ANNOTATION

dissertation work for the degree of Doctor of Philosophy

**Topic:** “Morphofunctional characteristics of neutrophil extracellular traps in colorectal cancer”

**Specialty:** 6D110100 Medicine

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# Relevance of the research topic

Colorectal cancer is considered an extremely common pathology: the individual risk of developing this disease reaches 5-6%. About 1200,000 new cases of colon cancer and 700,000 deaths from it are registered annually in the world [1, 2].

In the Republic of Kazakhstan, in the structure of oncological morbidity, colon cancer occupies the 6th position, accounting for 5.5% (2019 - 5.3%), compared to 2019, there is an increase in the incidence. Rectal cancer in the structure of malignant neoplasms of both sexes ranks 7th in terms of rank with a specific gravity of 5% (2019 - 5%). Colon cancer in the structure of causes of death of the population of both sexes from cancer in 2020, retained the 5th place, with a share of 5.4% in the structure of causes of death. Rectal cancer in the structure of causes of death of the population of both sexes from malignant neoplasms in 2020 takes the 6th place with a specific weight of 5.22% [3].

An important feature of malignant neoplasms is their relatively autonomous growth, regulated by locally produced factors, which include "microenvironmental factors" of tumors, produced both by the tumor cells themselves and by the cells of the surrounding stroma. Neutrophil granulocytes are a constant component in the structure of the tumor microenvironment. However, their role in oncogenesis has not been fully established. Evidence has appeared that, in addition to antitumor activity, neutrophils can also demonstrate protumor activity, provoking metastasis [4, 5]. In response to microbial and nonmicrobial stimuli, neutrophils actively form in the extracellular space network-like structures consisting of nucleic acids and enzymes - neutrophil extracellular traps (NEТs). The fibrous structures of NEТs are the main DNA chain containing histones and neutrophil proteins. The main protective function is the destruction of pathogens [4, 5].

Ideas about the role of NEТs in oncology vary greatly. On the one hand, there are data on the anticarcinogenic properties of NEТs associated with the direct destruction of tumor cells and stimulation of the immune system. Cytotoxicity towards tumor cells is exhibited by the components of NEТs (myeloperoxidase, proteinases, and histones), while DNA strands are considered as a kind of scaffold for capturing tumor cells and limiting their further spread [6]. On the other hand, network structures of traps can promote migration and immune avoidance of tumor cells or create a physical barrier between tumor cells and immunocompetent cells [7].

It should be recognized that at present there is a clear lack of evidence on the formation of NEТs in various types of cancer, stage, localization and treatment regimens, and the available results are rather contradictory.

Currently, much attention is paid to the personalization of prescribed treatment. The need for individualization is associated with the need to improve diagnostic methods and timely treatment of patients, which should lead to an increase in five-year and relapse-free survival. Despite the improvement of surgical technique and chemotherapy, the disease has not yet been completely overcome due to metastases. Again, the emergence of more accurate additional prognostic factors could greatly facilitate the choice of the most appropriate treatment regimen and improve the treatment of specific patients with colorectal cancer.

# Purpose of the study

# To give a morphofunctional characteristic of neutrophil extracellular traps in colorectal cancer.

# Research objectives

# 1. Determine the ability to generate neutrophil extracellular traps in the blood of patients with colorectal cancer before and after treatment.

# 2. To study the ability to generate neutrophil extracellular traps from smears of tumor imprints in patients with colorectal cancer after treatment.

# 3. To assess the correlation dependence of the formation of neutrophil extracellular traps in blood and tumor tissue on the degree of morphological differentiation of the tumor and the prevalence of the tumor process

# 4. To study the role of NEТs in the blood, as well as in the center and periphery of the tumor tissue in the prognosis and course of colorectal cancer, incl. in life expectancy of patients with colorectal cancer.

# Scientific novelty

For the first time, the features of the generation of neutrophil extracellular traps in peripheral blood in patients with colorectal cancer before and after specialized treatment were determined.

It was shown for the first time that the median blood NEТs in patients with colorectal cancer in the compared groups at the first stage after surgery in the group without neoadjuvant radiation therapy is higher than in the group with radiation therapy (χ2=5.6565, p=0.0174). At stages 2, 3, and 4, there were no differences in the median blood NEТs values between groups of patients with colorectal cancer with and without radiation therapy.

