Correlation of clinico-pathologic data with inflammatory cells infiltration in colorectal cancer

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ABSTRACT

Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide. At every phase of cancer development, the inflammatory process has an important impact. Accurate assessment inflammatory cells in the tumour environment in conjunction with clinico-pathologic features can be a relevant prognostic or predictive parameter.
Purpose: To analyse inflammatory cell infiltration in CRC tumour mass and correlate with chosen clinico-pathologic parameters.
Materials and methods: The study group consisted of 160 patients (64 women, 96 men) diagnosed with colorectal cancer who underwent surgery. Tissue material obtained from routine histopathological diagnosis was stained with H&E and used to assess the type of inflammatory cells in the invasive front and centre of the tumour. Results were subjected to statistical analysis with the age and gender of patients, tumour localization, tumour growth and size, TNM stage, adenocarcinoma type, fibrosis, necrosis, metastasis and tumour invasion (by the Spearman's correlation coefficient test).
Results: The presence of neutrophils in the invasive front of tumour mass was associated with fibrosis and inflammatory cell infiltration in the invasive front of tumour. Macrophages in the invasive front of tumour were found to correlate with tumour growth (expanding and infiltrate). Macrophages and eosinophils were associated with inflammatory cell infiltration in the invasive front and in the centre of tumour.
Conclusions: The type of inflammatory cells in the invasive front or centre of the tumour may be useful to prognoses clinical features of colorectal cancer.
Keywords: Colorectal cancer, inflammatory cells, fibrosis

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INTRODUCTION

Colorectal cancer (CRC) was the third most common cancer worldwide, representing 10.6% of the total number of new cases diagnosed in 2018. Despite therapeutic progress it causes more than 500000 deaths every year [1].

Currently, prognostic factors in colorectal cancer include TNM classification (T - size of the **t**umour, N - involved lymph **n**odes, M – **m**etastasis), tumour grade, lymph node involvement, tumour expanding, lymphatic invasion, angiogenesis, furthermore genetic factors (including the presence of mutations in the KRAS, NRAS, BRAF gene) [2–4]. The TNM classification is frequently used for prognosis, although even patients with tumours of the same disease stage can have very different remote clinical outcome [5]. It seems reasonable to look for new prognostic or predictive markers in CRC, which could help to guide treatment decisions.

Tumour mass is comprised of not exclusively malignant cells but either many other non-malignant cell types such as miscellaneous infiltrating immunological cells. The main purpose of the inflammatory reaction is to defend the body against pathogens and to repair tissues that have been damaged. The cells of the immune system migrate to damaged tissues where the infiltration is formed. In the last decades, the interaction between tumours and the immunology system became important aspect of tumour biology. Therefore, it is essential to search for correlation between inflammatory cells infiltration in tumour mass and development and invasiveness of this cancer.

The tumour microenvironment, as already mentioned, is formed by many distinct cell populations, including immune cells. At every stage of cancer development, the inflammatory process has a significant impact [6]. It is well known that during carcinogenesis, the inflammatory response is impaired. This is due to the low antigenic immunogenicity of tumour cells [7]. Thorough identification of inflammatory cells in the tumour environment in conjunction with clinical targets can be an important prognostic or predictive parameter. In CRC tumour microenvironment, the inflammatory infiltration consists of cells such as neutrophils, monocytes or eosinophils [8].

Neutrophils are the main element of the inflammatory response. In CRC, the role of neutrophils infiltration in patient prognosis has been the subject of arguable discussion. Whereas some researchers indicate the protective role of neutrophils, others show their aggravating function [9,10]. Macrophages in tumour mass frequently constitute a meaningful part of infiltrating immune cells. Macrophages can contribute to cancer promotion by producing angiogenic factors, growth factors, reactive oxygen and nitrogen radicals [11]. Eosinophils are usually associated with allergic diseases or parasitic infections [12]. Some types of cancer are also associated with eosinophilia, within the tumour or circulating in the blood. A number of earlier studies has demonstrated the biological role of macrophages and eosinophils in the modulation of tumour growth [13,14].

In the present study, routine histopathological preparations were used to detect inflammatory cells in the invasive front and centre of the tumour. The aim was to examining the correlation of inflammatory cells infiltration in the invasive front of tumour mass to patients' clinicopathologic features and disease-free survival (DFS).

