



**MEDICAL UNIVERSITY – PLEVEN
FACULTY OF MEDICINE
DEPARTMENT OF NEPHROLOGY, HAEMATOLOGY AND
GASTROENTEROLOGY**

DR DILIANA STEFANOVA PETROVA, MD

**COLORECTAL CARCINOMA –
CLINICOMORPHOLOGICAL,
LABORATORY AND IMAGING CORRELATIONS
WITH PROGNOSTIC VALUE
IN DIFFERENT LOCALIZATIONS**

A U T O R E F E R A T

OF DISSERTATION

**for awarding an educational and scientific degree
„DOCTOR”**

Doctoral Program: Gastroenterology

Scientific supervisor: Assoc. Prof. Dr. Zornitsa Gorcheva, MD, PhD

Pleven, 2026

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Official Reviewers

**Prof. Dr Rosen Evgeniev Madjov, MD, DScs
Prof. Dr Vladimir Nikolov Andonov, MD, PhD**

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The official defence of the dissertation will take place on 10.11.2026 at 13.00 in the "Ambroise Pare" hall, Medical University - Pleven according to Order of the Rector of MU-Pleven No. 2872/15.09.2026, in front of scientific jury composed of:

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The defense materials are published on the website of MU-Pleven www.mu-pleven.bg

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Abbreviations:

CRC – Colorectal Carcinoma
IARC – International Agency for Research on Cancer
WHO – World Health Organization
HDI – Human Development Index
ACS – American Cancer Society
FAP – Familial Adenomatous Polyposis
DNA – Deoxyribonucleic Acid
RNA – Ribonucleic Acid
PJS – Peutz-Jeghers Syndrome
MAP – MYH-Associated Polyposis
BMI – Body Mass Index
FIT – Fecal Immunochemical Test
FOBT – Fecal Occult Blood Test
IBD – Inflammatory Bowel Disease
CIBD – Chronic Inflammatory Bowel Disease
DCRC – Right-Sided Colorectal Carcinoma
LCRC – Left-Sided Colorectal Carcinoma
MSI – Microsatellite Instability
CIN – Chromosomal Instability
AJCC – American Joint Committee on Cancer
CT – Computed Tomography
NMR – Magnetic Resonance Imaging
TRUS – transrectal ultrasound
UC – ulcerative colitis
CD – Crohn's disease
GIT – gastrointestinal tract
ESR – erythrocyte sedimentation rate
CRP – C-reactive protein
FCP – fecal calprotectin
M2-PK – M2-pyruvate kinase
MPO – myeloperoxidase
MMP – matrix metalloproteinases
FL – fecal lactoferrin

INTRODUCTION

Colorectal cancer (CRC) is the second most deadly and the third most commonly diagnosed cancer worldwide. CRC is a significant public health challenge. In 2020, CRC accounted for 9.4% of cancer-related deaths. Due to the significant increase in cases among the elderly population, the global incidence of CRC is projected to more than double by 2035, with the largest increase expected in less industrialized countries. By 2040, the burden of CRC will increase to 3.2 million new cases per year (a 63% increase) and 1.6 million deaths per year (a 73% increase). The incidence of CRC is progressively escalating worldwide. Genetic and environmental factors have a significant influence on an individual's susceptibility to CRC. Furthermore, the risk of CRC increases with age and in people suffering from chronic ulcerative colitis and Crohn's disease. Studies have shown that factors predisposing individuals to colorectal cancer include dietary habits, lifestyle, family medical predisposition and persistent inflammation. The development of CRC is a gradual process, usually taking more than ten years for a polyp to transform into a malignant tumor. Therefore, early detection and removal of polyps through regular screening are essential for the prevention of colorectal cancer. Currently available diagnostic methods can detect less than 30-40% of cases in the early stages, with a risk of recurrence after surgery and treatment. Although chemotherapy drugs target cancer cells, they also damage healthy cells. This can lead to resistance in most CRC patients, reducing the effectiveness of anticancer drugs and ultimately leading to chemotherapy failure. It is therefore of utmost importance to engage in a comprehensive dialogue on the epidemiology, risk factors and preventive measures for colorectal cancer, taking into account the latest evidence-based knowledge. This will allow us to effectively address the upcoming challenges associated with this disease.

To effectively minimize the occurrence of CRC and reduce mortality on a larger scale, screening of individuals at average risk is the most successful approach. Population-based screening initiatives have been introduced in a number of European countries, Canada and parts of Asia, Oceania, North and South America. Eligibility for screening for CRC is determined by age and geographical location. Through population-based screening (population-based screening), it becomes possible to identify individuals at average risk (with hidden diseases), allowing timely treatment and reduction of risks for both individuals and communities.

Screening of people at average risk is the most effective way to prevent CRC and lower mortality overall. As a result, some European countries, Canada, and certain regions of Asia, Oceania, and North and South America have initiated population-based screening programs. Eligibility for screening for CRC depends on life expectancy and geographic location. Microsimulation modeling results indicate a decline in CRC morbidity and mortality in the United States due to the implementation of screening programs. In addition, population-based screening seeks to detect latent disease in the average-risk population, facilitating timely interventions and mitigating risks to individuals and communities. The most effective way to reduce CRC and lower mortality on a larger scale is through individual assessment. The goal of population-based screening is to identify undetected disease in average-risk individuals, allowing for rapid interventions and minimizing risks to both individuals and society. In addition, it is essential to adopt complementary successful approaches that include identification and surveillance of high-risk groups. These groups include patients with inflammatory bowel disease, families with hereditary CRC syndromes, individuals with a familial predisposition to CRC but without identifiable genetic markers, and those with phenotypic markers indicating increased risk. The metabolome of patients suffering from cancer and recurrent infections has been found to exhibit significantly higher homocysteine/methionine and homocysteine/thiodiglycolic acid ratios compared with age-matched healthy controls. Therefore, the metabolome has played a crucial role in uncovering key biological processes affected by genetic variation. It is also important to consider that the metabolome varies with time, pathology, developmental stage, progression, drug treatment, dietary intervention, environmental factors, and even the microbiome. It is acknowledged that simultaneous and accurate analytical quantification of the metabolome remains a challenge.

Currently, cancer treatment has been significantly transformed by immunotherapeutic approaches using immune checkpoint inhibitors and adoptive cell therapy (ACT), in addition to chemotherapy. Neoadjuvant therapy involves the administration of radiotherapy, chemotherapy, and a combination of

different treatment methods before surgery. Adjuvant systemic therapy can reduce relapses and improve survival by killing cells that have escaped from the primary tumor bed through the lymphatic and blood vessels. This has led researchers to suggest that the administration of systemic therapy before surgery (neoadjuvant) may improve outcomes, as it would destroy undetectable microscopic circulating cells. This approach aims to downgrade the tumor stage, thereby reducing the likelihood of local recurrence and improving the overall prognosis.

In recent years, there has been an unfavorable trend in the epidemiological indicators of CRC. Screening for CRC is one of the preventive measures aimed at reducing mortality. Crucial to the creation of an effective CRC prevention program is its comprehensive nature, including information on risk factors for CRC and optimizing lifestyle and nutrition to prevent the development of the disease. Screening for CRC is developing rapidly, increasingly based on innovative technologies, requiring significant organizational and financial costs, but can significantly reduce mortality from CRC. The topic of early screening for CRC (convenient for clinical practice), aimed at improving prognosis, is also the subject of the present work.

AIM:

To study clinical laboratory, morphological and imaging characteristics of colorectal carcinoma and establish correlations with prognostic value in different locations.

TASKS:

1. Study of demographic and clinical characteristics of patients with colorectal carcinoma of different localizations.
2. Study of basic laboratory indicators in patients with colorectal carcinoma of different localizations (blood count – hemoglobin, leukocytes; C-reactive protein).
3. Study of fecal calprotectin and fecal M2-pyruvate kinase in patients with colorectal carcinoma of different localizations.
4. Study of results of morphological, instrumental and imaging diagnostic methods in patients with colorectal carcinoma of different localizations.
5. To analyze the results of the above studies in order to establish correlations with prognostic value in different localizations.
6. Development of a diagnostic algorithm for screening patients with colorectal carcinoma, specifying the need for fibrocolonoscopy and facilitating early diagnosis and prognosis.

DESIGN:

1. Retro-prospective study of demographic, clinical, laboratory indicators, results of morphological, instrumental and imaging diagnostic methods in patients with colorectal carcinoma of different localizations (group 1 – target group), patients with Crohn's disease and ulcerative colitis (group 2) and patients with irritable bowel syndrome (colon irritable) (group 3). Patients from group 2 and group 3 were used as controls for patients from group 1.

1.1. Retro-prospective study of demographic, clinical, laboratory indicators, results of morphological, instrumental and imaging diagnostic methods in patients with colorectal carcinoma of different localizations (group 1 – target group)

1.2. Retro-prospective study of demographic and clinical indicators in patients from group 2 and group 3

1.3. Retro-prospective study of laboratory indicators (PCK – hemoglobin, leukocytes; C-reactive protein) in patients from the three groups

1.4. Prospective study of results of fecal calprotectin and fecal M2-pyruvate kinase tests in patients from the three groups

2. Comparative studies of demographic, clinical, laboratory indicators (PCK – hemoglobin, leukocytes; C-reactive protein; fecal calprotectin, fecal M2-pyruvate kinase) in the target group:

2.1. By age and sex

2.2. By localization of colorectal carcinoma

2.3. By stage of colorectal carcinoma

3. Comparative studies of laboratory indicators (PCK – hemoglobin, leukocytes; C-reactive protein; fecal calprotectin, fecal M2-pyruvate kinase) in patients with colorectal carcinoma (group 1) with 1) patients from group 2; 2) patients from group 3.

MATERIALS:

1. Subjects of the study – 90 patients, divided into 3 groups (listed above) of 30 patients per group.

The study period is 3 years – covering 2018-2020. The study is retro-prospective, with the retrospective part covering a period of two years – 2018-2019 inclusive, and the prospective part – a one-year period – 2020. The study is monocentric and covers patients over the age of 18, hospitalized as an emergency or planned in the clinics of Gastroenterology, Hematology, Oncosurgery, First Surgical Clinic of the University Hospital for Active Treatment “Dr. G. Stranski” EAD – Pleven and who underwent diagnostic **fibrocolonoscopy** in the Gastro-diagnostic Department of the Gastroenterology Clinic of the University Hospital “Dr. G. Stranski” EAD – Pleven. The team has permission from the hospital ethics committee to conduct the study, and no ethical problem has been identified regarding the design of the study and the patients included in the study.

2. Criteria for inclusion in the study:

2.1. Study (target) group – patients with colorectal carcinoma of various stages and localizations: 30 individuals

- age over 18 years;
- hospitalized urgently or planned in the clinics of Gastroenterology, Hematology, Oncosurgery, First Surgical Clinic of the University Hospital for Active Treatment "Dr. G. Stranski" EAD – Pleven;
- underwent diagnostic fibrocolonoscopy in the Gastro-diagnostic Department of the Gastroenterology Clinic of the University Hospital "Dr. G. Stranski" EAD – Pleven;
- lack of established current pregnancy among the female subjects of reproductive age;

2.2. Control groups – group 2 (30 patients) and group 3 (30 patients) – total 60

- age over 18 years;
- absence of established current pregnancy among the studied female subjects of reproductive age.

3. Demographic, clinical and laboratory indicators studied; imaging and pathoanatomical characteristics.

History and objective status – collection of information about the onset, course and development of the disease and the presence of a family history. The main sources of this information are hospital history of the disease, epicrisis and hospital information system Gamma Code master. We used a questionnaire created according to the classification of Rasmussen S (2015), and the symptoms reported by the patient were divided into two groups: specific and non-specific. Specific symptoms were defined as abdominal pain, change in stool consistency, stool frequency, rectal bleeding. Nonspecific symptoms of concern were defined as: diarrhea, constipation, abdominal bloating, weight loss, fatigue. Patients were asked whether they had one or more of these symptoms in the last 4 weeks. When interpreting the results in relation to clinical characteristics, we assumed the following anatomical sections of the large intestine for the right colon – cecum, ascending colon and hepatic flexure; transverse colon (colon transversum) and left colon – covering the left flexure, descending colon and sigmoid colon, while the rectum remains an independent anatomical end of the lower intestinal tract.

In the clinical laboratory, microbiology laboratory, imaging diagnostics, pathoanatomy units located on the territory of the University Hospital “Dr. Georgi Stranski” EAD – Pleven, all necessary basic laboratory, imaging diagnostics and pathoanatomical examinations are performed, as provided for in the methodological guidelines of the Ministry of Health, according to the characteristics and needs of the patient in a specific situation. These include examination of general clinical-laboratory, imaging and immunological indicators: PKC, liver (AST, ALT), renal indicators (urea, creatinine), lipid profile, CRP, glucose, urine; microbiological examination of a fecal sample for pathogenic bacteria and fungi; instrumental examinations – fibrocolonoscopy, ultrasound of abdominal organs, CT of abdominal organs; pathoanatomical examinations – specimens in standard paraffin blocks, stained with hematoxylin and eosin, immunohistochemical examinations. The financing of routine laboratory indicators and instrumental examinations is through the relevant clinical pathways within the framework of the hospital treatment of patients.

The funding of the studies of fecal calprotectin and fecal M2-pyruvate kinase in the three groups of patients was after an approved research project No. D2/ 2024 of the Medical University of Pleven, and the studies were performed in the Clinical Laboratory of the University Hospital "Dr. Georgi Stranski" – EAD, Pleven.