# For the first time, the features of neutrophil extracellular traps in tumors from surgical material in patients with colorectal cancer were determined. A multidirectional release of NEТs from the center and periphery of the tumor, and healthy tissue of the colon, depending on the type of neoadjuvant treatment and the degree of prevalence of the tumor process, was revealed (certificate of entering information into the state register of rights to objects protected by copyright No. A., Maratkyzy M., Kabildina N. A., Sirota V. B. “Determination of extracellular neutrophil traps in tumor tissues in colorectal cancer”).

# For the first time, the quantitative effect of neutrophilic extracellular blood traps on the survival of patients with colorectal cancer before and after specialized treatment was determined (certificate of entering information into the state register of rights to objects protected by copyright No. 24947 dated April 11, 22, Maratkyzy M., Zhumalieva V.A. , Beisenaeva A.R. "Influence of quantitative regeneration of neutrophilic extracellular blood traps on the survival rate of patients with colorectal cancer").

# For the first time, the quantitative effect of tissue neutrophil extracellular traps on the survival of patients with colorectal cancer was determined (certificate of entering information into the state register of rights to objects protected by copyright No. 24948 dated April 11, 22. Maratkyzy M., Zhumalieva V. A., Beisenaeva A. R. "Influence of quantitative release of tissue neutrophil extracellular tumor traps on the survival of patients with colorectal cancer").

# Relationship of the thesis with other research papers

# The research work was carried out within the framework of the research program of project-targeted financing No. BR05236771 "Personalized approach in the management of a number of significant diseases." This scientific project was approved by the National Scientific Council of the Ministry of Health of the Republic of Kazakhstan (02/03/2018) as part of the Competition for program-targeted funding for scientific, scientific and technical programs for 2018-2020. Implementation of this project in accordance with clause 3.10 of the Agreement on joint activities No. 121 dated 09/01/2017, concluded between " ROD " of Karaganda and the "KSMU" of the Ministry of Health of the Republic of Kazakhstan (under the project).

# The main provisions for defense:

# The median NEТs of blood in patients with colorectal cancer changes after surgical treatment, which contributes to its increase.

# The release of tissue NEТs in the center, periphery of the tumor and the surrounding healthy tissue in colorectal cancer is not the same, depending on the neoadjuvant radiation therapy.

# The formation of neutrophilic extracellular blood traps in the group of patients with colorectal cancer treated with neoadjuvant radiation therapy has a direct correlation according to Spearman with the degree of morphological differentiation of the tumor.

# The generation of NEТs of the tumor center in all patients has a Pearson-like relationship with NEТs of the tumor periphery and NEТs of healthy tissue. NEТs of healthy tissue directly correlates with the degree of morphological differentiation of cancer.

# Quantitative release of NEТs blood and tumor tissue have a direct impact on the life expectancy of patients with colorectal cancer.

# Practical significance

The results of the study made it possible to expand the understanding of the role of NEТs of blood, tumor and healthy tissue around the tumor in the prognosis and course of colorectal cancer.

A statistically significant quantitative effect of tissue NEТs in the periphery of the tumor on the survival of patients with colorectal cancer was revealed. The greater the number of NEТs in the tumor, the higher the life expectancy. If during the operation a low amount of NEТs is detected in smears in the center and periphery of the tumor, this is a reason for the attending physician to think about further continuation of treatment in the adjuvant regime, that is, after surgical radiation therapy or chemotherapy

**Author's personal contribution**

The dissertation student independently performed the selection of patients, obtained informed consent for the study, as well as under the supervision of Doctor of Medical Sciences, Professor Sirota V.B. a smear-imprint was made using the method “Determination of extracellular neutrophil traps in tumor tissues in colorectal cancer” (Zhumaliyeva V. A., Maratkyzy M., Kabildina N. A., Sirota V. B., 2020) with a dissected in the center , the periphery of the tumor and healthy tissue in the postoperative material on glass slides. Together with the staff of the Department of Biomedicine of the NJSC KMU under the supervision of Doctor of Biological Sciences, Professor Muravleva L.E. staining and counting the number of extracellular DNA networks were carried out. After counting the NEТs, the glass preparations were photographed by the dissertator. As well as the dissertator, the data obtained were processed, analyzed, the results were presented in the form of publications and scientific reports.

