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ABSTRACT
Of the PhD DOCTORAL DISSERTATION

Topic title: **"Diagnostic and prognostic significance of the tumor microenvironment in colorectal adenocarcinoma"**

According to the educational program 8D10102 "Medicine"
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ABSTRACT

Imanbayeva N. M. on the topic "Diagnostic and prognostic significance of the tumor microenvironment in colorectal adenocarcinoma" submitted for the degree of Doctor of Philosophy (PhD), specialty 8D10102 "Medicine".

Scientific consultants: Candidate of Medical Sciences, Associate Professor Iztleuov E. M., Doctor of Medical Sciences, Professor Bekmukhambetov E. Zh.

Foreign consultant: Doctor of Medical Sciences, Professor Rasulov A. O.

Relevance of the study:

Colorectal cancer (CRC) is a disease with high morbidity and mortality, which is the third most common in the world. According to the International Agency for Research on Cancer, approximately 1.93 million new cases of CRC were reported worldwide in 2020, and the age-standardized incidence rate of CRC was 19.5 per 100,000 people.

Currently, one of the bases for clinician decision-making on the management of cancer patients, including patients with CRC, is the TNM classification. However, in patients with the same stage of CRC according to the TNM classification, clinical outcomes may differ. This actualizes the search for additional criteria and factors for stratification of patient groups.

Traditionally, in histological studies of malignant tumors, the leading place was occupied by the epithelial component, but modern research paradigms have gradually shifted from the tumor epithelium to the stroma. The prognostic value of the stroma of the invasive edge of the tumor was demonstrated by Ueno H., who isolated a mature, immature and intermediate desmoplastic reaction. The intermediate desmoplastic reaction is characterized by a large heterogeneity of the stroma, including both a small amount of kelloid-like collagen (KPC) in the fibrotic stroma, and thick bundles of kelloid-like collagen circularly surrounding cancer nests and remodeling the extracellular matrix.

Pathologists can identify the components of the stroma, but their description and quantification is not a routine procedure and stromal factors are not always taken into account when making treatment decisions. Therefore, it is necessary to identify prognostic factors to improve patient survival. A separate problem is to determine the optimal threshold level (cut-off point) with the maximum discriminating ability for kelloid-like collagen in the stroma of the invasive edge of the tumor. Based on the above, it follows that histophenotyping of collagen fibers of the extracellular matrix of the CRC is an important applied clinical and scientific value.

The aim of this study is to evaluate the CRC in the stroma of the CRC interpreted on micro-preparations stained with hematoxylin and eosin and Masson's trichrome, and to conduct a comparative analysis of these parameters with traditional prognostic factors to establish their prognostic significance.

Objective: to evaluate the diagnostic and prognostic significance of the microenvironment (kelloid-like stroma collagen) of the invasive margin of colorectal adenocarcinoma.

Research objectives:

1. Determine regional features of colorectal cancer in the Aktobe region;

2. To establish a prognostically significant level of kelloid-like stroma collagen of the invasive margin for assessing the outcomes of colorectal adenocarcinoma;

3. To study the relationship of kelloid-like collagen in the invasive region with histopathological characteristics of the tumor, clinical data, and probable outcomes (overall and disease-free survival).

4. To perform a comparative analysis of CD3, CD4, CD8, CD20, CD56, and CD163 positive immune cells with the percentage of KPC in the microenvironment and the relationship with the outcomes of colorectal adenocarcinoma.

Scientific novelty:

1. Histochemical analysis was used to determine a prognostically significant threshold level of the relative amount of colloid-like collagen in the stroma of the invasive edge of colorectal adenocarcinoma with an intermediate desmoplastic reaction based on the maximum discriminative ability for overall and disease-free survival.

2. It was found that the presence of a diffuse histophenotype of kelloid-like collagen in the stroma of the invasive edge of colorectal adenocarcinoma with an intermediate desmoplastic reaction correlates with such signs as T-stage, venous and lymphatic invasion.

