TIL's lymphocyte expression in patient with Colorectal cancer

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

ABSTRACT

Purpose: Colorectal cancer cells are infiltrated by different types of immune cells. They are scattered throughout the medulla, stroma, and glands of the tumor, as well as in the invasive margin and in organized lymphoid follicles distant from the cancerous lesion. The aim of the study was to presence of CD8+ T lymphocyte infiltration in the tumor and its front in correlation with clinicopathological parameters.

Materials and Methods: The study included a group 62 of patients operated on due to colorectal cancer. The histopathological results of the patients were analyzed, including the assessment of the expression of CD8+ T lymphocytes in the main mass of the tumor and its front, and an analysis of correlation with the patient's age, sex, histological malignancy stage, presence of metastases to lymph nodes and distant metastases was performed.

Results: Statistical significance was demonstrated for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the presence of distant metastases ($p = 0.041$). Statistical significance was demonstrated for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the depth of tumor infiltration ($p = 0.042$).

Conclusions: The immune response expressed by CD8+ T lymphocyte infiltration increases with the depth of tumor infiltration. An immune response expressed by a strong expression of CD8+ T lymphocytes may be an indicator of the absence of distant metastases.

Keywords: CD8, Lymphocytic infiltration, Colorectal cancer, TIL's, CRC

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INTRODUCTION

Colorectal cancer cells are infiltrated by different types of immune cells [1]. They are scattered throughout the medulla, stroma, and glands of the tumor, as well as in the invasive margin and in organized lymphoid follicles distant from the cancerous lesion [2]. Tumor tissues may contain neutrophils, mast cells, natural killer cells, dendritic cells (DCs), and tumor-associated macrophages [1]. The adaptive immune response is antigen-specific and requires the recognition of specific foreign antigens upon presentation. The specificity of the antigen allows for the generation of responses tailored to specific pathogens. The ability to create these tailored responses is maintained in the body by so-called memory cells. The cells of the adaptive immune system are special types of leukocytes - lymphocytes [2,3]. The degree of invasion of primary tumors is a strong independent predictor of recurrence and an estimate of survival. In most cases, abundant lymphocyte infiltration is a positive prognostic factor [4].

Therefore, the aim of our study was to analyze the assessment of the presence of CD8 + T lymphocytes in colorectal cancer in correlation with selected anatomically parameters.

MATERIALS AND METHODS

The study group consisted of 62 patients operated on due to CRC in the 2nd Department of General and Gastroenterological Surgery of the University Clinical Hospital in Bialystok. The research on which this paper focuses was based on the analysis of histopathological results carried out in patients, together with the immunohistochemical assessment of CD8+ T lymphocyte expression in histopathological preparations used in routine diagnostics. Consent of the Bioethics Committee (No. R-I-002/130/2018) to conduct this research.

Immunohistochemistry of T CD8 + lymphocyte

The following antibodies were used for immunohistochemical staining of CD8+ T cells: SP57 anty-CD8 using an automated technique according to the XT ultraView DAB v3 procedure. Diaminobenzidine (DAB) was used as the chromogen. The assessment was made by diagnosing pathologists using an Olympus CX41 light microscope at 400x magnification. The results of the counts were summed up and compiled for evaluation in the form of the true value of the number of lymphocytes in the inflammatory neoplastic infiltration in 5 fields of view. The results were statistically analyzed using Statistica 13.3 PL (StatSoft Polska). The age and sex of the patients, the location of the neoplastic lesion and its histological type, the degree of histological malignancy, the depth of tissue infiltration, as well as the presence of metastases to lymph nodes and metastases to distant organs were correlated. The significance level of $p < 0.05$ was considered statistically significant.

RESULTS

Table 1. Characteristics of the study group

		N
Age	**≤ 60**	17
	> 60	45
Sex	**Female**	24
	Male	38
localization	**colon**	38
	rectum	24
Histopathology	**Adenocarcinoma**	54
	Adenocarcinoma with mucinous part	8
G	**Low grade**	60
	High grade	2
pT	**pT1**	1
	pT2	1
	pT3	55
	pT4	5
pN	**absent**	35
	present	27
pM	**Absent**	49
	present	13

