high inflammatory cell infiltrate had 1- and 2-year DFS of 83 and 80%. DFS did not differ in the inflammatory cell infiltrate in the centrum of the tumor mass, TSP and GMS (P=0.252, P=0.447, P=0.902, P=0.418) (Fig. 4). Low TSP group had 1-year DFS of 76% and 2-year DFS of 56%, whereas patients with high TSP group had 1-year DFS of 78% and 2-year DFS of 35%. Univariate analysis showed that preoperative treatment (P=0.048), type of lymph nodes (P=0.039), number of tumor deposits (P=0.025) have prognostic values. Moreover, the multivariate Cox-analysis proved that inflammatory cell infiltrate in the invasive front was an independent predictive factors in CRC (P=0.041) (Table III).

Discussion

Inflammatory infiltration located in both the invasive front and in the center of the primary tumor may play a significant role in the development of malignant tumors. In our study, we noted the lack of weak inflammatory infiltration in the invasive front and in center of the tumor in 50% of cases comparable to a medium or large infiltration in both locations (45-46%). Richards *et al* (18) reported a low-grade inflammatory cell infiltrate in 48% and high-grade inflammatory cell infiltrate in 52% cases in peritumoral stroma. We also noted low-TSP in 65% of cases and high-TSP in 35% of cases. Also,

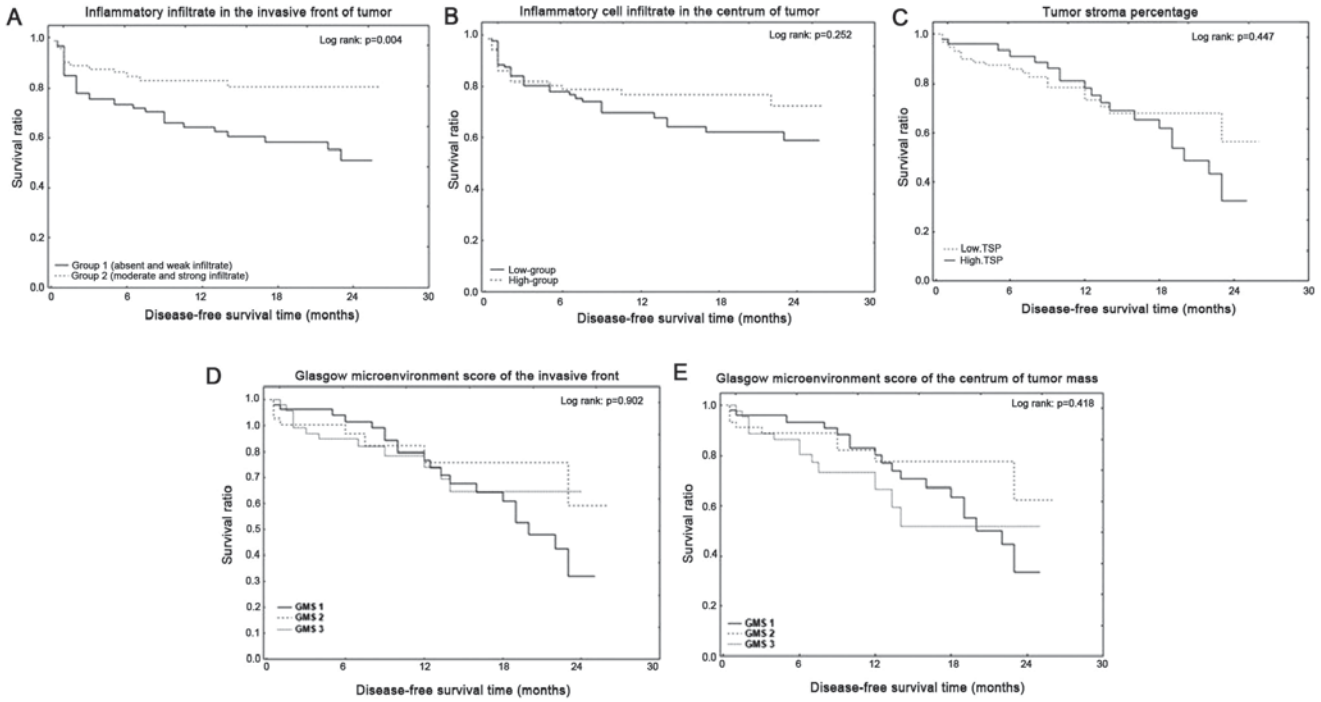

Figure 4. Postoperative disease-free survival of patients with colorectal cancer. Kaplan Meier curves of inflammatory cell infiltration in the invasive front (A) and in the main mass of primary tumor (B), tumor stroma percentage (C), Glasgow Microenvironment Score of the invasive front (D) and centre of primary tumor (E).