3. It was found that colorectal adenocarcinomas with focal kelloid-like collagen in the stroma of the invasive margin and an intermediate desmoplastic reaction have a higher frequency of cases with a high number of CD3 and CD8 positive immune cells and a higher frequency of cases with a low number of CD163 positive immune cells compared to tumors with diffuse kelloid-like collagen.

Theoretical significance:

1. The results of the research presented in the dissertation expand the existing understanding of the histophenotype of the extracellular matrix of the invasive edge of colorectal adenocarcinoma by the type of "kelloid-like collagen-reticulin".

2. The effect of the relative amount of kelloid-like collagen on immune cell proliferation established in this study may represent a new immunosuppressive mechanism in the tumor microenvironment, which is manifested by the established relationship between the density of kelloid-like collagen in tumors with an intermediate desmoplastic reaction and prognosis for cancer patients.

Practical significance:

Determining the relative amount of keloid-like collagen in colorectal adenocarcinomas with an intermediate desmoplastic reaction in accordance with the established threshold value is a new addition to the system for predicting outcomes in cancer patients, which contributes to improving risk stratification, which is important in practice for oncologists and pathologists.

The main points of the dissertation submitted for defense:

1. The intermediate desmoplastic reaction in colorectal adenocarcinomas is a heterogeneous group that can be further stratified by the relative proportion of kelloid-like collagen in the stroma of the invasive tumor front.

2. The level of kelloid-like collagen greater than 30% (diffuse) of the extracellular matrix of the invasive tumor front with an intermediate desmoplastic reaction is an independent prognostic factor for an unfavorable outcome (relapse and

death) in patients with colorectal adenocarcinoma, and is also associated with the presence of unfavorable signs (higher T-stage, the presence of venous or lymphatic invasion).

3. The level of kelloid-like collagen less than 30% (focal) of the extracellular matrix of the invasive tumor front with an intermediate desmoplastic reaction is associated with a high degree of lymphocytic infiltration and a large number of CD3 and CD8 positive immune cells and a smaller number of CD163 positive immune cells.

Implementation in practice. Act of implementation in practice on the basis of the MC of the NAO of the Marat Ospanov West Kazakhstan State Medical University No. 1 dated 26.06.2023 " Determination of the tumor microenvironment (CD3, CD4, CD20, CD56, CD163)". Act of implementation in practice on the basis of the MC of the NAO of the Marat Ospanov West Kazakhstan State Medical University No. 2 dated 26.06.2023 "Determination of the parenchymal-stromal ratio in colon tumors in colorectal cancer". Act of implementation in practice on the basis of the MC of the NAO of the Marat Ospanov West Kazakhstan State Medical University No. 3 dated 26.06.2023 " Determination of microsatellite instability (MSI) as a screening test for selecting patients with suspected Lynch syndrome."

Personal contribution of the author. The study was conducted independently: recruitment, primary and statistical processing of materials, analysis, generalization of research results and their description, obtaining author's certificates, implementation certificates, as well as working with publications from writing the material to submitting it to scientific journals. The author was directly involved in all surgical procedures for patients, supervised after the operation, and examined them in the immediate and long-term postoperative periods. A histological, histochemical, and immunohistochemical study of the surgical material was conducted under the supervision of the supervisor, describing microscopic patterns of type I and type III collagen. Morphometric analysis with counting of stromal and cellular structures with description of histological material and subsequent microphotography of the obtained objects was performed independently. All the material is systematized, documented, and arranged in chapters of the dissertation work personally by the author.

Testing the work

The main points of the dissertation were reported and discussed at:

- The XIII Congress of Oncologists and Radiologists of the CIS and Eurasia countries made a report on April 27-29, 2021. Kazakhstan. Almaty - "Epidemiological aspects of the structure of colorectal cancer on the example of border regions".