MATERIALS AND METHODS

The present study was performed in accordance with the Declaration of Helsinki for Human Experimentation, and the protocol was approved by the Bioethics Committee of the Medical University of Bialystok (approval no. R-I-002/351/2016). Written informed consent was obtained from all participants.

Patients

The present study included 160 patients diagnosed with CRC, underwent surgery in the Oncological Surgery Department (Oncology Centre in Bialystok, Poland) between April 2014 and December 2016. Some medical information was collected before surgery. All patients received routine diagnostic tests, including basic diagnostic laboratory tests (blood panel and lipid profile), electrocardiography, spirometry, arterial blood gas, X-ray and computerized tomography (CT) imaging of the chest. Clinical efficiency was evaluated with the response criteria of the Eastern Cooperative Oncology Group [15]. The clinical stage of CRC was evaluated according to the TNM classification [16] and the Dukes staging systems [17]. Preoperatively, patients with tumours localized in other regions did not receive inflammatory or immunosuppressive therapy. The response to preoperative therapy was estimated according to the Response Evaluation Criteria in Solid Tumours [18].

Histopathological examination of CRC tumour tissue

All tissues was obtained from surgical resection. Sections (4 μm-thick) were cut from paraffin blocks and stained with H&E (POCH S.A.; Avantor Performance Materials Poland, Gliwice, Poland) according to the manufacturers' protocol. Slides were deparaffinized in a heating oven at 60°C for 5 min. Subsequently, slides were rehydrated in xylene (3 washes at 10 min respectively) and in a gradual concentration of ethanol (100, 95, 85, and 75% for 1 min at each concentration). Routine histopathological assessment of the sections was performed to determine the type of tumour growth, tumour size, histological type, percentage of muci-

nous components, grade of malignancy, TNM and Dukes stages. We also analyzed localization of cancer (right-side, transverse, left-side, sigmoid or rectum). Venous, lymphatic and perineural invasions were also analyzed. Characteristic features of lymph node invasion, including the 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 were examined. Tumour cells were required to be present at all borders of the image field; necrotic areas and mucinous components were excluded. The extent of the inflammatory cell reaction in the invasive front of the tumour and the centre of the tumour mass was also observed and classified according the Klintrup-Makinen criteria [19]. The extent of necrosis and fibrosis in the central tumour was evaluated according to Richards *et al.* [20] and graded as: Absent, none; focal, <10% of tumour area; moderate, 10-30%; or extensive, >30%.

Morphological examination of inflammatory cells in invasive front and in the centre of CRC tumour tissue

Tissue material obtained from routine histopathological diagnosis was stained with H&E and used to assess inflammatory cells in the invasive front and centre of the tumour by light microscopy under a high-power magnification, x400 (Leica DM6 B). For the counting of inflammatory cells we examined multiple tumour sections and averaged. The tissue sections contain tumour mass and margins of tumour. The analysis was evaluated by two independent pathologists who were blinded to the clinical information. Cells were counted and defined as a percentage of examined cells. Neutrophils are divided into three groups: 1-(0%), 2-(<20%), 3-(> 21%). The macrophages and eosinophils were evaluated as absent (lack of cells) or present (more than 5 cells observed in the examined area). Count of each group cells was followed in our previous study [8].

Statistical analysis

Statistical analysis was conducted using the STATISTICA 10.0 program (Statsoft). The results were analysed with the method for assessing normal distribution (the Shapiro–Wilk test). The parameters did not have a normal distribution. Correlations between the parameters were calculated by the Spearman's correlation coefficient test. A p-value of <0.05 was considered statistically significant. DFS time was calculated from the date of diagnosis to the date of disease progression, including local or distant relapse. DFS rate was estimated using the Kaplan-Meier method and the survival curves were compared using log-rank tests.

RESULTS

Clinical characteristics of studied patients.

The present study included 160 patients (64 females) who underwent surgery in the Department of Oncological Surgery, Comprehen sive Cancer Centre (Bialystok, Poland) between April 2014 and December 2016. The mean age was 67.5 years, including 40 patients <60 and 120 patients ≥60 years old.