The analyzed data in individual groups presented the following results

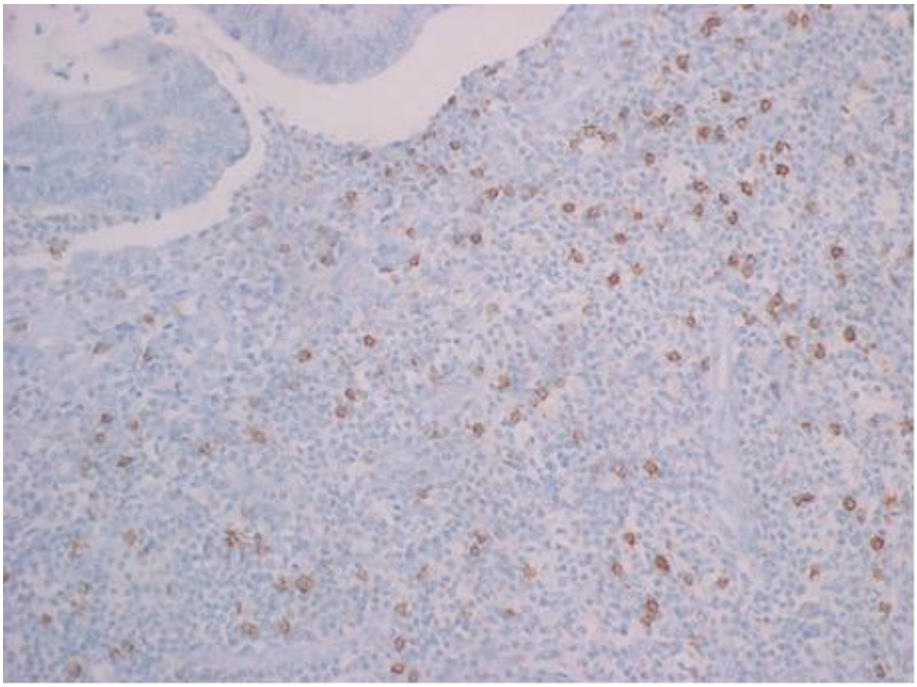

Figure 1. Expression of lymphocyte T CD8 + (400x)

The analyzed data in individual groups presented the following results.

Expression of CD8+ T lymphocytes in the main mass of the tumor.

There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the age of the patients (p = 0.379). There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the sex of the patients. There was no statistical significance for the correlation between the differentiation of TCD8+ tumor infiltration and tumor location. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the histological type of the tumor . There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the histological malignancy of the tumor. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the depth of tumor infiltration. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the involvement of lymph nodes by the tumor. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the presence of distant metastases.

Expression of CD8+ T lymphocytes in the tumor invasion front

There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the tumor and the age of the patients. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the invasion front in relation to the sex of the patients. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the location of the tumor. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the histological type of the tumor. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the histological malignancy of the tumor. There was no statistical significance for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the involvement of lymph nodes by the tumor (p = 0.205).

Statistical significance was demonstrated for the correlation between the differentiation of TCD8+ infiltration in the invasion front and the depth of tumor infiltration (p = 0.042).

Statistical significance was demonstrated for the correlation between the differentiation of

TCD8+ infiltration in the invasion front and the presence of distant metastases (p = 0.041).

DISCUSSION

Lymphocytic inflammatory infiltration is a common aspect of malignancy. Cancer cells are eliminated by the host's immune system before a detectable tumor develops. This process is called tumor immune surveillance [5]. The push of the host's immune system towards immune selection may simultaneously result in the formation of malignant cells with the ability to evade elimination. Dysregulation of the immune response can often be noticed already in the early precancerous stages - in colorectal adenoma, as a decrease in the activity of Th1 helper lymphocytes [6]. Väyrynen et al. noticed that as the disease progresses to the advanced stage associated with the presence of distant metastases, the immune response of the system weakens [7].

In the examined histopathological material, a more abundant infiltration of CD8+ T lymphocytes was observed in patients not burdened with the presence of distant metastases, which may be an exponent of a stronger immune response to the disease at this stage.

Lymphocytes located in the immediate vicinity of a malignant lesion have the ability to recognize tumor antigens and induce tumor cell lysis. They can also release specific cytokines with chemotactic and pro-inflammatory properties [8]. In tumor cells in direct contact with tumor infiltrating lymphocytes (TILs), destruction of the cell membrane and cytoplasm has been observed, and in some cases penetration into the interior of the tumor cell and destruction of the cell nucleus [9].

The site of the invasion front is a key defensive area against cancer metastases. Without adequate immune stimulation, tumor structures - including blood and lymphatic vessels, as well as perineural spaces - may allow the invasion of selected clones of tumor cells to surrounding tissues and distant organs.

Immunotherapy developed for patients with colorectal cancer is becoming a realistic clinical approach [10,11]. Over the last 20 years, there have been many publications on tumor immunology and the prognostic impact of various types of immune and inflammatory cells on the cancer microenvironment, which may provide promising results both in vitro and in direct patient diagnosis and therapy. 128 A large number of tumor-infiltrating lymphocytes, especially intraepithelial and CD4+ and CD8+ antigens, appear to have a favorable prognosis [11,12,13]. The abundance of these cells infiltration is likely to correlate positively with a reduced rate of local recurrence after surgery, as well as with a longer survival time, both in patients without metastases and in those with distant metastases who underwent liver resection [11,12,14]. Also, Galon et al., examining the type, density and location (tumor / invasion front) of lymphocytic infiltration, including CD3+ and CD8+ T cells, showed an independent, positive effect of the presence of these lymphocytes on both disease recurrence and experience [15].

The intensity of expression of CD3+ and CD8+ T cells in the immune infiltration may be considered a factor in determining the number of lymph nodes indicated for surgical removal, however, Kim et al. suggest that inflammatory cell infiltration is a more reliable predictor than T cell markers alone. 170 Makkai-Popa et al. hypothe-sized that a lower abundance of CD8+ T cell infiltration may be a strong predictor of a longer period of tumor remission, although this topic still requires careful study [16].

This study also showed that the depth of tumor invasion is related to the presence of an immune response in the form of CD3+ T cell infiltration, and that the immune response mediated by CD8+ T cells increases with the depth of tumor invasion.

CONCLUSION

The immune response expressed by CD8+ T lymphocyte infiltration increases with the depth of tumor infiltration. An immune response expressed by a strong expression of CD8+ T lymphocytes may be an indicator of the absence of distant metastases.

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