- The XIII Congress of Oncologists and Radiologists of the CIS and Eurasia countries together with the Association of Directors of Institutes of Oncology and Radiology of the CIS and Eurasia (ADIOR) made a report on April 29, 2022. Russia. Moscow. (online) - "Structure of colorectal cancer in Aktobe region".

- X Annual International Scientific and Practical Conference "TOPICAL ISSUES of MEDICINE" April 27-28, 2023, Baku, Azerbaijan - "The state of the

parenchymal-stromal ratio of the tumor depending on the localization in colorectal cancer".

- IX Congress of Oncologists and Radiologists of the Republic of Kazakhstan with international participation. October 26-27, 2023, Astana - " Nomogram of survival in patients with colorectal cancer. Retrospective analysis for 2019-2021".

List of scientific papers published on the topic of the dissertation

Based on the materials of the dissertation, 6 scientific papers were published, including:

1 publication in scientific publications recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan: "Parenchymal-stromal ratio in colorectal cancer tumors as an indicator of metastasis" / / Oncology and Radiology of Kazakhstan. - №3(69). - 2023. – P. 34-37.

5 publications in international scientific publications included at the time of publication of articles in the Scopus and PubMed information base:

1. «Colorectal cancer and microbiota: systematic review» // Gastroenterology Review. – 2024. - Vol. 19(1). 2023 IF-1,3. Q-1 Medicine. CiteScore 2023-2, 2.The 90th percentile.
2. «Parenchymal-Stromal Ratio in Colorectal Cancer Tumors: A Mini Review» // Journal of Family and Reproductive Health. – Vol. 18(1) (March 2024). 2023 IF-0,0. Q-2. Medicine. CiteScore 2023-0. 0.Percentile-55th.
3. «Diagnostic and prognostic significance of keloid-like collagen remodeling patterns in the extracellular matrix of colorectal cancer» // Pathology and Oncology Research. 2024. – Vol.30. CiteScore 2023-6,3. Q-1. Процентиль-80-й.
4. «Evolution of Colorectal Cancer Trends and Treatment Outcomes: A Comprehensive Retrospective Analysis (2019-2023) in West Kazakhstan» // Asian Pacific Journal of Cancer Prevention (APJCP). 2024. - Vol. 25(8). CiteScore 2023-2.8. Процентиль-46-й. Q-3.
5. «Brief review of colorectal cancer and its microenvironment» // Medical Journal of the Islamic Republic of Iran. – 2024. Vol. 38(108). 2023 IF-2,4. Q-2. Medicine. CiteScore 2023-2, 4.The 54th percentile.

Materials and methods of research:

The main research work was carried out on the basis of the Medical Center of the NAO West Kazakhstan Medical University named after Marat Ospanov in the Department of Pathomorphology and at the Department of Oncology. The study was conducted in accordance with the ethical principles established by the local ethics committee (Protocol No. 9 of 19.11.2021).

For the first task, the study is a retrospective cohort study conducted to analyze the demographic and clinical characteristics of patients with colorectal cancer (CRC) in the Aktobe region for the period from 2019 to 2023 on the basis of the Medical Center of the NAO West Kazakhstan Medical University named after Marat Ospanov located at 8 Zhanakonys microdistrict, Aktobe. The medical documentation of patients with CRC was studied.

The data set provided complete information about patients, which allowed for a comprehensive analysis of their condition, risk factors, treatment, and outcomes. All data were obtained from the Comprehensive Medical Information System "Damumed", as well as after studying outpatient patient records.

To achieve the subsequent objectives of the dissertation, a retrospective blind study of the histochemical pattern of the extracellular matrix of colorectal adenocarcinoma was performed to determine the prognostically significant level of kelloid-like collagen in the invasive edge of the tumor with an intermediate desmoplastic reaction.

The structure of the study included 3 main stages:

Stage 1 of the study: study of prognostically significant levels of kelloid-like collagen in the invasive edge of a colon tumor with an intermediate desmoplastic reaction.