3.1. Tested indicators and characteristics in the three groups:

3.1.1. Gender

3.1.2. Age

3.1.3. Registration of subjective complaints

3.1.4. Blood count

3.1.5. C-reactive protein values

3.1.6. Fecal calprotectin and fecal M2-pyruvate kinase values

3.1.7. Descriptions of results from abdominal ultrasounds

3.1.8. Descriptions of results from fibrocolonoscopies

3.1.9. Morphological characteristics of colorectal tumors, determined by histological studies

3.2. Investigated indicators and characteristics only in the colorectal carcinoma group

3.2.1. Morphological characteristics of colorectal tumors established by histological studies

METHODS:

1. Sociological method for collecting information.

1.1. Interview – an additional questionnaire was developed to collect data on demographic characteristics of the subjects studied. Questionnaires and an informed consent form were designed, tailored to the patients from the three study groups (*Appendices 1, 2, 3*).

2. Documentary method (medical documents) for collecting data on the health status of patients – filling in demographic, clinical, laboratory indicators and family burden in the questionnaires by the patients in the three study groups.

3. Clinical method – the studied patients were clinically examined (taking a detailed history and physical examination).

4. Laboratory methods – venous blood testing for non-specific hematological and biochemical indicators (complete blood count, C-reactive protein, fecal calprotectin and fecal M2-pyruvate kinase) using relevant laboratory methods – for the target and control groups.

4.1. Complete blood count (CBC) – using a MEDONIC M32 hematology analyzer, Boule. Reference values: hemoglobin (Hg) – men 135-180 g/L; women 120-160 g/L] erythrocytes (Er) – men 4.4-5.9 x 10¹²/L; women 3.7-5.3 x 10¹²/L] leukocytes (WBC) – 3.5-10.5 x 10⁹/L; platelets (PLT) – 130-360 x 10⁹/L. Regarding the definition of anemia criteria, we have adhered to the definitions of the World Health Organization for anemia – in women (non-pregnant) – hemoglobin level below 120 g/L, and for men – below 130 g/L. Hemograms were made on a hematology analyzer Advia/Simens/.

4.2. Biochemical parameters – examined with a biochemical analyzer Cobas 6000, Roche.

4.2.1. C-reactive protein – by immunoturbidimetric test. Human CRP agglutinates with latex particles coated with anti-CRP antibodies. The precipitate is determined turbidimetrically and the absorption is proportional to the concentration of CRP. Reference values: 0.00-5.00 mg/L

4.2.2. Fecal calprotectin – by latex-turbidimetric test for quantitative determination in human feces samples Reference values: 0-50 U/mL – negative result; 50-200 U/mL – borderline result; >200 U/mL – presence of inflammation of the gastrointestinal tract.

4.2.3. Fecal M2-pyruvate kinase – by enzyme-linked immunosorbent assay (ELISA). Reference values: norm <4 U/mL.

4.3. Pathoanatomical studies of specimens in standard paraffin blocks, stained with hematoxylin and eosin, immunohistochemical studies.

4.4. Instrumental and imaging methods: abdominal ultrasound, fibrocolonoscopy with biopsy, abdominal organs with contrast.

4.4.1. Abdominal ultrasound (in all patients) Two Aloka ProSound Alpha 7 devices with a convex transducer with a frequency of 3.5-5 mHz were used.

4.4.2. Fibrocolonoscopy – was performed with an endoscope model TYPE CF-Q165L from the company "Olimpys".

4.4.3 Computed axial tomography (CAT) – performed with two Multidetector /4 rows/ computed tomograph “CT Bright Speed SE”, General Electric.

5. Statistical methods

The data were entered and processed with the statistical packages IBM SPSS Statistics 21.0 and MS Excel v. 2010. The following methods of medical statistics were used:

5.1. Descriptive statistical methods

5.1.1. Determination of relative shares of qualitative characteristics;

5.1.2. Determination of indicators of central tendencies for quantitative variables – calculation of arithmetic averages (mean); calculation of mode (mode) and median (median) for uneven variation series.

5.1.3. Determination of dispersion indicators – standard deviations (sd) and confidence intervals (CI);

5.2. Comparative statistical analyses

5.2.1. Comparison of means and qualitative features (t-test). Statistical significance – at $p < 0.05$.

5.2.2. Kruskal–Wallis test – 3-group comparison

5.2.3. One-way analysis of variance (ANOVA) with unequal complex. Statistical significance – at $p < 0.05$.

5.2.4. Mann-Whitney U test, Bonferroni corrected – comparison of groups in pairs

5.2.5. Correlation heat map (Pearson).

5.2.6. Boxplot by groups

5.2.7. Violin plots by groups

5.2.8. Bland–Altman: Calprotectin vs CRP (log10); M2-pyruvate kinase vs CRP (log10) and calprotectin vs M2-pyruvate kinase (log10)

5.2.9. Spearman_r test

5.2.10. Spearman_p test

5.2.11. Dunn post-hoc

5.2.12. Tables and graphs were used to illustrate the statistical results.

RESULTS

Chapter I. Studies of demographic, topographic, clinical, laboratory, imaging and histological characteristics of patients with colorectal carcinoma (general group)

For the period 2018-2020, 3795 patients underwent diagnostic fibrocolonoscopy at the gastrodiagnostic department (GDO). Colorectal carcinoma (CRC) was proven in 206 of them, representing 5.4% of all endoscopic patients (*Table 1*).

Table 1. Fibrocolonoscopies findings

	Type of pathology in FCS	Number of patients (n)	%
1	Colon polyps	1025	27%
2	Ulcerative colitis	165	4.4%
3	Crohn's disease	53	1.4%
4	Diverticulosis	873	23%
5	Irritable bowel syndrome	702	18.5%
6	Colorectal carcinoma	206	5.4%
7	Normal fibrocolonoscopy	771	20.3%
	Total	3795	100%

Of the patients with proven malignant disease of the colon and rectum, the youngest was 39 years old, and the oldest was 89 years old. The average age of the studied patients with CRC was 67 ± 5.7 years.

In terms of gender structure, men with proven CRC were 118 (57.3%), and women – 88 (42.7%).

Regarding the localization of the tumor process along the lower intestinal tract, the distribution was as follows – carcinoma of the cecum – 22 patients (10.6%); carcinoma of the ascending colon – 33 (16.0%); carcinoma of the hepatic flexure – 12 (5.8%); of the transverse colon – 16 (7.8%); flexure of the splenic flexure – 18 (8.7%); colon descent – 17 (8.2%); sigmoid colon – 84 (40.8%); rectum – 4 (1.9%) ($p < 0.0005$). The distribution of the respective localization of the tumor process along the course of the colon among both sexes is shown in **Fig. 1** and **Fig. 2**.

According to the topographic criterion, the male sex prevails over the female sex in terms of localization of CRC in the left colon, the result being statistically significant ($p < 0.01$). This relationship of prevalence of male over female sex is most pronounced in terms of localization of the tumor process in the sigmoid colon ($p < 0.01$). In women, more frequent localization of CRC is found in the cecum and in the flexure of the splenic flexure, but the result is not statistically significant ($p > 0.05$). With equal share of involvement in both sexes is the localization of CRC in the transverse colon and rectum ($p > 0.05$).

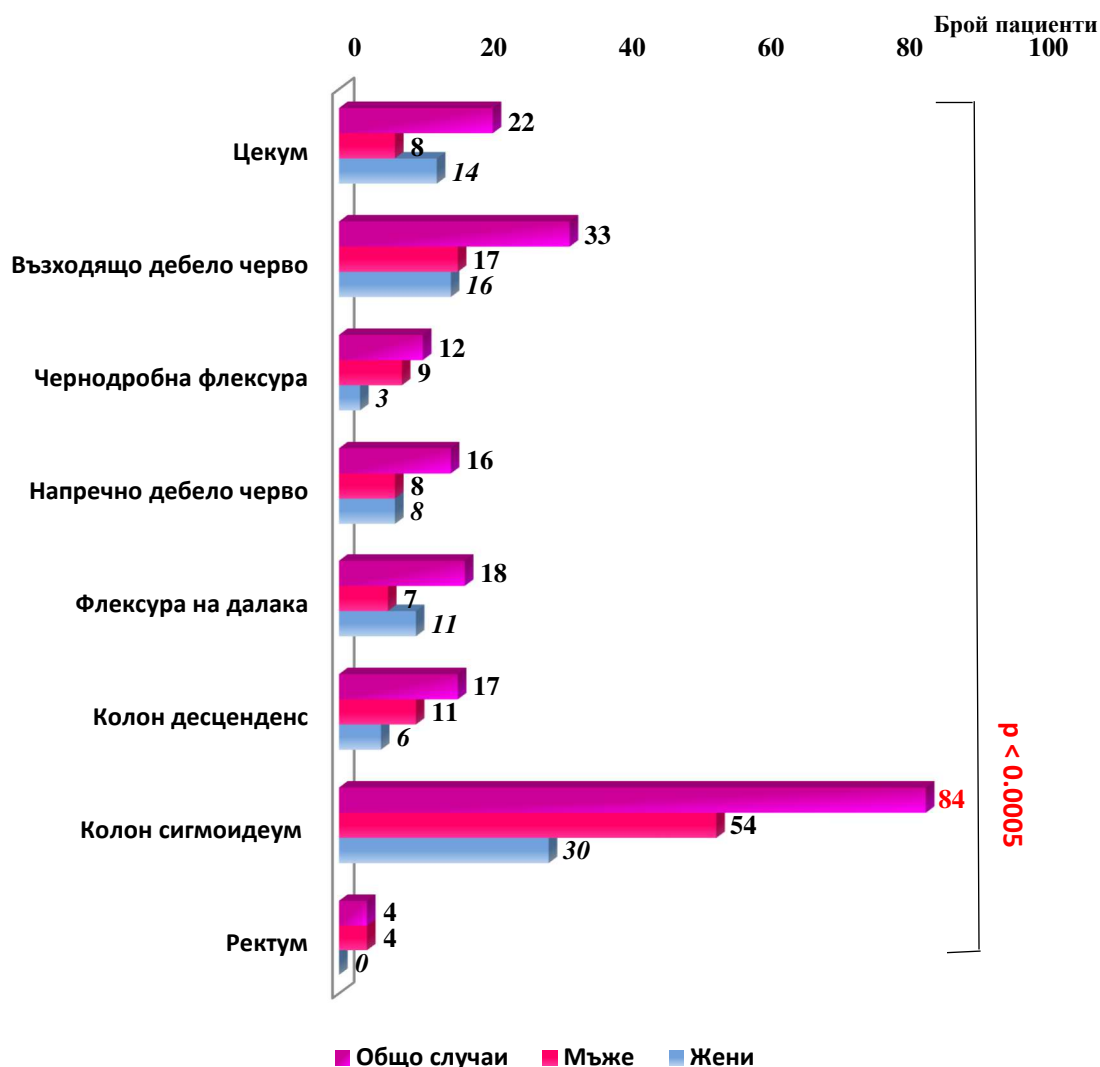


Fig. 1. Distribution of patients with CRC by localization in the lower intestinal tract and by gender

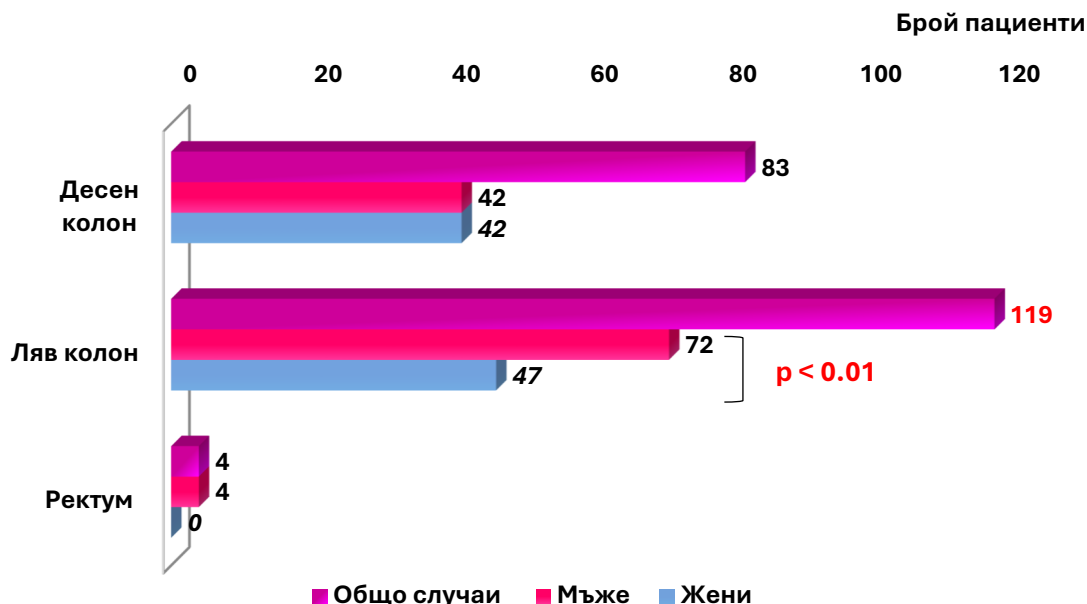


Fig. 2. Distribution of CRC by localization in the lower intestinal tract and by gender – generalized topogram

Regarding the anamnestic data, the most common symptoms that led patients to hospital treatment were included in a special questionnaire, focusing on the presence of the following specific and non-specific symptoms:

- Specific symptoms: colicky abdominal pain, change in stool consistency, frequency of defecation, presence of blood in the stool.
- Non-specific symptoms: constipation, diarrhea, flatulence, weight loss, adynamia, feeling of incomplete bowel emptying, dyspeptic syndrome with manifestations of nausea and vomiting.

The results regarding the localization of the process along the course of the colon and the manifestations of specific and non-specific symptoms among the patients are presented in **Fig. 3**, **Fig. 4** and **Fig. 5**.