# Approbation of work

# Approbation of work. The main results of the dissertation work were reported and discussed at:

# - Republican scientific and practical conference with international participation “Modern trends in the development of clinical and radiation oncology. Multidisciplinary approaches”, 05/22/2019, Semey Medical University;

# Modern Molecular- biochemical Markers in Clinical and Experimental Medicine -2019” during 07 – 09 November 2019 which will be held in Prague, the Czech Republic;

# - at a meeting of the Department of Oncology and Radiation Diagnostics of the NJSC KMU, protocol No. 8 dated 04/21/2022

# Publications on the topic of dissertation.

17 publications on the research topic were published: in the proceedings of international conferences - 5, in the proceedings of foreign conferences - 1, publications in scientific journals of Kazakhstan recommended by the Control Committee in the field of education and science of the Ministry of Education and Science of the Republic of Kazakhstan; - 4, publications recommended by the Russian Science Citation Index - 1, in an international scientific publication included in the Scopus database - 3 Received a certificate of entering information into the state register of rights to objects protected by copyright - 3.

# Design and research methods

The study design was based on a prospective clinical study.

On the basis of the "Regional Oncological Dispensary" in Karaganda, 323 patients were examined under the project for 2018-2020. Of these, NEТs was determined in 97 patients, they were divided into 2 groups: group 1 - patients without treatment (n=60), group 2 - patients with neoadjuvant radiation therapy (n=37). The study was approved by the Clinical Research Ethics Committee of the NJSC KMU No. 16 dated April 29, 2019. All patients were familiar with the essence of the study and gave informed voluntary consent to the sampling of material: peripheral blood, a tumor fragment from the surgical material.

The selection of patients included in the study was made according to the inclusion and exclusion criteria presented in Table 1.

Table 1 - Criteria for inclusion and exclusion

|  |  |
| --- | --- |
| Inclusion Criteria | Exclusion Criteria |
| Male and female;  Age from 31 to 60 years;  A verified diagnosis of CRC  Patient consent to participate in the study  No significant comorbidity  No history of oncological pathology of other localizations | patients with decompensated chronic diseases  patients with synchronous malignancies  patients in an inoperable condition  receiving palliative chemotherapy  patients in whom the diagnosis of colorectal cancer was not confirmed after surgical treatment  refusal of patients to participate in the study |

Table 2 - Distribution of patients with colorectal cancer by stages

|  |  |  |  |
| --- | --- | --- | --- |
| Clinical and morphological indicators | Mean M±m | 95% CI | 95% CI |
| Stage of the tumor process | | | |
| 1 Stage | 21,6±3,96 | 18,4 | 22,99 |
| 2 Stage | 54,6±4,99 | 45,57 | 65,54 |
| 3 Stage | 17,5±3,79 | 16,42 | 17,94 |
| 4 Stage | 6,1±2,4 | 5,59 | 6,55 |

**Determination of extracellular neutrophil blood traps in patients with colorectal cancer**

All patients included in the study underwent sampling No. 1 (preoperative) for the determination of NEТs, 100% (n=97) of patients. Sample No. 2 (postoperative) was taken on the 7-10th day after the operation. This stage was carried out by the paramedical personnel of the "ROD". Blood from the cubital vein for examination in the amount of 5 ml was collected by a system for taking blood into vacuum tubes with sodium citrate. Further, the vacutainers with venous blood were transported to the Department of biomedicine of the NJSC KMU

## Evaluation of the number of induced NEТs in the blood was carried out according to the method of Dolgushin I.I. [8] by adding pyrogenal on glass slides and stained with azure - eosin according to the May-Grunwald method, followed by microscopy at a magnification of 100 times. Counting was carried out per 100 neutrophils

## Determination of tissue extracellular neutrophil tumor traps in patients with colorectal cancer

## A smear-imprint was made using the method “Determination of extracellular neutrophil traps in tumor tissues in colorectal cancer” (Zhumaliyeva V. A., Maratkyzy M., Kabildina N. A., Sirota V. B., 2020) with a dissected in the center , the periphery of the tumor and healthy tissue in the postoperative material on glass slides. This stage was carried out in the ROD immediately after the removal of the macropreparation during surgical treatment. The smear-imprint was transported to the Department of Biomedicine of the NJSC KMU and also stained with azure - eosin according to the May-Grunwald method [9] with microscopy in a light microscope at a magnification of 400 times with the calculation of extracellular DNA. Counting was carried out in 10 fields of view. It was evaluated both free-lying DNA strands and in contact with tumor cells.