Stage 2 of the study: etablation of the method of histophenotyping of kelloid-like collagen in the invasive edge of a colon tumor with an intermediate desmoplastic reaction.

Stage 3 of the study: standardization of the method by comparative analysis of the number of CD3, CD4, CD8, CD20, CD56 and CD163 positive immune cells in the microenvironment (kelloid-like stroma collagen) of the invasive edge of colorectal adenocarcinoma.

Endpoints and definitions. The primary endpoint of the study was disease-free survival. Relapse-free survival was defined as the time in months between the date of surgery and the date of cancer recurrence (defined as the first date of X-ray or histological diagnosis of local tumor recurrence or colon cancer metastasis) or the date of last follow-up (with a maximum period of 5 years). Overall survival was defined as the duration from surgery to death or the last follow-up. Patients who were alive at the time of the last observation were recorded as censored events.

Histological examination стандартным методом окрашиванияby standard hematoxylin and eosin staining.

Histochemical study. *Masson trichrome staining procedure.* Bundles of collagen fibers were stained and distinguished from cellular components using Masson trichrome dye. For Masson trichrome staining, a commercial kit (Trichrome dye (Masson)) was used Bio-Optica (Italy)) using the standard protocol. Collagen fibers were defined as dark blue fibers with black cores. Procedure *Gomori silvering procedure.* Histological evaluation of reticulin fibers was performed using *Homori silvering histochemical staining*, a set of Reticulum dyes (modified by Gomori's), Bio-Optica (Italy) according to the standard protocol. Reticulin fibers were defined as black or dark brown fibers with gray cores.

Immunohistochemical study. Immune cells infiltrating the tumor were detected immunohistochemically using antibodies against CD3, CD4, CD8, CD20, CD56, and CD163. The dilution of each antibody is 1: 100 for CD3, 1: 50 for CD4 and CD8, and 1: 200 for CD20, CD56, and CD163. Lymph nodes were used as controls.

Morphometric assessment of the relative amount of kelloid-like collagen. Micro-products were anonymized prior to the start of the study, and micro-products

were reviewed without information about clinical data or patient outcomes. *Keloid-like collagen* was evaluated on the invasive tumor site of micro-preparations stained with hematoxylin and eosin and Masson's trichrome. For each case, the most invasive part of the removed tumor was determined ("invasive front"), defined as the area with the deepest tissue infiltration by the tumor or the area where the tumor tissue borders on non-tumor tissue. On the micro-preparation of the most invasive edge of the tumor, 10 fields of view were selected at magnification ×40. Areas of the stroma with localized inflammation, presumably caused by external pathogens, for example, around microscopic abscesses due to intracancer perforation were not taken into account. Stromal cells in areas with artifacts of crushing, necrosis, and inflammation were not evaluated. The field of the microscope minus the tissue to be visually ignored was set to 100%. The amount of kelloid-like collagen in the stroma was estimated in increments of 10% (10%, 20%, 30% etc.) in the field of view. The relative amount of keloid-like collagen for each case was calculated as the arithmetic mean of all obtained values

Morphometric assessment of the histopattern of reticulin fibers. Reticulin fibers were defined as black or dark brown fibers with gray cores. When evaluating reticulin fibers, their localization and location were evaluated. The entire surface of each slice was examined at 100x magnification. Histopathological analysis of reticulin assessed its presence in accordance with a semi-quantitative assessment system ("<5% " - minimal," 6-30% "– focal, " >30%" - diffuse) and native structure ("thin filamentous fibers / thick, unfolded fibers", "clear / blurred borders","ordered / chaotic fiber distribution", "discontinuous / continuous").