According to the topographic criterion, the most common specific symptoms that led patients to a diagnostic FCS were localized in the left colon, and the result was statistically significant in our study ($p < 0.01$). Colic-like pain and the appearance of blood in the stool are leading and statistically significant data from the anamnesis of patients with CRC from the left parts of the colon ($p < 0.01$), and the change in the consistency of the stool and the changes in the intervals and frequency of defecation are the most frequently reported when CRC is localized in the right parts ($p < 0.02$). In the localization of CRC in the rectum, symptoms such as the presence of rectoragia, changes in the consistency of the stool, tenesmus were reported equally often and in combination.

The laboratory monitoring that we have carried out is in all patients with proven CRC, by examining the PBC. The presence of leukocytosis (highest relative proportion in the sigma) and variously pronounced anemic syndrome (highest relative proportions in the sigma) was found, and in terms of a marker for a nonspecific inflammatory reaction, we examined C-reactive protein (CRP) (also the highest relative proportion in the sigma) ($p < 0.02$) (**Fig. 6**).

In terms of the manifestation of laboratory data for leukocytosis, anemia and elevated CRP values – all three laboratory indicators prevail and show their highest value in the group of patients with CRC in the left colon, which is a statistically significant result ($p < 0.01$). In this localization, the sigmoid colon is most often affected ($p < 0.01$). In the localization of CRC in the right colon, the most frequent laboratory abnormality is anemic syndrome ($p < 0.01$), followed by elevated CRP levels ($p < 0.02$) and to a lesser extent the manifestation of leukocytosis ($p < 0.03$) (**Fig. 7**).

In all patients with CRC, we performed abdominal ultrasound, and in the different localizations of the tumor process along the colon, we recorded cases with normal ultrasound and one patient proving dissemination of the main process in the liver parenchyma. Out of a total of 206 abdominal ultrasounds performed, in 41 of them (19.9%) metastatic lesions in the liver were visualized. In a detailed analysis,

according to the different localization of CRC, we obtained the following results – in tumors of the cecum, the proportion of positive findings for metastases from abdominal ultrasound was 12.2%; in tumors of the ascending colon – 9.76%; in the hepatic flexure – 4.88%; in the transverse colon – 14.63%, in the splenic flexure – 9.76%; in the descending colon – 7.32%; in the sigmoid colon – 41.46%. In all diagnosed cases of rectal cancer, no liver metastases were detected by ultrasound (**Fig. 8**). The structure of ultrasound scans with metastases regarding CRC localizations is presented in **Fig. 9**.

Regarding the results of the ultrasound scans performed on the right colon, 11 out of 56 ultrasound scans showed liver metastases (19.6%). In transverse colon carcinomas, 6 out of 10 ultrasound scans showed metastases (60%), and on the left colon, 24 out of 95 ultrasound scans showed positive findings for liver dissemination (21.3%).

Of all 206 cases with proven neoplasia of the lower intestinal tract, 203 (99%) had colorectal adenocarcinoma. Regarding its differentiation, we obtained the following results – in 87 patients, highly differentiated adenocarcinoma was proven – 42.2%, in 66 – moderately differentiated (32.0%), in 50 – poorly differentiated (24.3%). In three patients, a neuroendocrine malignant tumor was proven (1.5%). A detailed picture of the histological results and sub-localization of adenocarcinoma along the colon is shown in **Fig. 10** and **Fig. 11**.

When summarizing the data according to the topographic principle, we obtain a statistically significant result in the group of carcinomas with localization in the left colon, where the relative shares of cases with highly and poorly differentiated adenocarcinomas prevail significantly compared to the groups of CRC with other localization ($p < 0.01$). In CRC with right localization, the highest relative share is the moderately differentiated and highly differentiated histological variants ($p < 0.01$). In CRC with left localization, the highest relative share is the highly differentiated tumors, followed by poorly differentiated ones. Rectal tumors are only moderately differentiated G2.

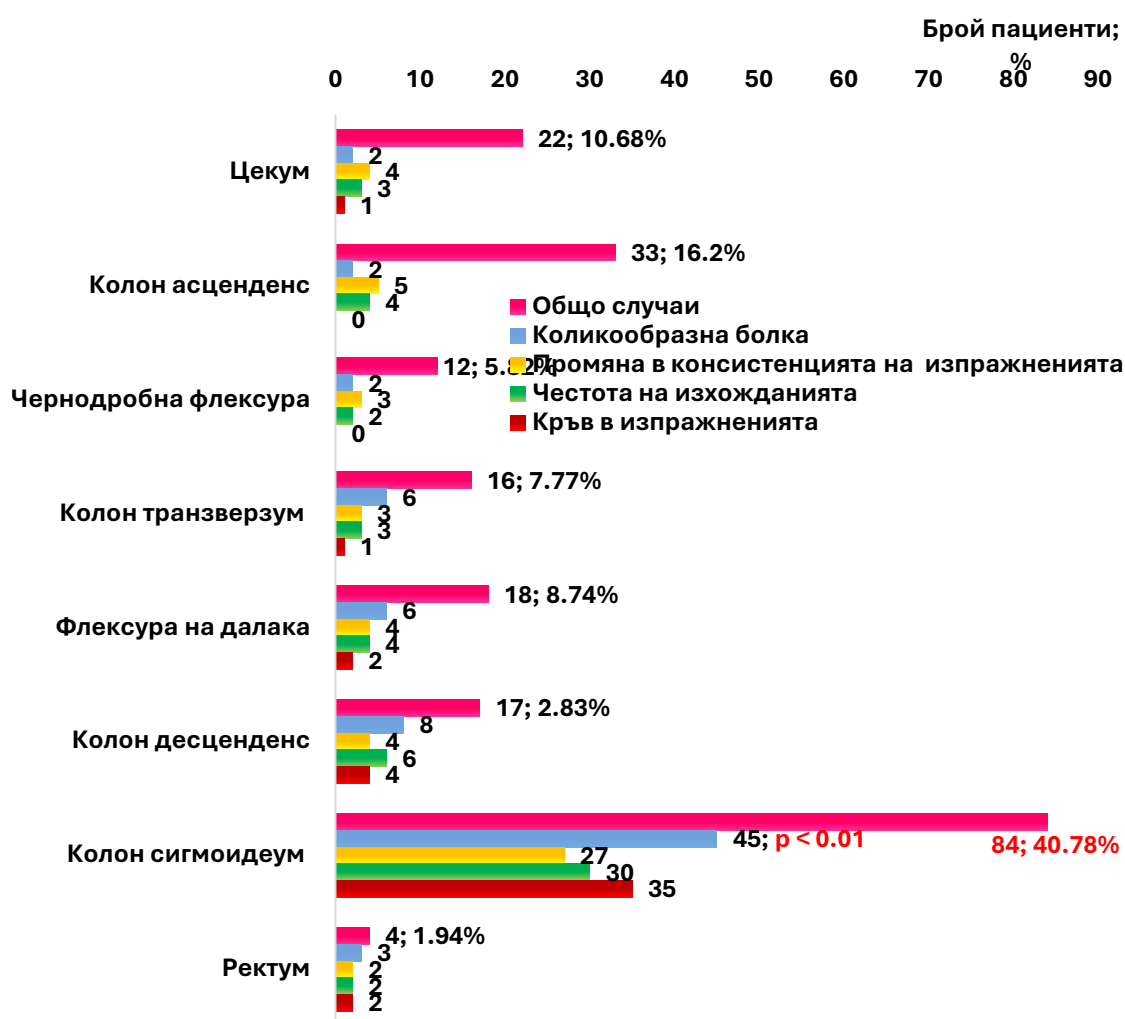


Fig. 3. Specific symptoms in different locations of colorectal carcinoma

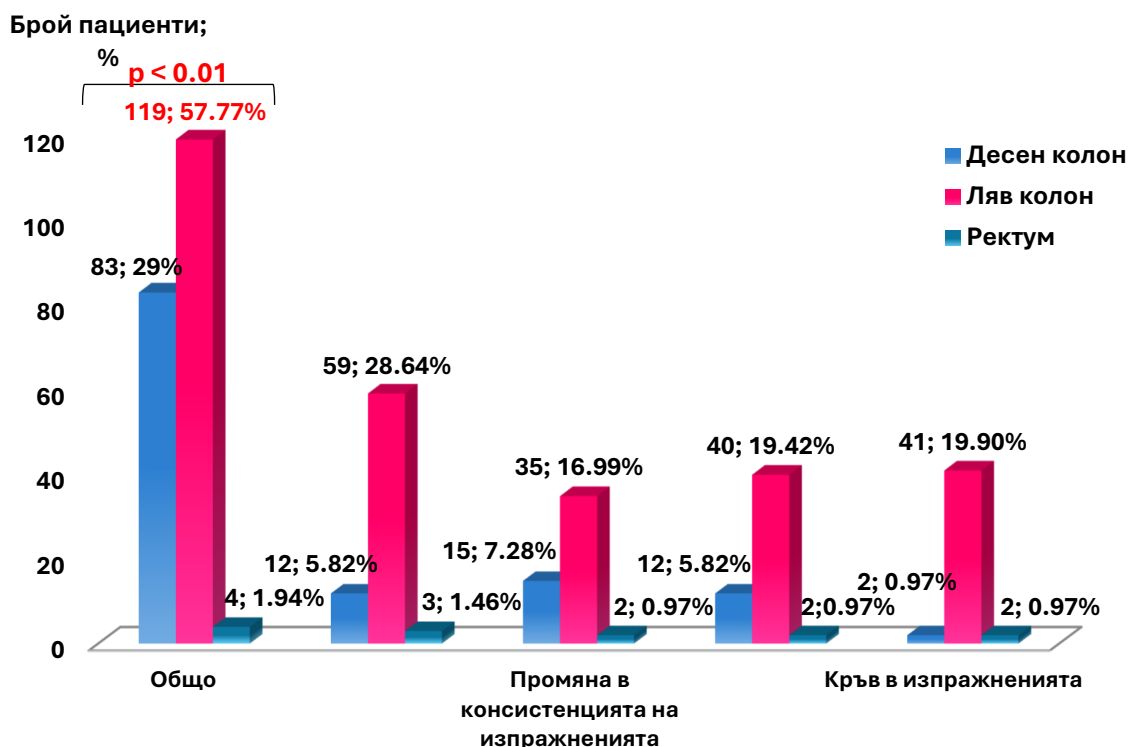


Fig. 4. Specific symptoms at different localization of CRC – generalized topogram