Correlation between neutrophils macrophages and eosinophils in the invasive front of tumour mass, and clinico-pathologic features

Neutrophils present in the invasive front of tumour mass were correlated with fibrosis R= (-0.191; p= 0.021) and inflammatory cell infiltration in the invasive front of the tumour (R=0.350; p<0.001). Macrophages in the invasive front of the tumour were found to correlate with tumour growth (expanding and infiltrate, R=0.190; p=0.022), inflammatory cell infiltration in the invasive front and in centre of tumour mass (R=0.284; p=0.001, R=0.178; p=0.032). Eosinophils in the invasive front of the tumour were associated with inflammatory cell infiltration in the invasive front and in centre of tumour mass (R=0.304, p<0.001; R=0.260, p=0.002) (Table 1 and 2).

Table 1. Correlation between a particular population of inflammatory infiltrating cells located in the invasive front of the tumour mass and clinico-pathologic parameters in patients with colorectal cancer

Parameter		**N** 160	**Neutrophils** Rho p	**Macrophages** Rho p	**Eosinophils** Rho p
Age	<60	40	0.064	0.074	-0.078
	≥60	120	0.443	0.374	0.351
Gender	Female	64	0.035	-0.141	-0.019
	Male	96	0.669	0.091	0.813
Localization	Right-side	20			
	Transverse	14			
	Left-side	15	-0.040	-0.117	-0.094
	Sigmoid	29	0.643	0.178	0.276
	Rectum	82			
Tumour growth	Expanding	133	0.070	**0.190**	0.023
	Infiltrate	27	0.402	**0.022**	0.778

Tumour size	<2.5cm 2.5-5.0cm >5.0cm	27 106 27	0.089 0.287	0.051 0.543	-0.063 0.450
TNM stage	1 2 3 4	42 31 69 18	-0.015 0.859	-0.080 0.339	-0.146 0.080
Adenocarcinoma Type	Partially mucinous Non-mucinous	30 130	-0.071 0.396	-0.122 0.143	-0.036 0.662
Percentage of mucinous component	10-30% 30-50%	15 15	-0.053 0.525	-0.112 0.180	0.017 0.837
Grade of malignancies	2 3	148 12	-0.062 0.454	0.014 0.863	-0.035 0.671
pT stage	1 2 3 4	3 62 91 4	0.046 0.582	-0.045 0.587	-0.089 0.288

Nonparametric Spearman correlation. A p-value less than 0.05 (typically ≤ 0.05) is statistically significant.

Table 2. Correlation between a particular population of inflammatory infiltrating cells located in the invasive front of the tumour mass and clinico-pathologic parameters in patients with colorectal cancer

Parameter		**N**	**Neutrophils** Rho p	**Macrophages** Rho p	**Eosinophils** Rho p
Venous invasion	Absent Present	113 46	-0.067 0.421	-0.078 0.353	-0.105 0.208
Lymphatic invasion	Absent Present	121 38	-0.080 0.339	-0.050 0.544	-0.065 0.439
Perineural invasion	Absent Present	143 17	-0.094 0.261	-0.084 0.316	-0.053 0.524
Lymph node Metastasis	Absent Present	81 79	-0.036 0.665	-0.038 0.644	-0.061 0.466
Distant metastasis	Absent Present	143 17	-0.112 0.179	-0.093 0.268	-0.107 0.199
Necrosis	Absent Focal Moderate Extensive	45 61 36 18	0.041 0.625	0.003 0.963	0.062 0.454
Fibrosis	Absent Focal Moderate Extensive	11 72 43 34	**-0.191** **0.021**	-0.138 0.098	-0.087 0.296
Inflammatory cell infiltrate in the invasive front of tumour	Absent Weak Moderate Strong	20 66 48 26	**0.350** **<0.001**	**0.284** **<0.001**	**0.304** **<0.001**
Inflammatory cell infiltrate in the centre of tumour mass	Absent Weak Moderate Strong	11 76 51 22	0.074 0.377	**0.178** **0.032**	**0.260** **0.002**

Nonparametric Spearman correlation. A p-value less than 0.05 (typically ≤ 0.05) is statistically significant

Disease- free survival time in correlation with examined cells in the invasive front of the tumour mass

The mean DFS time was 12.1 months for all patients. The Kaplan-Meier curve analyses of neutrophils, macrophages and eosinophils in the invasive front of tumour mass were not statistically significant (Figure 1).