Morphometric assessment of histopathological prognostic factors. The stroma-parenchyma ratio (TSR) was evaluated on hematoxylin-and eosin-stained sections of primary tumor tissue with a thickness of 4 microns and analyzed by light microscopy. Micro-preparations of the primary tumor were selected from the most invasive part of the colon adenocarcinoma. The amount of stromal tissue was estimated in increments of 10% (10%, 20%, 30% etc.) on the image field. For statistical analysis, the stromal ratio groups were divided into groups with high and low stroma. A high stroma level is defined as >50% of the stroma area, and a low stroma level is defined as < 50% of the stroma area in the histological section, which is defined a priori as the value that has the maximum discrimination ability. The desmoplastic reaction was evaluated using sections of hematoxylin and eosin-stained tissues, differentiating as mature, intermediate, and immature, as described by Hideki Ueno et al. A desmoplastic reaction was considered immature if myxoid changes were present in the fibrous stroma independently of keloid-like collagen. Otherwise, it was classified as intermediate if the stroma contained keloid-like collagen but no mixoid changes, or mature if the stroma contained neither keloid-like collagen nor mixoid changes. Tumor budding was defined as an isolated cancer cell or a cluster containing <5 cells on the invasive front, and classified as grades BD1, BD2, and BD3 according to international criteria. Vascular invasion was defined as the presence of tumor cells in the muscle layer of blood vessels or invasion in the muscle layer of a vessel or endothelium, invasion of lymphatic vessels was defined as the presence of nests of tumor cells in the lymphatic vessels.

vessels. Perineural invasion was defined as the presence of tumor cells in three layers of the nerve sheath or in the immediate vicinity of the nerve, affecting at least 33% of its entire circumference.

The Klintrup-Mäkinen index was evaluated according to the criteria established by Klintrup K. The inflammatory response was assessed as low if immune cells were absent or there was a slight increase in them on the invasive front. The inflammatory response was assessed as high if there was a ribbon-like or cup-shaped infiltration of inflammatory cells at the invasive edge of the tumor with the destruction of cancer cells.

The percentage of tumor-infiltrating lymphocytes was evaluated by visually assessing the area occupied by mononuclear cells over the stroma area in 10 visual fields, on micro-preparations stained with hematoxylin and eosin, using a threshold of 42% as the limiting percentage of the area occupied by lymphocytes in accordance with the cut-off point established by Iseki Y.

Morphometric assessment of immune cells. For statistical analysis, all tumors were divided into cases with high or low levels of CD3, CD4, CD8, CD20, CD56, and CD163 according to the median ratio. Representative images were obtained by light microscopy using a Zeiss AxioLab 4.0 microscope with the "Image" software.

Methods of statistical data processing. All statistical procedures were performed using Statistica 10.0 and IBM SPSS Statistical 27.0 software (Inc., Chicago, IL, USA, an IBM Company). The analytical framework was developed under the guidance of the Union for International Cancer Control and the Guidelines for Clinical Oncology. To ensure the validity of the conclusions, a power analysis was performed to determine the required sample size to identify significant demographic associations with the desired results, while meeting the usual statistical power threshold of 0.80 or higher. Qualitative and quantitative variables were compared using the $\chi 2$ or Student criteria, respectively (Mann-Whitney or Kruskal-Wallis criteria, when the confidence conditions of the Student and $\chi 2$ criteria were not met). A p-value<0.05 was considered statistically significant.

Survival analysis was performed using Kaplan-Meier curves, and statistically significant differences were determined using a log-rank test. The Kaplan-Meier method was used to calculate overall and relapse-free survival. Univariate and multivariate analyses using the Cox Proportional Hazards regression model were performed to calculate HR and 95% CI.

Main results of the study:

The study included 650 patients diagnosed with colorectal cancer, which allowed us to get a complete picture of the socio-demographic and clinical characteristics of this population. There is a moderate predominance of men (59.7%) in comparison with women (40.3%). The age distribution shows that the majority of patients (63.1%) are in the age group from 24 to 65 years, while the remaining 36.9% are older than 65 years. No gender differences were found in the age structure (p=0.965). In addition, analysis of the KRAS gene mutation status revealed that the vast majority of patients (76.0%) do not have this mutation. There were also no significant differences in the prevalence of the mutation between men and women

(p=0.86). These results demonstrate the relative homogeneity of the study group in the main socio-demographic and clinical parameters.