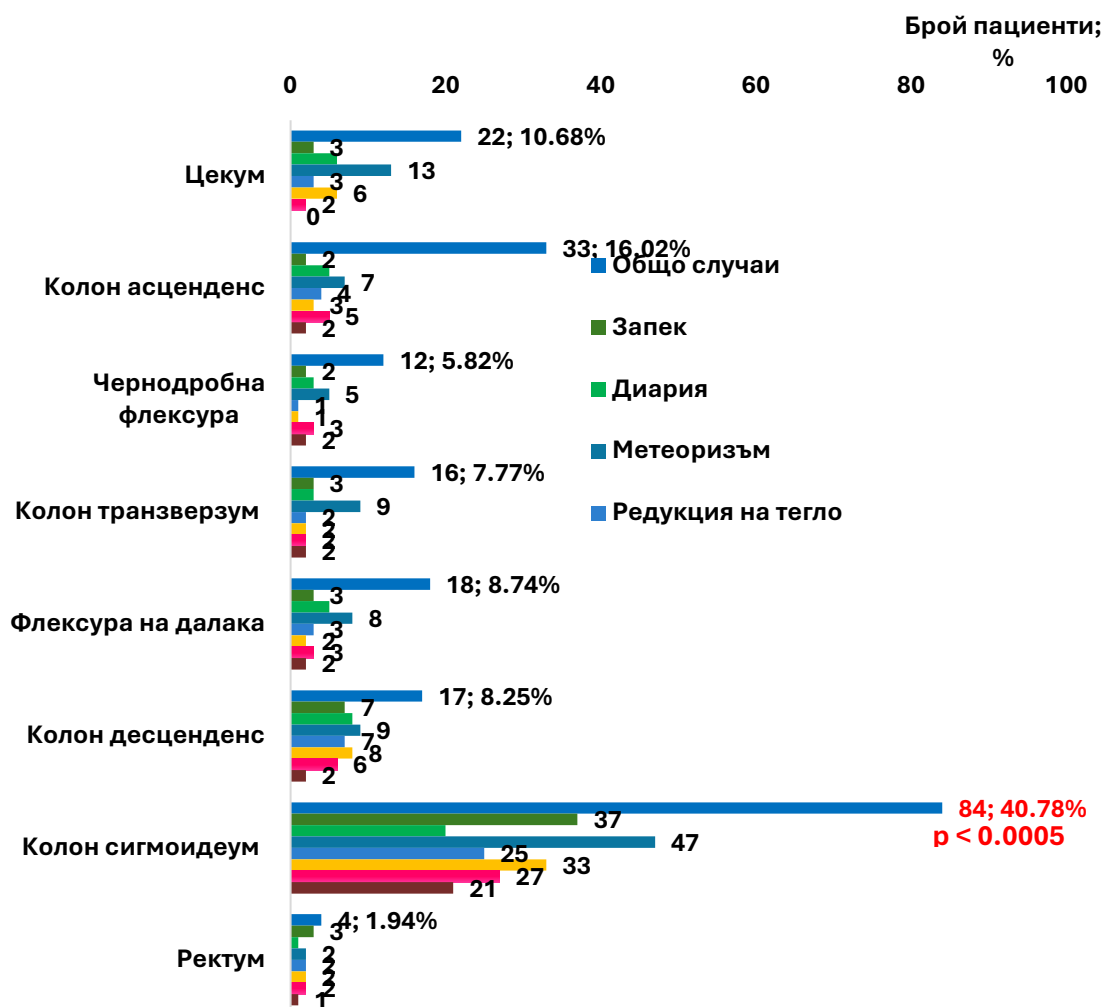


Fig. 5. Nonspecific symptoms in different localization of CRC along the colon

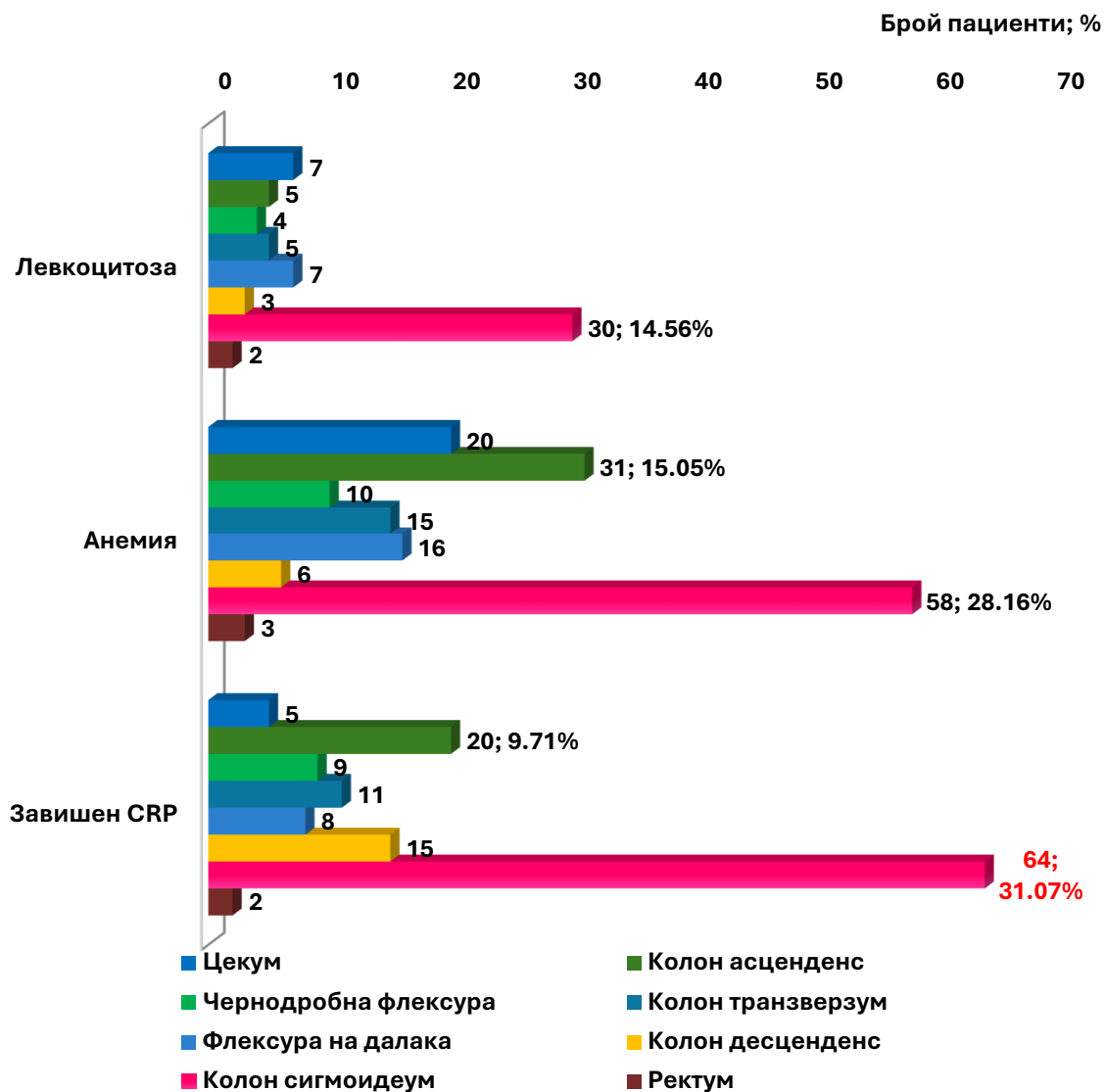


Fig. 6. Laboratory characteristics of different localization of CRC along the lower intestinal tract

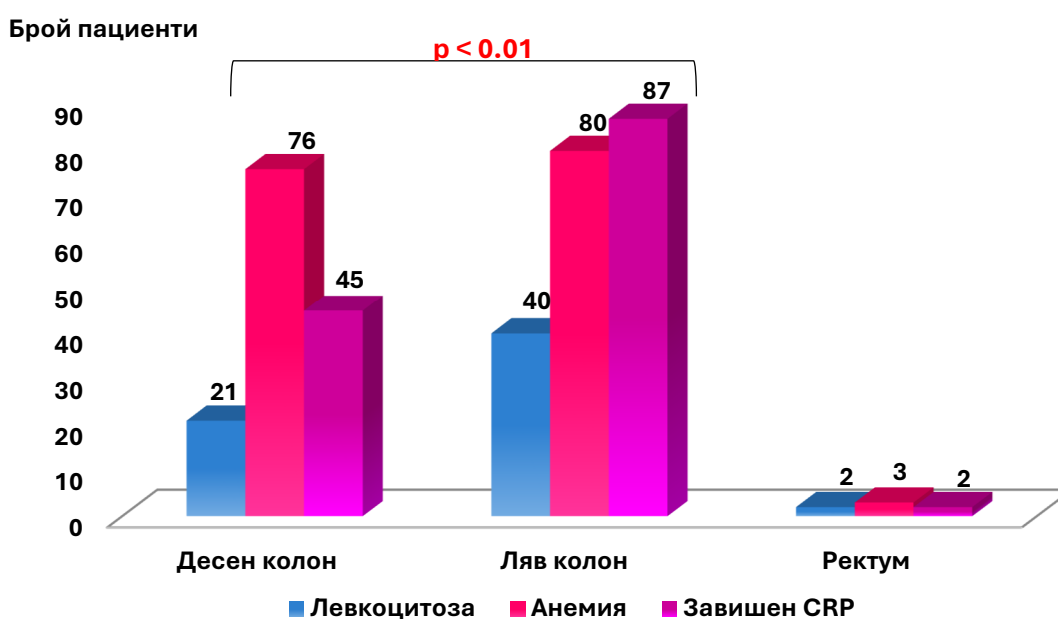


Fig. 7. Laboratory characteristics in different localization of CRC along the lower intestinal tract – generalized topogram

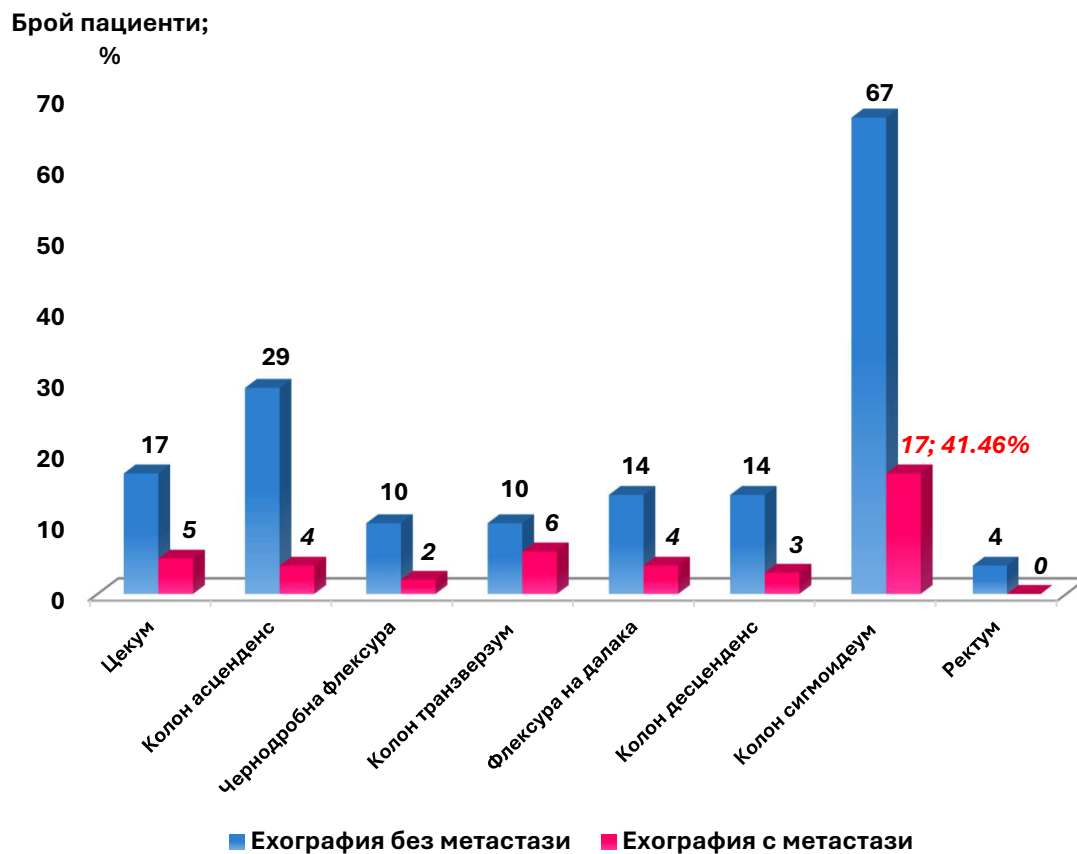


Fig. 8. Abdominal ultrasound findings in different localization of CRC along the colon