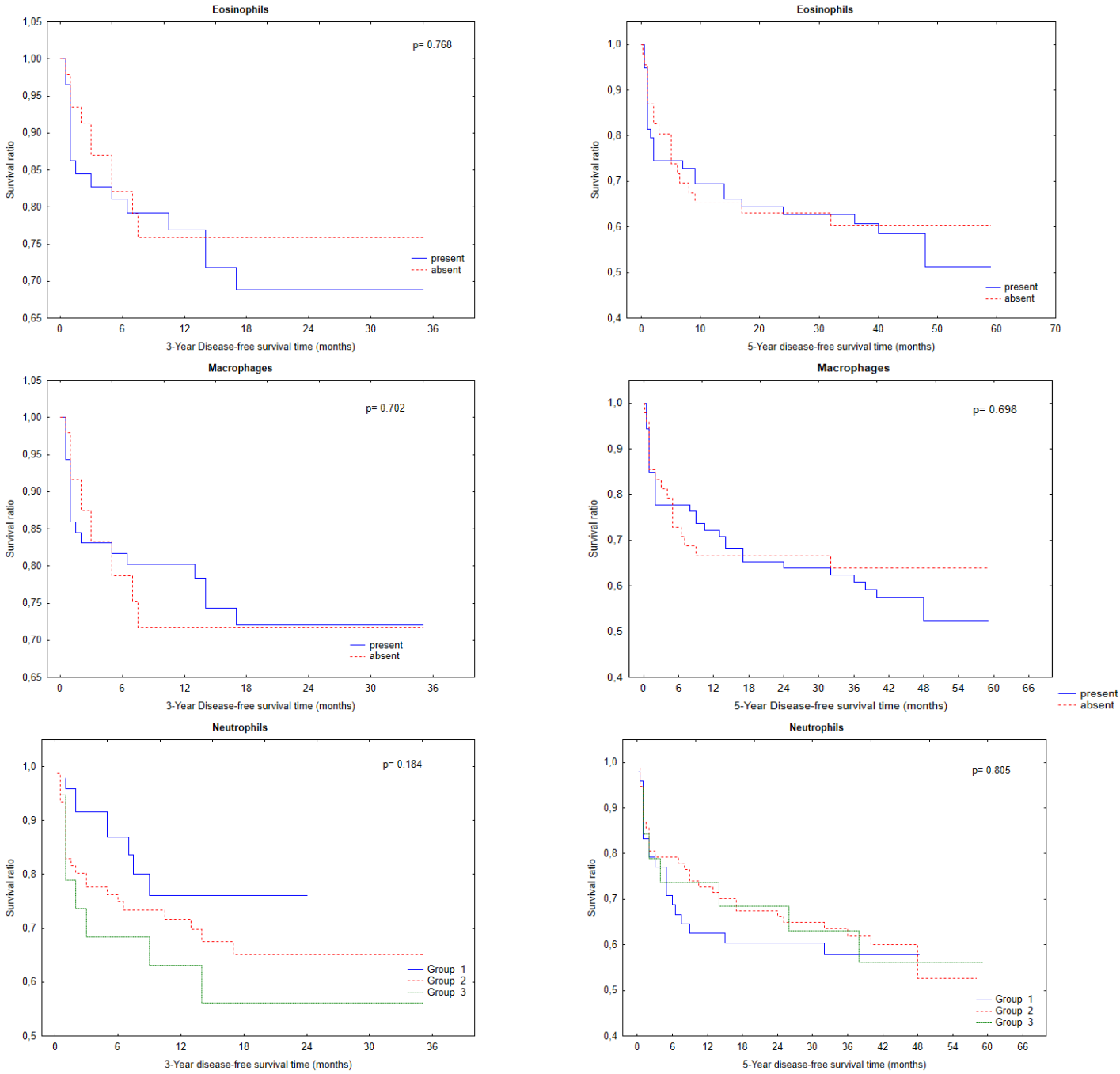

Figure 1. Postoperative disease-free survival curves of patients with colorectal cancer. The Kaplan-Meier plots of eosinophils, macrophages and neutrophils in the invasive front of the tumour mass