Results of univariate logistic regression. Age was an important factor influencing cancer progression, with individuals aged 24 to 65 years showing a reduced risk (p=0.04) compared to those over 65 years of age. Thegender marker did not show a statistically significant relationship; men and women had an OR of 0.745 (95% CI=0.253-1.425, p=0.42). KRAS mutations showed a slightly significant association (p=0.02), with positive mutations yielding an OR of 1.024 (95% CI=0.774-1.345). In individuals aged 24-65 years, the probability coefficient (0.977) of exposure to cancer treatment is slightly lower than in those over 65 years of age (OR=1.038). Gender did not have a significant impact on cancer treatment, with the odds ratio of men and women being 0.936 and 1.103, respectively. Age is a crucial factor in cancer outcomes, and in the 24-65-year-old age group, OR=1.038 (95% CI=0.913-1.180) with a slight p-level of 0.566, while As a group of 65+ years, they have OR=0.942 (95% CI=0.769-1.154) with a p-valueof 0.624. Gender does not significantly affect mortality, since men have OR=1.017 (95% CI=0.890-1.162), and women have OR=0.975 (95% CI = 0.890 – 1.162).CI=0.802-1.185). Notably, KRAS mutations, a genetic factor, have no significant association (p=0.776). The influence of tumor morphology is significant: in adenocarcinoma, OR=1.086 (CI=0.887-1.329) and p=0.02, while other morphologies have a lower OR=0.948 (95% CI=0.833-1.078) with a p value pof 0.007.

Results of multivariate logistic regression and showed that the odds ratio for adenocarcinoma is 1.032 (95%CI=0.745-1.429, p=0.03). This suggests a slight association with adenocarcinoma, although the result is not statistically significant. As for the tumor stage, the odds ratio for the first stage is 0.935 (95%CI=0.658-1.327, p=0.02). This indicates a lower probability of being in the first stage compared to the last stage, and the relationship is statistically significant. For tumor infiltration, the odds ratio for low-grade infiltration is 1.216 (95%CI=0.880-1.680, p=0.04). Although the odds ratio indicates an increased probability of low infiltration, the result is not statistically significant. Finally, for lymph node involvement, the odds ratio for < 3 lymph nodes is 0.824 (95%CI=0.582-1.167, p=0.03). The probability coefficient for men is 0.969 (95%CI=0.701-1.339) with p=0.09, which indicates a slight downward trend in mortality compared to women. Tumor morphology, in particular adenocarcinoma, shows a odds ratio of 1.037 (95% CI=0.749-1.434) and p=0.07, indicating a borderline non-certified association with mortality. The tumor stage presents interesting findings, with the first stage showing OR=0.934 (95% CI=0.658-1.324) and p=0.01, indicating a potential protective effect against mortality. Conversely, the last stage does not reach statistical significance, which indicates a more complex relationship between the stage of the tumor and mortality. Tumor infiltration shows a odds ratio of 1.195 (95%CI=0.867-1.648) with a p valueof 0.03 for the "Low" category, which indicates a weak tendency to increase mortality compared to the "High"category.

Regression analysis of overall survival and relapse-free survival using the Cox proportional hazards model showed that for patients in whose tumor the relative amount of keloid-like collagen in the tumor stroma was more than 30%, the risk ratio

of relapse was 3.16 (95%CI=1.480-6.749; p=0.003) and death was 2.01 (95%CI=1.198-3.369; p=0.008) compared to patients whose tumors detected less than 30% kelloid-like collagen. For the cut-off threshold of kelloid-like collagen in the tumor stroma, other than 30%, no statistically significant differences were found in the overall and disease-free survival of patients in the study sample (p>0.05). The results showed that the presence of more than 30% kelloid-like collagen in the stroma of the invasive edge of the tumor correlates with the presence of such an aggressive clinical sign as T-stage (p=0.009).