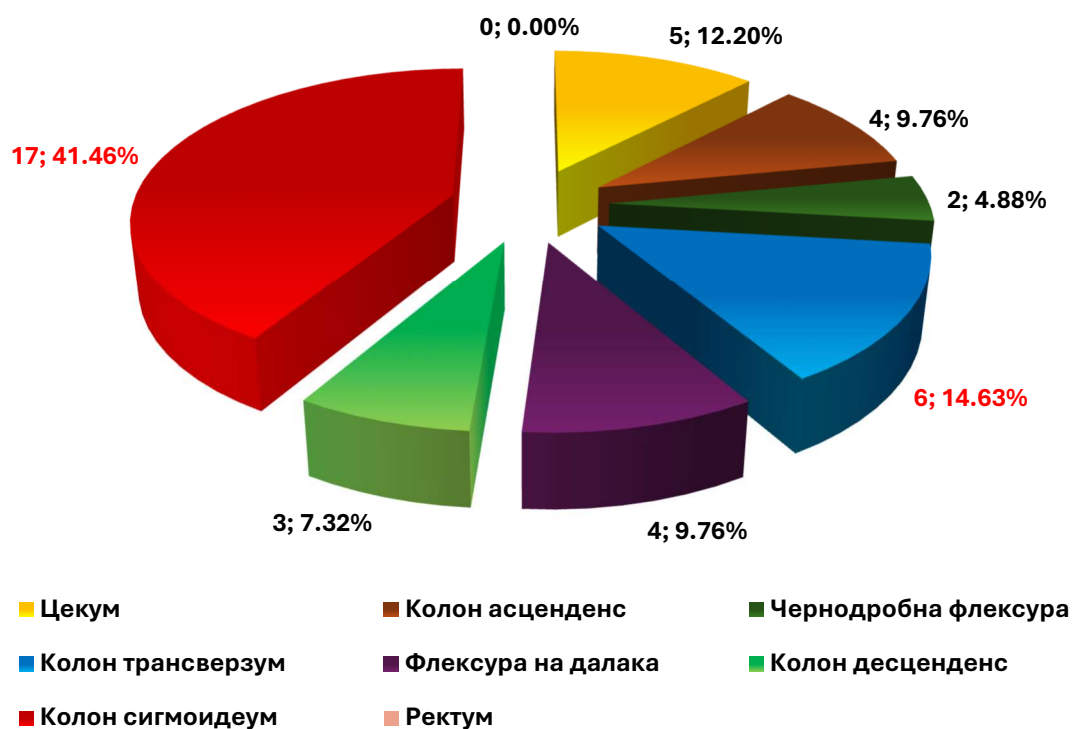


Fig. 9. Structure of abdominal ultrasounds with established metastases

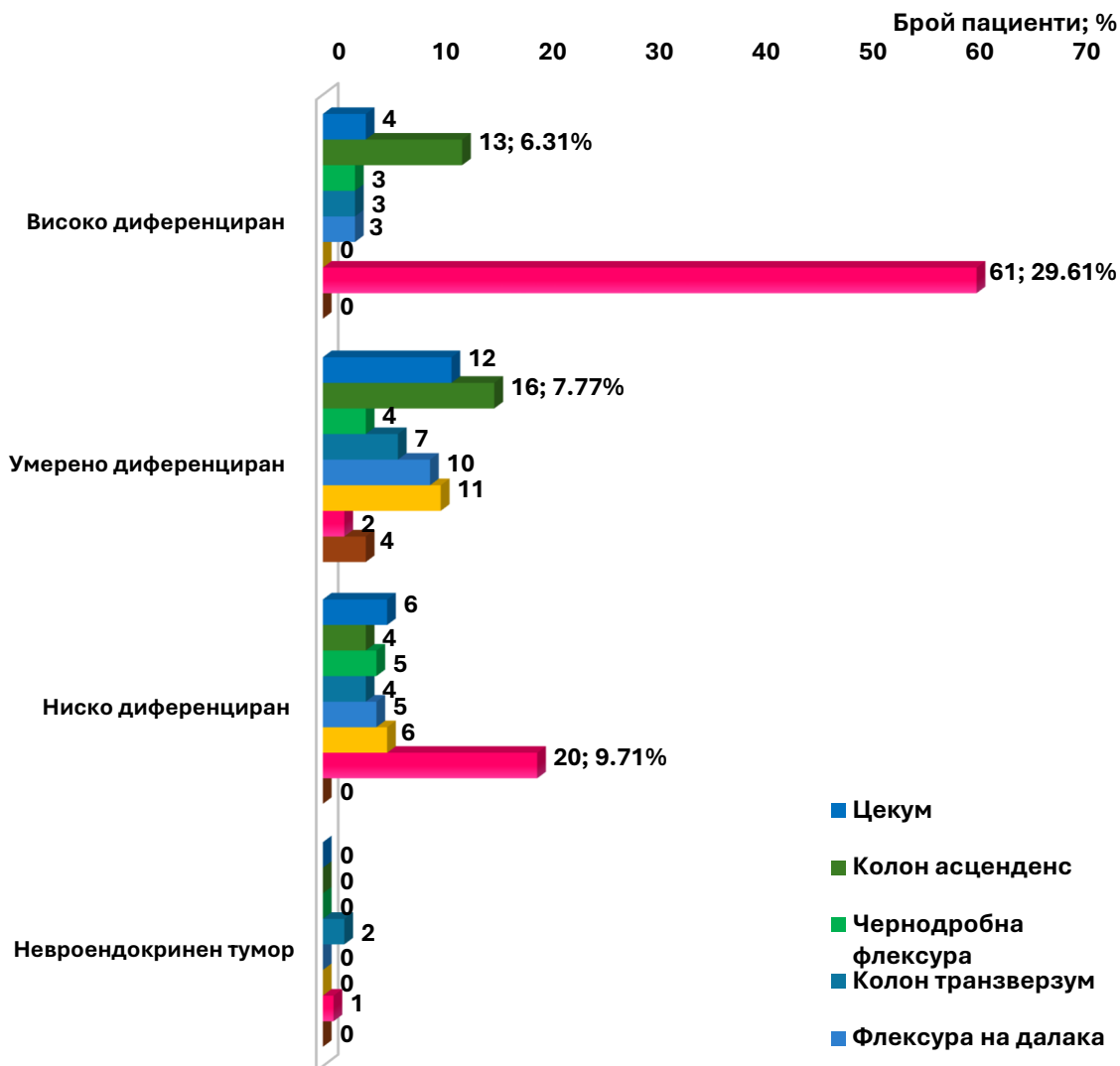


Fig. 10. Histological type of adenocarcinoma at different locations along the lower intestinal tract

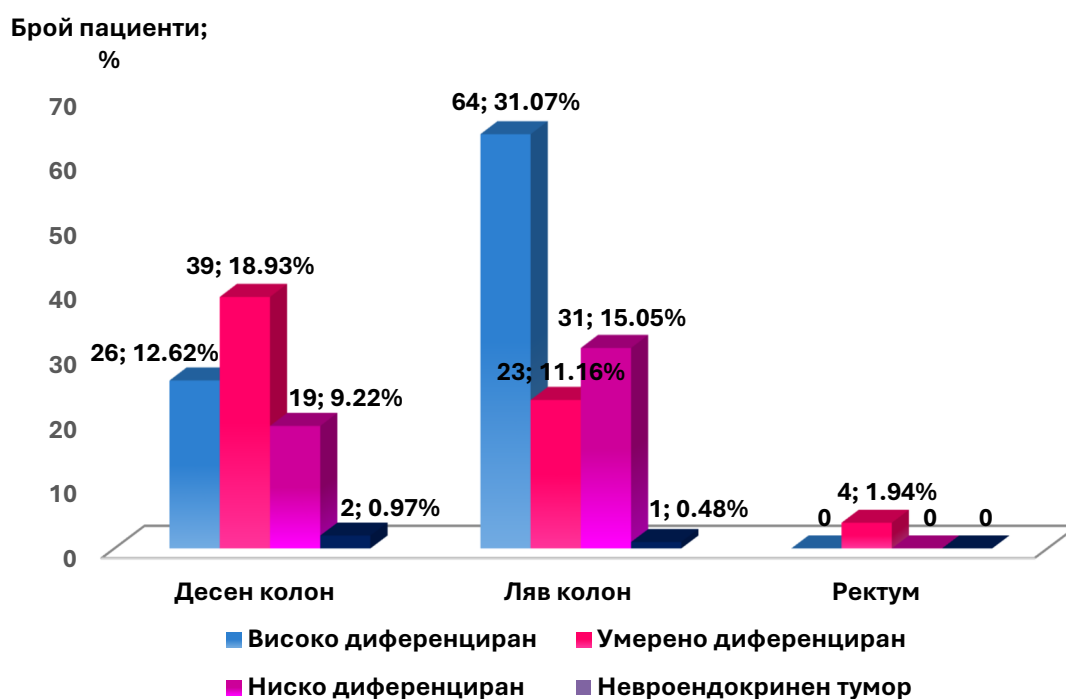


Fig. 11. Histological type of adenocarcinoma in different locations along the lower intestinal tract – generalized topogram

Chapter II. Studies of demographic, topographic, clinical and laboratory characteristics of patients with colorectal carcinoma (target group) and control groups

1. Studies of demographic indicators in patients from the target group with colorectal cancer (N₀=30)

The **age** of the studied subjects was from 48 to 89 years (mean 70.57 ± 10.11 years; median age 71 years). The distribution by age groups was uneven - the largest number of people aged 60-69 years (9/30; 30%) and 70-79 years (9/30; 30%), followed by people aged 80-89 years (7/30; 23.33%), 50-59 years (4/30; 13.33%) and 40-59 years (1/30; 3.33%) ($p < 0.05$). The age structure is presented in **Fig. 12**.

Gender structure – men predominate (17/30; 56.67%); women are 13 (43.33%) ($p < 0.05$) (**Fig. 13**).

The **age distribution by gender** is as follows: the age of men with CRC is from 56 to 89 years (mean 74.12 ± 9.68 years; median age 75 years). The predominant age group is 70-89 years (total 12/17; 70.59%) ($p < 0.05$), followed by 60–69 years (3/17; 17.65%) and 50-59 years (2/17; 11.76%).

Women with CRC are aged 48 to 81 years (mean 65.92 ± 8.99 years; median age 67 years). The largest relative proportion of women is aged 60-69 years (6/13; 46.15%) ($p > 0.05$), followed by 70-79 years (3/13; 23.08%); 50-59 years (2/13; 15.38%). In the age intervals 40-49 years and 80-89 years there are 1 woman each (respectively 7.69%) (**Fig. 14**).

2. Demographic studies of patients with Crohn's disease (CD) and ulcerative colitis (UC) (group 2 = control group 1)

The **age** of patients in group 2 ranged from 33 to 89 years (mean 61.3 ± 16.35 years; median age 63 years). The distribution by age group was uneven – the largest number of individuals was aged 60-69 years (7/30; 23.33%), followed by the age groups 40-49, 70-79 and 80-89 years (5/30; 16.67%, respectively), 50-59 years (4/30; 13.33%) and 30-39 years (4/30; 13.33%, respectively) ($p > 0.05$). The age structure is presented in **Fig. 15**.

Gender structure – men predominate (21/30; 70.00%); women are 9 (30.00%) ($p < 0.025$) (**Fig. 16**).

The **age distribution by gender** is as follows: the age of men with BC and UC is from 33 to 89 years (mean 61.10 ± 16.18 years; median age 64 years). The age distribution in men is as follows: 60-69 years (5/21; 23.81%), followed by 70–79 years (4/21; 19.05%), the groups 80-89 years, 50-59 years 40-49 years and 30-39 years (respectively 3/21; 14.29%) ($p > 0.05$).

Women with BC and UC were aged 37 to 88 years (mean 61.78 ± 17.72 years; median age 62 years). In women, the age distribution was as follows: in the groups 60-69 years, 80-89 years and 40-49 years there were a total of 6 (respectively 2/13; total 57.15%), followed by 70-79 years (3/13; 23.08%); 50-59 years and 30-39 years – a total of 3 patients (1/13; total 33.33%) ($p > 0.05$) (**Fig. 17**).

3. Demographic studies of patients with irritable bowel syndrome (group 3 = control group 2)

The **age** of patients in group 3 ranged from 35 to 80 years (mean 58.2 ± 13.61 years; median age 61 years). The distribution by age group was uneven – the largest number of individuals was aged 60-69 years (10/30; 33.33%), followed by the age groups 40-49 and 70-79 years (6/30; 20%, respectively), 30-39 years (4/30; 13.33%), 50-59 years (3/30; 10%) and 80-89 years (1/30; 3.33%, respectively) ($p < 0.05$). The age structure is presented in **Fig. 18**.

Gender structure – men predominate (17/30; 56.67%); women are 13 (43.33%) ($p > 0.05$) (**Fig. 19**).

The **age distribution by gender** is as follows: the age of men with irritable bowel syndrome is from 38 to 80 years (mean 60.24 ± 12.96 years; median age 62 years). Men are predominantly aged 60-69 years (7/17; 41.18%), followed by 70-79 years (3/17; 17.65%), 50-59 years, 40-49 years and 30-39 years (2/17 each; total 35.29%) and 80-89 years (1/17; 5.88%) ($p > 0.05$).

Women with irritable bowel syndrome were aged 35 to 78 years (mean 55.54 ± 14.49 years; median age 59 years). The largest proportion of women was aged 40-49 years (4/13; 30.77%), followed by 60-69 years and 70-79 years (3/13 each; total 46.15%), followed by 30-39 years (2/13; 15.38%); 50-59 years (1/13; 7.69%) ($p > 0.05$) (**Fig. 20**).

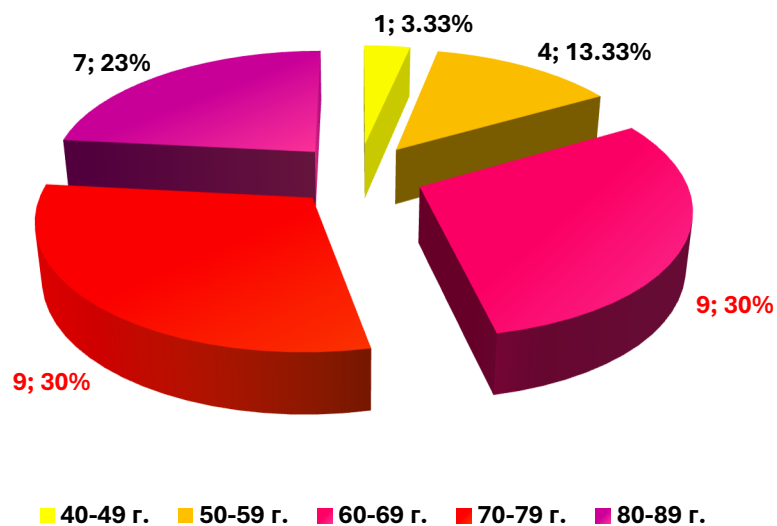


Fig. 12. Age structure of patients with colorectal carcinoma ($N_1=30$)

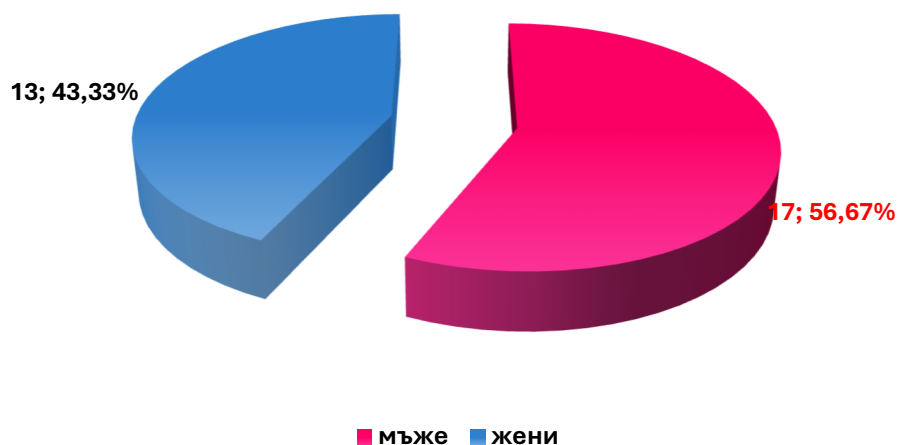


Fig. 13. Gender structure of patients with colorectal carcinoma ($N_1=30$)

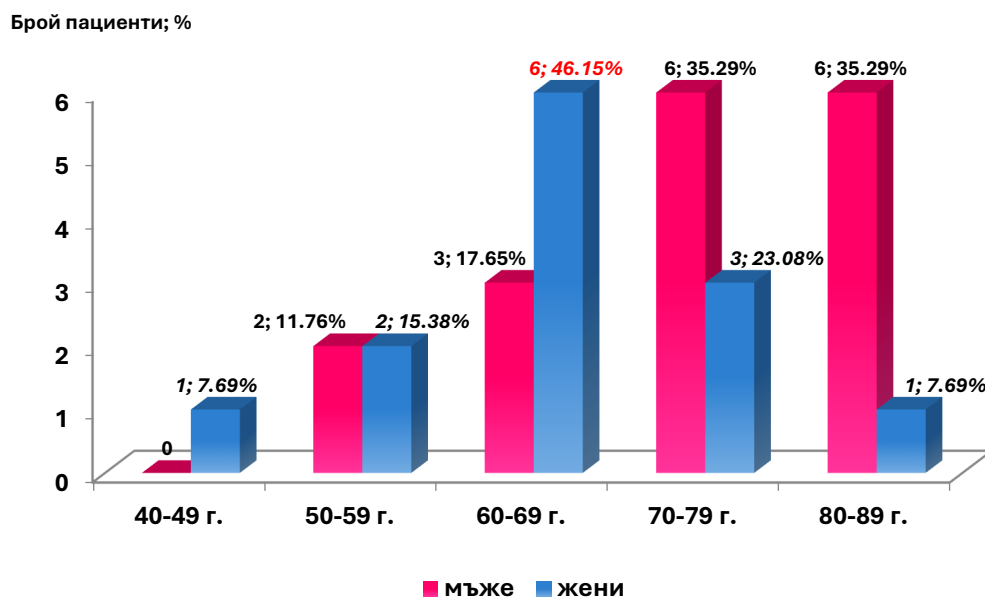


Fig. 14. Distribution by gender and age in the target group ($N_1=30$)