## The assessment of the number of extracellular DNA networks was carried out jointly with the staff of the Department of Biomedicine of the NJSC KMU under the supervision of Doctor of Biological Sciences, Professor L.E. Muravleva. After counting the NEТs, the glass preparations were photographed by the author. Photos on a portable hard drive indicating the number assigned to the patient of the sample, as well as glass slides of blood smears and tumors, are stored in one copy at the Department of Oncology and Radiation Diagnostics of the NJSC KMU, confidentiality is maintained.

## Methods of Statistical Analysis. For statistical processing of the obtained results, the procedures of mathematical statistics were used, implemented in the application programs "STATISTICA 10.0" and EXСEL. Test χ2 was used to prove statistical differences when comparing pairs. Differences were considered significant at the level of statistical significance p<0.05. The statistical significance of different values for binary and nominal indicators was determined using Pearson's Chi-square test. Correlation analysis was carried out on the basis of Spearman's nonparametric rank correlation. Comparative characteristics of the parameters of the normal distribution was carried out by determining the coefficient according to Student's t-test. Survival of patients with colorectal cancer, depending on the amount of NEТs in the blood and tissue, was determined by the non-interval method of E. Kaplan - P. Meier using the Gehan-Wilcoxon, F-Cox, Cox-Mentel, Log-Rangov, Wilcoxon-Peto criteria.

## Conclusions

## In the general group of patients with colorectal cancer, a statistically significant increase in NEТs was observed in the blood from 6.56±8.80 before surgery to 9.41±11.64 after surgery (p≤0.05). A similar picture is observed in the group of patients without neoadjuvant radiation therapy: an increase in NEТs from 5.81±8.37 before surgery to 9.38±11.28 after surgery (p≤0.05).

## In all patients with colorectal cancer, the same release of NEТs from the center and periphery of the tumor (10.86±1.03, 10.72±1.16, respectively; p≥0.05), in the surrounding healthy tissues of the tumor, single NEТs (3.14 ±0.61). The quantitative difference between NEТs from tumor tissue and healthy tissues is statistically distinct (p≤0.05). Carrying out neoadjuvant radiation therapy contributes to the release of only NEТs of healthy tissue around the tumor (χ2=5.0000, p=0.0253).

## The formation of NEТs of blood in patients with colorectal cancer at the first stage after surgery in the group without neoadjuvant radiation therapy is higher than in the group with radiation therapy (χ2=5.6565, p=0.0174). At stages 2, 3, and 4, there were no differences in median indicators of blood NEТs between groups of patients with colorectal cancer with and without radiation therapy.

## In the group of patients with colorectal cancer treated with neoadjuvant radiation therapy, NEТs of blood after surgery had a direct correlation according to Spearman with the degree of morphological differentiation of the tumor (rr=0.39; p≤0.05).

## When conducting a Pearson correlation, the NEТs of the tumor center in all patients had a direct relationship with the NEТs of the tumor periphery (rp=0.39; p≤0.05) and the NEТs of healthy tissue (rp=0.297; p≤0.05). NEТs of healthy tissue directly correlated with the degree of morphological differentiation of cancer (rр= 0.21; p≤0.05).

## 4. There is a quantitative effect of NEТs of blood on survival in the general group of patients with colorectal cancer with and without neoadjuvant radiation therapy before surgery (WW p=0.0443, F-Cox p=0.0156, Cox-Mentel p=0.0484, Log-Rank p=0.0478) and after surgery (F-Cox p=0.0158, Cox-Mentel p=0.0493, Wilcoxon-Peto p=0.0489, Log-Rank p=0.0485). Statistically significant quantitative effect of NEТs of blood on survival in the group of patients with colorectal cancer without neoadjuvant radiation therapy before (F-Cox p=0.0196) and after surgery (F-Cox p=0.0220).

## A statistically significant quantitative effect of tissue NEТs in the periphery of the tumor on the survival of patients with colorectal cancer was revealed. Survival is higher in patients with a large release of tissue NEТs at the tumor periphery (WW p=0.0309, F-Cox p=0.0316, Wilcoxon-Peto p=0.0415).

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