Univariate and multivariate regression analysis of prognostic factors for overall survival and disease-free survival showed the following: the p-risk of death in patients with diffuse kelloid-like collagen was 3.3 (95% CI=1.9-5.72) times higher than in patients with focal kelloid-like collagen (p<0.001).

Disease-free survival was significantly lower among patients with diffuse kelloid-like collagen compared to patients with focal kelloid-like collagen (HR=2.92, 95%CI=1.88-4.53, p<0.001).

In the group with kelloid-like collagen comprising Type A<30% of the stroma, the following indicators were determined: one – year overall survival was 86.5%, three – year overall survival was 78%, five – year overall survival was 76.3%; one – year disease-free survival was 86.5%, three-year disease-free survival was 71.5%, and five-year disease-free survival was 64.2%.

In the group with kelloid-like collagen, which is Type B>30% of the stroma, the following indicators were determined: one – year overall survival was 75.7%, three – year overall survival was 54.6%, five – year overall survival was 45.9%; one – year relapse-free survival was 70.1%, three-year relapse-free survival was 40.2%, and five-year relapse-free survival was 30.4%.

Patients with high lymphocyte infiltration (HR=0.499, 95%CI = 0.318-0.785, p=0.003) compared to patients with lower levels of lymphocytic infiltration in their tumors. Peritumoral lymphocytic response (RR = 0.616, 95%CI=0.434-0.875, p=0.007), Crown-like lymphoid response (RR=0.686, 95%CI=0.475-0.990, p=0.044), Klintrup–Mäkinen index (RR=0.494, 95%CI=0.292-0.835, p=0.008) and the percentage of lymphocytes infiltrating the tumor (HR=0.570, 95%CI=0.363-0.894, p=0.014) were also significant prognostic factors for disease-free survival.

Patients with diffuse kelloid-like collagen in the tumor stroma (stroma histophenotype Type B (kelloid-like collagen >30%) had a markedly higher risk of recurrence (HR=2.741, 95%CI=1.753-4.286, p=0.004) than patients with focal kelloid-like collagen (stroma histophenotype type A (kelloid-like collagen ≤0.3).

Crown-like lymphoid response, Klintrup–MiKinep index, and the percentage of tumor-infiltrating lymphocytes were not predictive factors for an unfavorable outcome, in contrast to the structural pattern of venous invasion (HR=1.669, 95%CI=1.054-2.642, p=0.029) and the distribution of keloid-like collagen in the tumor stroma.

Multivariate regression analysis of prognostic factors for disease-free survival using the Cox proportional hazards model showed that diffuse distribution of collagen in the stroma of the invasive tumor margin (stroma histophenotype Type B

(kelloid-like collagen ≥0.3) is an independent factor significantly associated with disease-free survival (HR=2.019, 95%CI=1.201-3.395, p=0.008).

Multivariate regression analysis of predictive factors of overall survival using the Cox proportional hazards model showed that diffuse distribution of collagen in the stroma of the invasive tumor margin (stroma histophenotype Type B (kelloid-like collagen ≥0.3) is an independent factor significantly associated with overall survival (HR=2.325, 95%CI=1.241-4.354, p=0.008).

Thus, these results demonstrated that a large amount (more than 30%) of kelloid-like collagen in the stroma of colorectal adenocarcinoma is independently associated with a poor prognosis for patients.

Сравнительный aComparative analysis of CD3, CD4, CD8, CD20, CD56, and CD163 positive immune cells in groups with focal and diffuse colloid-like collagen content in the invasive edge of colorectal adenocarcinoma showed that theresults of histochemical studies of immune cells in the stroma of colorectal cancer stratified by the content of kelloid-like collagen in the invasive edge of the tumor, in the subgroup with focal content of CD3 – positive immune cells were higher than the median value of the group (n=175) in 44 (62.5%) tumors in the invasive edge of the tumor with an intermediate desmoplastic reaction, and lower in 29 (37.2%) tumors.