DISCUSSION

Colorectal cancer is one of the most frequently diagnosed cancers and the main reason for death as a result of cancer. However, the significance of inflammatory cell infiltration in CRC is poorly understood. In our research we studied immune cells infiltration in relation to clinico-pathologic features of patients with CRC. We studied the prognostic importance of different subsets of immune cells infiltrating the tumour (in tumour invasive front and centre). Neutrophils, macrophages and eosinophils play a significant role in the creation of the CRC tumour microenvironment [21]. There are reports that the inflammatory cell infiltrate in the invasive front of the tumour is a prognostic factor in patients with CRC. In addition, the authors suggest that extent of the inflammatory cell infiltration in the front of the tumour mass may influence the variables that determine disease progression [22].

Jakubowska et al. reported that neutrophils were chiefly observed in the invasive front of tumour [8]. Tumour cells secrete chemokines and cytokines.

This provides the recruitment of neutrophils to the tumour microenvironment. There has been a recent advance in determining the role of neutrophils in cancer progression, but that issue is still questionable. On the one hand, there is a neutrophil population correlating with poor prognosis (N2), on the other hand, some neutrophils have the ability to kill cancer cells (N1) [21]. In this study, we demonstrated the relationship between neutrophils present in the invasive front of the tumour with fibrosis. Fibrosis is a result of the persistent inflammation and can cause significant abnormalities in organs or systems. This process is associated with the production of fibroblast growth factor. Thus chronic immune reactions cause constant stimulation of fibrogenesis [23]. Further study of the interconnection neutrophils and fibrosis will be essential.

The current reports have presented inconsistent results. Some studies have reported that TANs and TAMs are correlated with poorly differentiated or undifferentiated types of CRC [24,25]. In turn, the different study showed TANs infiltrated low or moderately differentiated tumours [26].

A number of independent studies have reported that macrophages presence correlates with poor prognosis in cervical, breast or prostate cancer [11]. When it comes to colorectal cancer, Nakayama demonstrated that low infiltration of macrophages is associated with advanced stage of tumour [27]. Our study suggests that macrophages play a complex role in the regulation of cancer progression and tumour growth. We showed a correlation between infiltration of macrophages in the invasive front of tumour with tumour growth. There are conflicting results about the role of macrophages in tumorogenesis. Forssell et al. indicated that macrophage infiltration at the tumour front favourably impacted prognosis in CRC [28]. Thus, study about macrophages, their correlation with clinico-pathologic features and prognoses in cancer seem to be contradictory. Edin S. at el. describes two populations of macrophages: M1 and M2. The M1 macrophages are regarded as anticancer cell, while the M2 macrophages have a role of cancer promotion. The authors show that better prognosis in colorectal cancer is associated with infiltration of M1 macrophages [29].

There is evidence that eosinophils infiltrate tumours. Eosinophils have been described in many types of cancer, including squamous cell carcinoma of the lower colon, oral cavity or cervix. Correlation between the presence of eosinophils in the tumour mass and prognostic parameters have not been observed [30]. In our study, we did not observe any significant correlations between eosinophils in the invasive front of tumour mass and clinico-pathologic features. Although the absence or presence of eosinophils in the tumour mass does not appear to have a major impact on the prognosis, eosinophils may have a relevant role in the tumour biology.

McCoy et al suggest that some immune cells are associated with complete response to chemotherapy in patients with CRC [31]. Moreover, the number of infiltrating TANs and TAMs were used to constructed prognostic models in CRC patients [32]. This proves that studies such as our could facilitate the estimation of prognosis and have implications for treatment.

In our research we studied immune cell infiltration in relation to prognosis in CRC. The presence of neutrophils, macrophages or eosinophils in the invasive front of tumour mass was not associated with disease-free survival in the studied CRC patients. This study was conducted on a medium-sized group of patients. Further research on larger group is needed in order to confirmation our findings.

CONCLUSIONS

Based on the results from our research and other reports, we conclude that the inflammatory cells take actively participate in the defence mechanisms against the tumour. According to our study, the disease-free survival time is not associated with infiltration of inflammatory cells. These results have not confirmed all of the conclusions of other investigators, but extended them in essential directions.

Conflicts of interest

The authors declare that they have no conflict of interest.

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