Park *et al* (23) observed that the TSP was low in 75% of cases and high in 25% of cases. These observations confirmed that the presence of inflammatory infiltrate may be different in the cases of CRC patients. Probably, it is determined by the activity of the immune system, the speed of its reorganization during detection of tumor-associated antigen (TAA), the preoperative treatment modulating pathway of inflammatory response or the ability of tumor cells to produce specific antigens directly blocking immunocompetitive cells.

Inhibition or impartation of the inflammatory response allows malignant tumor cells to invade into the tissue. We confirmed such observation by correlations, in which together with the decrease in the inflammatory response increased tumor stage, TNM, and Duke's stage, including the primary tumor stage (pT), the presence of tumor cell emboli in blood and the lymphatic vessels, in perineutral spaces. Moreover, the degree of inflammatory infiltration was negatively correlated with the the presence of lymph node metastasis, its size, exceeding the lymph pouches and infiltration structures near to metastatic lymph nodes. Our results are consistent with the observations of Galon *et al* (12), Menon *et al* (24) and Väyrynen *et al* (25). They confirmed that patients with high TNM stage linked with presence of distant metastases were correlated with lower immune response. Moreover, authors showed that peritumoral inflammatory cell infiltrate was higher in advanced stage than in intratumoral densities. Also, Richards *et al* (18,26) demonstrated the presence of the relationship between the inflammatory cell infiltrate and pT status, positive lymph node status, TNM stage, venous invasion, necrosis and character of tumor growth.

Several studies confirmed an association between tumor inflammatory infiltrates and survival of patients with malignant neoplasms (27-29). In our study, we showed that patients with low inflammatory cell infiltrate located in the invasive front had a shorter DFS), which was 64% after 12 months and 51% after 24 months after the surgery. Mei *et al* (13) showed that a high level of $CD3^+$ cells in the invasive front was associated with good overall survival (OS) and DFS. Moreover, a high level of $CD8^+$ cells, but not $CD3^+$ or $FOXP3^+$ was correlated with better prognosis and longer OS. Also, Väyrynen *et al* (25) confirmed the relationship between the degree of inflammatory infiltration assessed in the basis of M-K criteria and the occurrence of relapse in patients with CRC. We also analyzed the relationship between TSP, GMS and DFS of patients with CRC. Patients with low-TSP had 1-year DFS of 76% whereas the 2-year DFS of patients with high TSP was 56%. Unfortunately, the differences were not statistically significant, in contrast to the results of Park *et al* (23) who reported a shorter cancer-specific survival (CSS) in CRC patients in stage I-III of the high-TSP compared to those in which low-TSP was found. In subsequent studies, the author confirmed that the 5-year survival of low TSP was 80% and in the high TSP group in 90% (4). We also assessed the overall parameters of inflammatory cell infiltrate and TSP by GMS, which did not confirmed statistically significant differences in DFS. Our observations are contrary to the results of Park *et al* (4). These differences may be due to sample size, nationality of the selected population and the scope of the TNM staging of patients enrolled in the study.

Multivariate analysis showed that the inflammatory cell infiltrate in the invasive front of the primary tumor is an independent prognostic factor in patients with CRC. Richards *et al* (26) presented that a low grade of local immune

response, TNM, venous invasion were associated indecently with reduced CSS. On the other hand, Park *et al* (23) demonstrated that low TSP in stage I to III of patients with CRC is associated with N0 status and those who received adjuvant chemotherapy had reduced CSS. It seems that the inflammatory cell infiltration is a very important part of the tumor, which, along with routinely assessed morphological, may provide additional prognostic factor in patients with CRC.

In conclusion, the degree of inflammatory cell infiltration in the invasive front of the primary tumor and especially TSP of patients with CRC affects significantly the variables that determine disease progression and DFS. Moreover, the routine, histopathological assessment of both parameters in the basis of tissue material stained with H&E may have potential diagnostic and prognostic values.

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