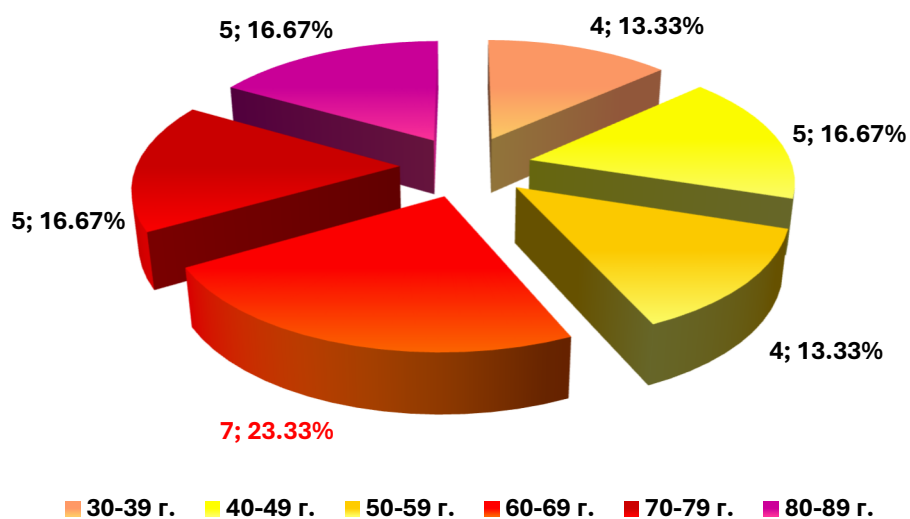


Fig. 15. Age structure of patients from group 2 ($N_2=30$)

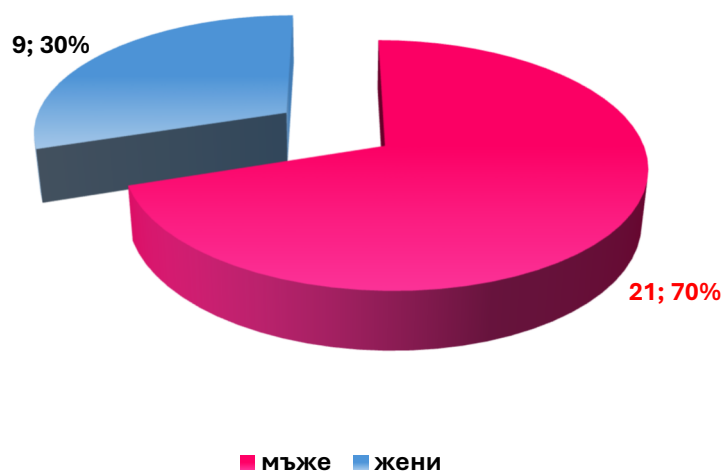


Fig. 16. Gender structure of patients from group 2 ($N_2=30$)

Брой пациенти; %

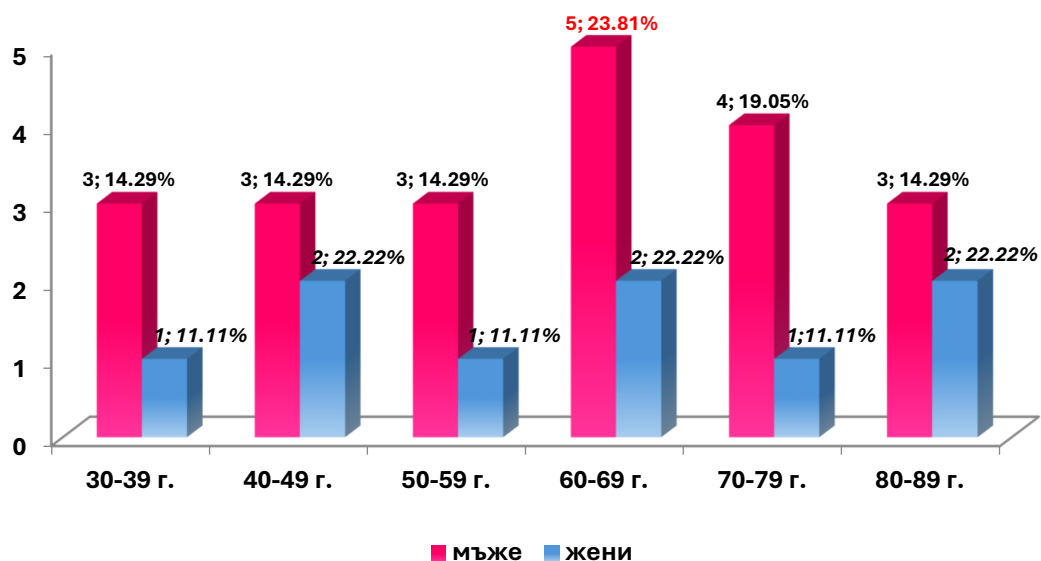


Fig. 17. Distribution by gender and age of patients from group 2 ($N_2=30$)

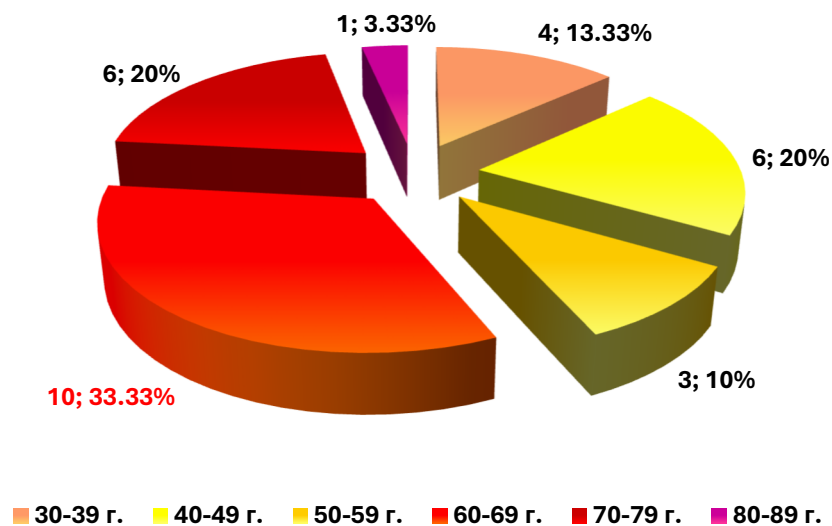


Fig. 18. Age structure of patients from group 3 ($N_3=30$)

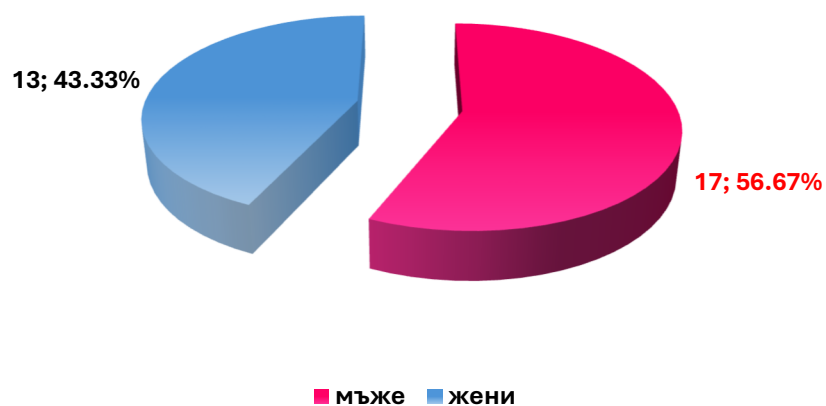


Fig. 19. Gender structure of patients from group 3 ($N_3=30$)

Брой пациенти; %

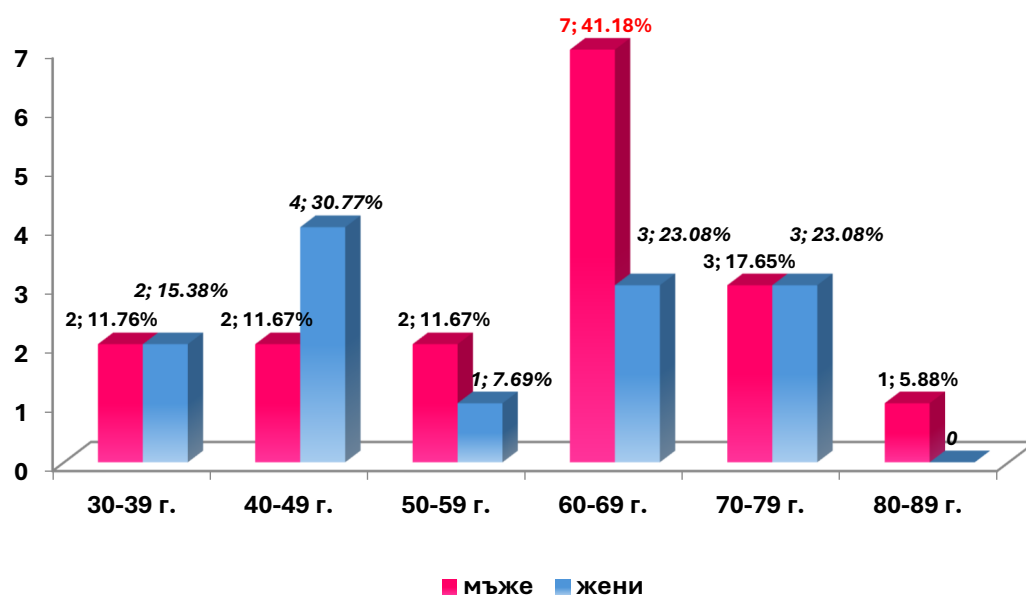


Fig. 20. Distribution by gender and age in group 3 ($N_3=30$)

4. Comparative study of demographic indicators in target (N1 = 30) and control groups (N2 = 30; N3 = 30)

The comparative study established a prevalence of male gender in the three groups without significant differences ($p > 0.05$) (*Fig. 21*).

Patients in the target group were significantly older than patients with irritable bowel syndrome (group 3) ($p < 0.05$), but there was no significant difference with patients with Crohn's disease and ulcerative colitis (group 2) ($p > 0.05$) (*Fig. 22*).

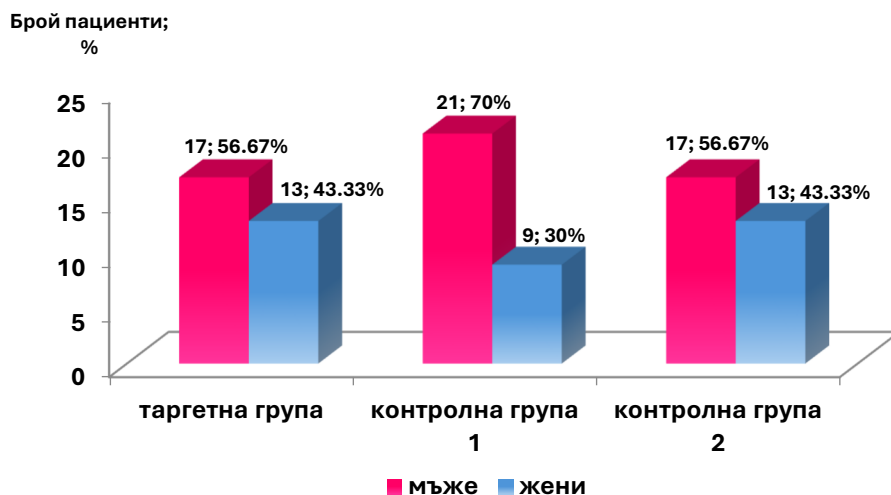


Fig. 21. Gender structure of patients from three groups

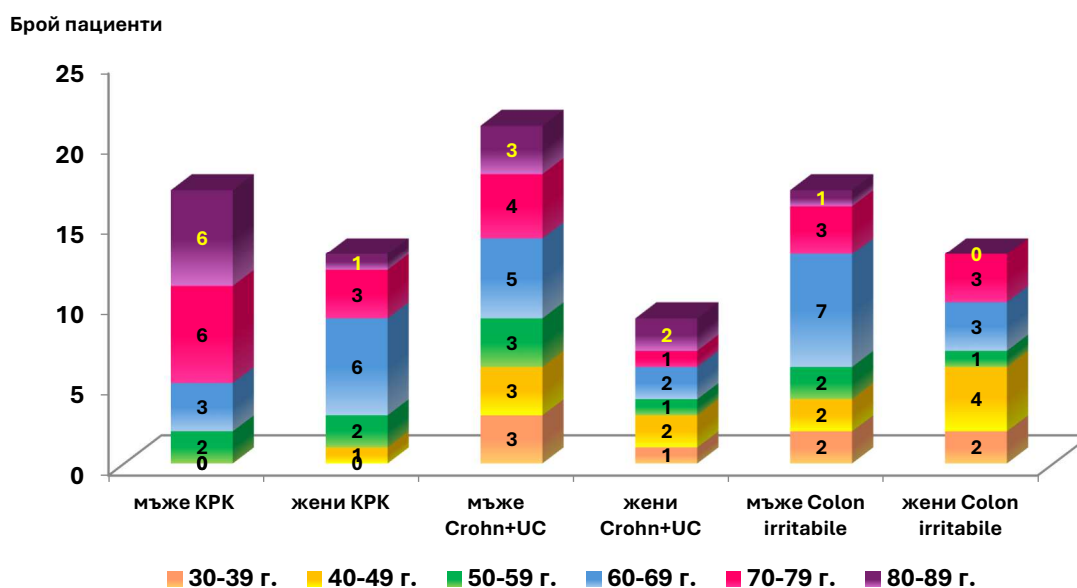


Fig. 22. Distribution by gender and age of patients from the three groups

5. Study of stages, localization of CRC and clinical symptoms in patients in the target group (N1 = 30)

5.1. Regarding the stage of CRC, the largest relative proportion of patients was in stage II of the disease (19/30; 63.33%) ($p < 0.05$) (*Fig. 23*). Ten patients were in stage I, one patient in stage III of CRC.