CD4 positive immune cells were higher than the median value of the group in 41 (52.6%) cases, lower – in 37 (47.4%) cases. CD8 positive immune cells were higher than the median value of the group in 59 (75.6%) cases, lower – in 19 (24.4%) cases. CD20 positive immune cells were higher than the median value of the group in 40 (51.3%) cases, lower – in 38 (24.4%) cases.CD56-positive immune cells were higher than the median value of the group in 9 (11.5%) cases, lower – in 8 (10.3%) cases. CD163 positive immune cells were higher than the median value of the group in 17 (21.8%) cases, lower – in 61 (78.2%) cases.

In the subgroup with diffuse content of kelloid-like collagen in the invasive edge of the tumor with an intermediate desmoplastic reaction, CD3 positive immune cells were higher than the median value of the group (n=175) in 39 (40.2%) tumors, lower – in 58 (59.8%) tumors. CD4 positive immune cells were higher than the median value of the group in 47 (48.5%) cases, lower – in 50 (51.5%) cases. CD8 positive immune cells were higher than the median value of the group in 29 (29.9%) cases, lower – in 68 (70.1%) cases. CD20 positive immune cells were higher than the median value of the group in 48 (49.5%) cases, lower – in 49 (50.5%) cases. CD56 positive immune cells were higher than the median value of the group in 8 (8.2%) cases, lower – in 9 (9.3%) cases, were not detected in 80 (82.5%) cases. CD163 positive immune cells were higher than the median value of the group in 71 (73.2%) cases, lower – in 26 (26.8%) cases.

Conclusions:

1. In Aktobe region, based on the data of 650 patients, 59.7% of them are men and 40.3% are women. Age (>65 years, p=0.04), tumor stage (stage IV, p=0.046), infiltration depth (p=0.001), and metastatic status (83.4% of patients had metastases, p=0.02) have significant effects on disease progression and mortality. The main methods of treatment were surgical resection in combination with chemotherapy

(49.1%) or radiation therapy (50.9%), while the absence of metastases increased the probability of successful treatment (OR=1.764, p=0.002). The results obtained emphasize the need for an individualized approach to the diagnosis and treatment of CRC, taking into account key prognostic factors.

2. Aprognostically significant threshold level of relative CPC content in the stroma of an invasive region with an intermediate desmoplastic reaction was established based on the maximum discriminative ability for overall survival and relapse - free survival-30%. A PDA of more than 30% is associated with poorer overall and disease-free survival rates compared to patients with tumors with less than 30% PDA.

3. Diffuse CCP histophenotype (>30%) is an independent risk factor for postoperative relapse (RR=2.019 95% CI=1.201-3.395, p=0.008); risk of death (RR=2.325, 95% CI=1.241-4.354, p=0.008). It correlates with T-stage (p=0.038), N-stage (p=0.002), G-stage (p=0.034), venous (p=0.007) and lymphatic invasion (p=0.006); it is inversely correlated with a high degree of lymphocytic (p=0.001), intra-tumor perigranular (p=0.009) and peritumoral lymphocytic infiltration (p=0.001), high Klintrup-Mäkinen index (p=0.01). Patients with focal kelloid-like collagen content in the tumor stroma (<30%) were characterized by a longer relapse-free period than patients with diffuse content (more than 30%) (p <0.01).

4. In the focal type of CCP (<30%), the percentage of high $CD3^+$ and $CD8^+$ cells is more common than in the diffuse type (p=0.003 and p <0.0001), which is characterized by longer overall and disease-free survival. In tumors with diffuse CCP type (>30%), the number of cases with a high content of^{CD163+} cells is more common compared to focal (p<0.0001), this is consistent with data on the association of high CD163 expression with greater macrophage infiltration and is associated with a worse prognosis.