The **distribution of men with CRC by stage** is as follows: **Stage I** – seven men (7/17; 41.18%); **Stage II** – ten (10/17; 58.82%). **Distribution of women by stage: Stage I** – three (3/13; 23.08%), **Stage II** – nine (9/13; 69.23%), **Stage III** – one woman (1/13; 7.69%).

Distribution of stages by age: Stage I (n = 10) – age from 56 to 89 years (mean 69.7 ± 10.66); median age 67 years; **Stage II** (n = 19) – age from 48 to 87 years (mean 71 ± 10.35); median age 71 years; **Stage III** – n = 1 woman aged 71 years.

Distribution of stages by age and gender: *Stage I* (n = 10) – seven men (70%) aged 56 to 89 years (mean 73 ± 11.18); median age 75 years; three women (30%) aged 59 to 65 years (mean 62 ± 3); median age 62 years; *Stage II* (n = 19) – ten men (52.63%) aged 56 to 87 years (mean 74.9 ± 9.02); median age 75 years; nine women (47.37%) aged 48 to 81 years (mean 66.67 ± 10.46); median age 68 years; *Stage III* – n = one woman aged 71 years.

5.2. The distribution of CRC localizations in the patients in the target group is presented in *Fig. 24*. The highest relative proportion of patients with CRC in the sigmoid colon (15/30; 50%) ($p < 0.05$).

The complex detailed analysis by gender, specific symptoms, stage and localization of CRC established the following: in stage I of CRC there were seven men and three women; in stage II – ten men and nine women ($p > 0.05$), in stage III – only one woman.

Regarding the **localization of CRC**: three patients (only men) (3/30; 10%) had tumor localization in the colon ascendens, three (one man and two women) (3/30; 10%) – in the colon transversum, two (one man and one woman) – in the colon descendens (2/30; 6.67%), fifteen (seven men and eight women) (15/30; 50%) – in the sigmoid colon and seven (five men and two women) (7/30; 23.33%) – in the rectum.

The **distribution of localizations by gender and age** is as follows: the three men with CRC localization in the colon ascendens were aged 56 to 77 years (mean 66.67 ± 10.5); median age 67 years; the patients (one man and two women) with CRC in the colon transversum were aged 53 to 73 years (mean 64 ± 10.15); median age 66 years; the patients (one man and one woman) with CRC in the colon descendens were aged 83 years and 69 years, respectively; in patients with CRC localization in the sigmoid colon (seven men and eight women), men were aged 56 to 87 years (mean 75 ± 10.44 ; median age 80 years), and women were aged 48 to 81 years (mean 68.12 ± 10.03 ; median age 69.5 years). In the subgroup with rectal carcinoma (five men and two women), men were aged 63 to 89 years (mean 75.8 ± 9.45 ; median age 75), and women were aged 59 to 65 years (mean 62 ± 4.24 ; median age 62).

5.3. The stages of CRC in different localizations are as follows: *Stage I* (ten patients) – four patients with localization in the sigmoid colon and two – with rectal carcinoma; *Stage II* (nineteen patients) – three with localization in the colon ascendens, three with localization in the colon transversum, two – in the colon descendens, ten in the sigmoid colon, one with rectal carcinoma. In stage III there is one patient with cancer of the sigmoid colon (*Fig. 25*).

The **distribution of localizations at different stages** of CRC is presented in *Fig. 26*.

5.4. The relative proportions of specific symptoms for CRC in the patients from the target group are as follows: presence of abdominal pain in all patients in the group (30/30; 100%), blood in the feces in 22 (22/30; 73.33%), disturbed defecation rhythm in 21 (21/30; 70%). Eight patients reported weight loss (8/30; 26.67%) (*Fig. 27*).

Regarding the frequency of specific symptoms, the following was found: abdominal pain was present in all patients in the target group (30/30; 100%), regardless of the stage and localization of the tumor process.

Blood in the stool was present in 22 patients in the target group (22/30; 73.33%); in all patients in stage I of CRC (10/10; 100%), in 11 patients in stage II (11/19; 57.89%) – in half of the men in this stage (5/10; 50%) and in six of the women in stage (6/9; 66.67%) and in the only patient in stage III (100%) ($p < 0.05$). Regarding the localization of CRC, blood in the stool was present in all patients with CRC of the sigmoid colon (15/15; 100%) and in all patients with CRC of the rectum (12/12; 100%). The symptom was absent in CRC of the colon ascendens, colon transversum and colon descendens ($p < 0.05$).

Disturbed defecation rhythm was present in 21 patients in the group (21/30; 70%); in half of the patients in stage I (5/10; 50%; four men and one woman), in fifteen of the patients in stage II (15/19; 78.95%; seven men and eight women) and in the only patient in stage III (100%) ($p < 0.05$). Regarding the localization of CRC, a disturbed rhythm of defecation was present in all three patients with CRC of the colon transversum, in two patients with CRC of the colon descendens, in fourteen patients with sigmoid cancer (14/15; 93.33%) and in two patients with CRC of the rectum (2/7; 28.57%). The symptom was absent in CRC of the colon ascendens ($p < 0.05$).

Weight loss was present in eight patients in the target group (8/30; 26.67%) – in one patient in stage I of CRC (1/10; 10%) and in seven patients in stage II (7/19; 36.84%). One of these patients had a

tumor localization in the sigmoid colon and seven – in the rectum. The symptom was absent in the proximal parts of the colon ($p < 0.05$).

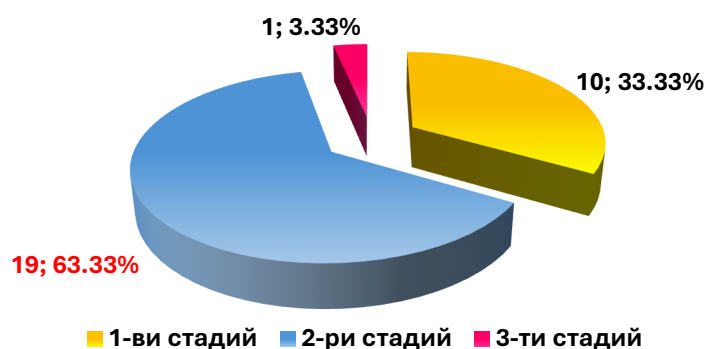


Fig. 23. Distribution by stage of patients from the target group ($N_1 = 30$)

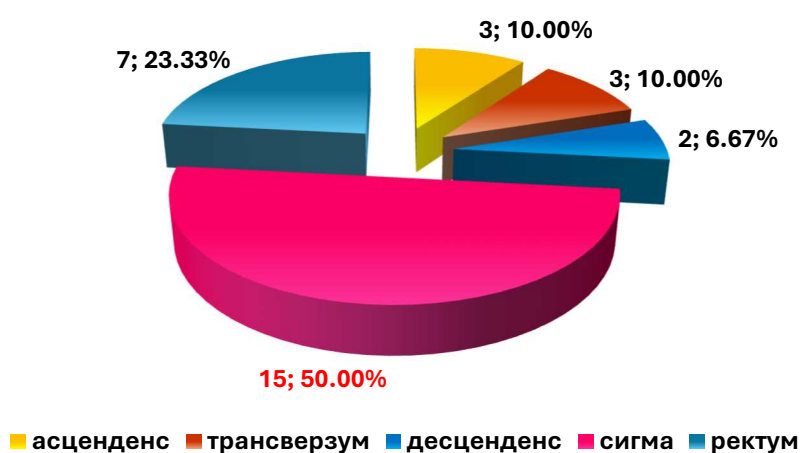


Fig. 24. Distribution by localization of CRC in patients in the target group ($N_1 = 30$)

Брой пацієнти; %

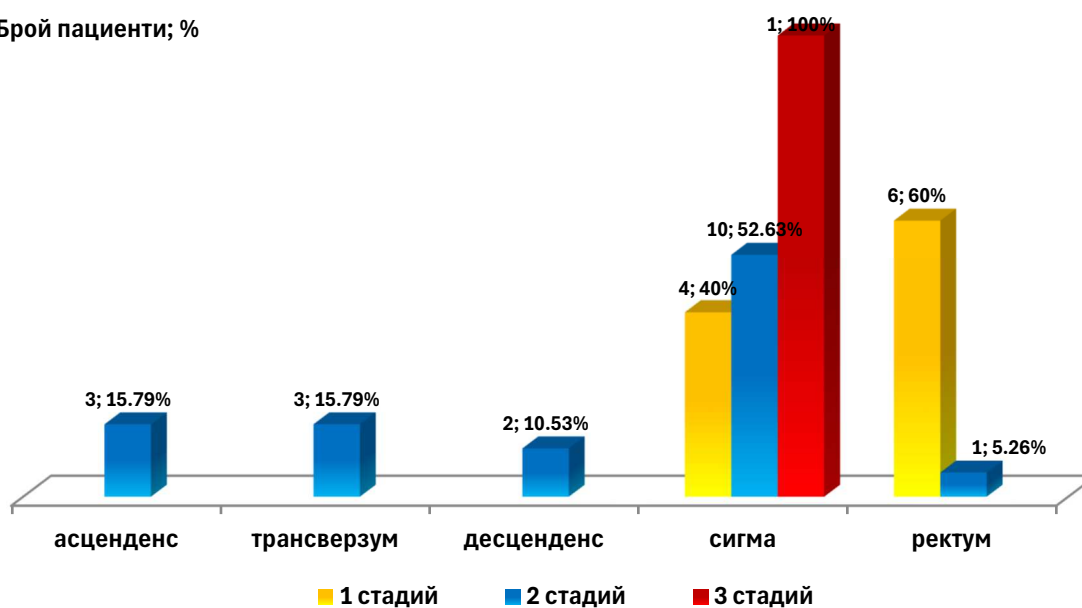


Fig. 25. Distribution of CRC stages at different locations

Брой пациенти; %

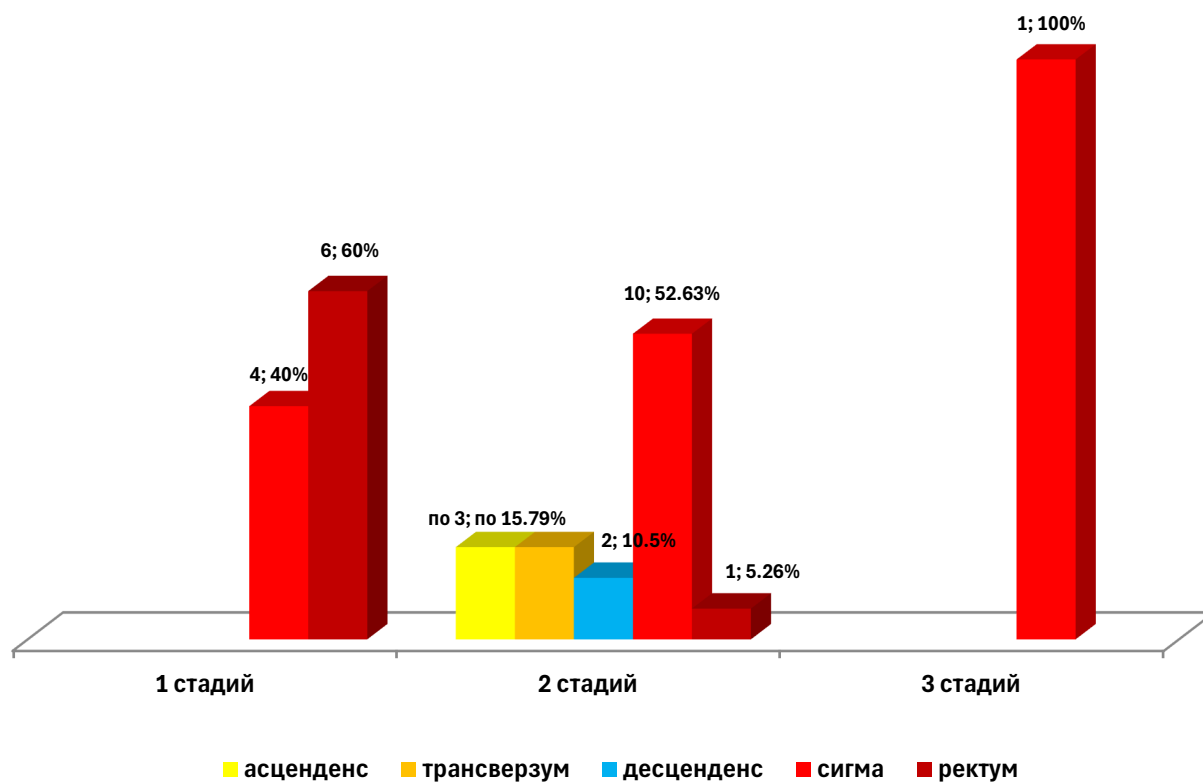


Fig. 26. Distribution of localizations at different stages of CRC

Брой пациенти; %

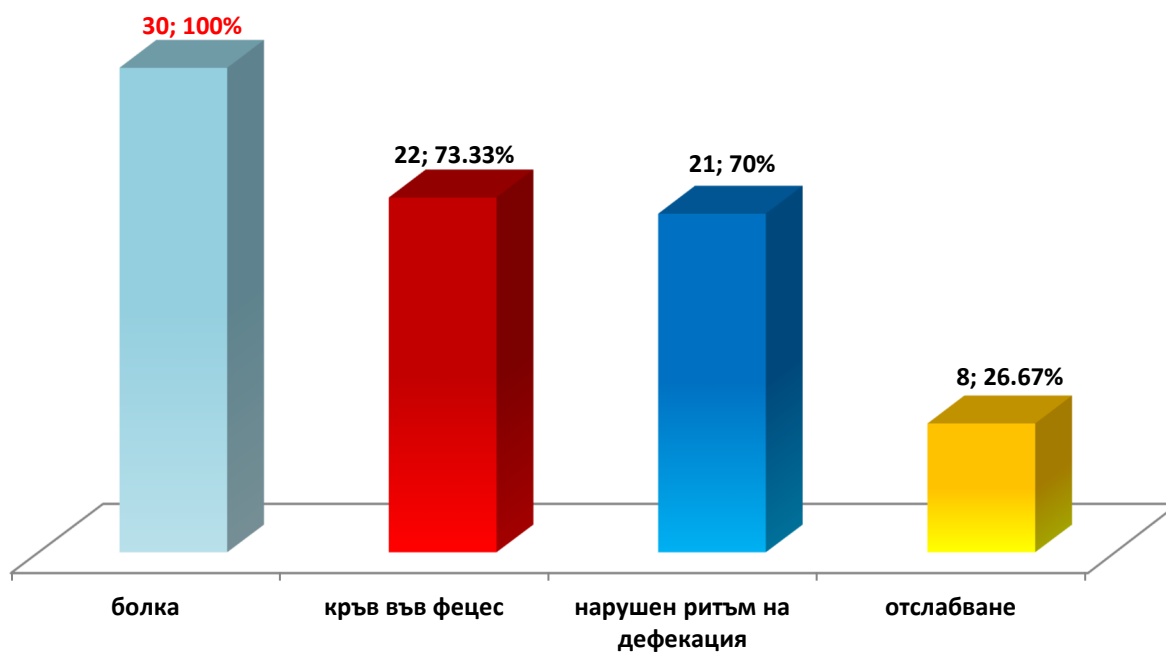


Fig. 27. Specific clinical symptoms in patients from the target group ($N_1 = 30$)

Chapter III. Laboratory tests

1. Laboratory tests in patients with colorectal cancer – target group (N1 = 30) (Table 2).

1.1. Complete blood count

1.1.1. Hemoglobin (Reference values: m.135-180 g/L; g. 120-160 g/L)

Hemoglobin values ranged from 79 to 127 g/L (mean 101 ± 10.89). All patients in the target group had hemoglobin below the lower reference limit (100%) ($p < 0.0001$).

1.1.2. Leukocytes (Reference values: 3.5-10.5 x 10⁹/L)

The leukocyte values ranged from 5.7 to 13.2 x 10⁹/L (mean 8.74 ± 2.14). There were 5 patients in the group with an increased number of leukocytes (16.67%), three of whom were men and two women.

Regarding the stage of the disease, four of the patients were in stage 1, one in stage 2.

Regarding the localization of CRC with an increased number of leukocytes, there were three patients with sigmoid carcinoma (two men and one woman) and two patients with rectal carcinoma (also two men and one woman).

1.2. C-reactive protein (Reference values: 0.00-5.00 mg/L)

The values of the indicator ranged from 18.5 to 67.1 mg/L (mean 38.39 ± 12.25). All patients in the target group had elevated C-reactive protein (100%) ($p < 0.0001$).

Regarding the stage of the disease, the highest average value of the indicator was in stage 1 (39.97 ± 11.72), followed by stage 2 (36.87 ± 12.65), but without a significant difference ($p > 0.05$).

Regarding the localization of CRC, the highest C-reactive protein is in carcinoma of the colon transversum (44.57 ± 19.59), followed by the rectum (40.11 ± 12.99), sigmoid colon (39.18 ± 11.57), colon ascendens (31.13 ± 9.42) and colon descendens (28.0 ± 1.41), but the differences are not significant ($p > 0.05$).

1.3. Fecal calprotectin (Reference values: 0-50 U/mL – negative result; 50-200 U/mL – borderline result; >200 U/mL – presence of inflammation of the gastrointestinal tract)

The values of the indicator are in the range from 233.5 to 1305.4 mg/kg (589.81 ± 267.79). All patients in the target group had elevated values of the indicator (100%) ($p < 0.0001$).

Regarding the stage of the disease, the highest average value of the indicator was in stage 1 (638.48 ± 204.21), followed by stage 2 (542.33 ± 285.31), but without a significant difference ($p > 0.05$).

Regarding the localization of CRC, the highest fecal calprotectin is in carcinoma of the colon transversum (665.67 ± 183.96), followed by the rectum (614.01 ± 234.57), sigmoid colon (601.11 ± 324.46), colon descendens (519.8 ± 185.97) and colon ascendens (447.67 ± 199.57), but the differences are not significant ($p > 0.05$).

1.4. Fecal M2-pyruvate kinase (Reference values: norm <4 U/mL)

The values of the indicator are in the range from 19.7 to 90.2 U/mL (52.30 ± 17.70). All patients in the target group have elevated values of the indicator (100%) ($p < 0.0001$).

Regarding the stage of the disease, the highest mean value of the indicator is in stage 2 (55.98 ± 17.49), followed by stage 1 (45.18 ± 17.63), but without a significant difference ($p > 0.05$).

Regarding the localization of CRC, the highest M2-pyruvate kinase is in carcinoma of the colon transversum (64.50 ± 30.97), followed by colon ascendens (59.3 ± 15.77), sigmoid colon (56.55 ± 16.72), colon descendens (40.8 ± 1.84) and rectum (40.01 ± 15.09), but the differences are not significant ($p > 0.05$).

2. Study of laboratory parameters in patients with Crohn's disease and ulcerative colitis - control group 1 (N2 = 30) (Table 3).

2.1. Complete blood count

2.1.1. Hemoglobin (Reference values: m.135-180 g/L; g. 120-160 g/L)

Hemoglobin values ranged from 121 to 162 g/L (mean 144.3 ± 10.21). There were five patients with hemoglobin below the lower reference limit (5/30; 16.67%) ($p > 0.05$).

In men, hemoglobin values ranged from 121 to 162 g/L (mean 143.95 ± 10.84). There were four patients with hemoglobin below the lower reference limit (4/21; 19.05%). In women, hemoglobin values ranged from 125 to 153 g/L (mean 145.11 ± 9.12). One patient (1/9; 11.11%) had hemoglobin below the lower reference limit.

2.1.2. Leukocytes (*Reference values: 3.5-10.5 x 10⁹/L*)

Leukocyte values ranged from 3.8 to 8.9 x 10⁹/L (mean 6.54 ± 1.27). All patients in the group had leukocytes in the reference range (30/30; 100%) (p > 0.05).

In men, the number of leukocytes ranged from 3.8 to 8.6 x 10⁹/L (mean 6.44 ± 1.22), in women – from 4.8 to 8.9 x 10⁹/L (mean 6.78 ± 1.40). There were no significant gender differences (p > 0.05).

2.2. C-reactive protein (*Reference values: 0.00-5.00 mg/L*)

The values of the indicator ranged from 1.5 to 5.0 mg/L (mean 3.46 ± 0.97). All patients in the group had normal C-reactive protein (30/30; 100%) (p > 0.05).

In men, C-reactive protein ranged from 1.5 to 5.0 mg/L (mean 3.41 ± 0.93), in women – from 2.0 to 5.0 mg/L (mean 3.57 ± 1.13). There were no significant gender differences (p > 0.05).

2.3. Fecal calprotectin (*Reference values: 0-50 U/mL – negative result; 50-200 U/mL – borderline result; >200 U/mL – presence of inflammation of the gastrointestinal tract*)

The values of the indicator ranged from 89.7 to 210.5 mg/kg (166.93 ± 29.25). There were 25 patients with borderline values (25/30; 83.33%), with elevated values – five patients (5/30; 16.67%) (p > 0.05).

In men, fecal calprotectin ranged from 118.9 to 210.5 mg/L (mean 168.47 ± 28.49). There were 17 patients with borderline values (17/21; 80.95%), with elevated values – four patients (4/21; 19.05%). In women, the indicator ranged from 89.7 to 203.7 mg/L (mean 163.32 ± 32.42). There were eight women with borderline values (8/9; 88.89%), with elevated fecal calprotectin there was one woman (1/9; 11.11%). There were no significant gender differences (p > 0.05).

2.4. Fecal M2-pyruvate kinase (*Reference values: normal <4 U/mL*)

The values of the indicator ranged from 2.7 to 12.1 U/mL (5.99 ± 2.51). There were 21 patients (21/30; 70.0%) with elevated values of the indicator (p < 0.05).

In men, fecal M2-pyruvate kinase ranged from 2.7 to 12.1 U/mL (5.86 ± 2.60), with thirteen patients (13/21; 61.90%) with elevated values of the indicator. In women, the indicator ranged from 3.8 to 11.5 U/mL (6.31 ± 2.43), with eight patients with elevated values (8/9; 88.89%). There were no significant gender differences (p > 0.05).

Table 2. Descriptive statistics of CRC patients – target group (N₁ = 30)

Променлива	n	Mean	SD	Median	F statistic	p	Значимост
Възраст (години)	30	70.57	10.11	71	6.72	0.001933	**
Фекален калпротектин	30	589.81	267.79	559.95	106.8622	0.000000	***
M2-РК	29	52.3	17.7	52.7	214.8067	0.000000	***
CRP	30	38.39	12.25	36.6	239.0889	0.000000	***
Левкоцити	30	8.74	2.14	8.5	22.5673	0.000000	***
Хемоглобин	30	101	10.89	101	139.0514	0.000000	***

*** p < 0.0001; ** p < 0.01; SD – standard deviation

Table 3. Descriptive statistics of patients with Crohn's disease and ulcerative colitis – control group 1 ($N_2 = 30$)

Променлива	n	Mean	SD	Median
Възраст (години)	30	61.3	16.35	63
Фекален калпротектин	30	166.93	29.25	166.8
M2-РК	30	5.99	2.51	5.8
CRP	30	3.46	0.97	3.5
Левкоцити	30	6.54	1.27	6.7
Хемоглобин	30	144.3	10.21	145

SD – standard deviation

3. Study of laboratory parameters in patients with irritable bowel syndrome – control group 2 ($N_3 = 30$) (Table 4).

1.1. Complete blood count

1.1.1. Hemoglobin (Reference values: m. 135-180 g/L; f. 120-160 g/L)

Hemoglobin values ranged from 121 to 161 g/L (mean 139.6 ± 11.94). All patients had hemoglobin within the reference range appropriate for gender ($p > 0.05$).

In men, hemoglobin values ranged from 140 to 161 g/L (mean 148.76 ± 6.36). There were no men with hemoglobin below the lower reference limit. In women, hemoglobin values ranged from 121 to 133 g/L (mean 127.61 ± 3.97). There were no women with hemoglobin below the lower reference limit.

1.1.2. Leukocytes (Reference values: 3.5-10.5 x 10⁹/L)

Leukocyte values ranged from 3.8 to 8.9 x 10⁹/L (mean 6.03 ± 1.43). All patients in the group had leukocytes in the reference range (30/30; 100%).

In men, the number of leukocytes ranged from 3.5 to 8.5 x 10⁹/L (mean 5.85 ± 1.60), in women – from 4.8 to 8.6 x 10⁹/L (mean 6.28 ± 1.18). There are no significant gender differences ($p > 0.05$).

1.2. C-reactive protein (Reference values: 0.00-5.00 mg/L)

The values of the indicator are in the range from 1.5 to 5.0 mg/L (mean 3.09 ± 1.02). All patients in the group have normal C-reactive protein (30/30; 100%) ($p > 0.05$).

In men, C-reactive protein ranges from 1.5 to 4.6 mg/L (mean 2.95 ± 1.03), in women – from 1.7 to 5.0 mg/L (mean 3.29 ± 1.02). There are no significant gender differences ($p > 0.05$).

1.3. Fecal calprotectin (Reference values: 0-50 U/mL – negative result; 50-200 U/mL – borderline result; >200 U/mL – presence of inflammation of the gastrointestinal tract)

The values of the indicator ranged from 12.5 to 47.1 mg/kg (25.38 ± 8.26). All patients in the group had normal values of the indicator (30/30; 100%) ($p > 0.05$).

In men, fecal calprotectin ranged from 12.5 to 41.5 mg/L (mean 25.27 ± 8.37). All men had fecal calprotectin in the reference range. In women, the indicator ranged from 17.6 to 47.1 mg/L (mean 25.52 ± 8.46). All women in the group had normal fecal calprotectin. There are no significant gender differences ($p > 0.05$).

1.4. Fecal M2-pyruvate kinase (Reference values: norm <4 U/mL)

The values of the indicator are in the range from 1.0 to 4.7 U/mL (2.95 ± 0.90). There are 6 patients with increased values of the indicator (6/30; 20.0%) ($p > 0.05$).

In men, fecal M2-pyruvate kinase is in the range from 1.0 to 4.2 U/mL (2.81 ± 0.91), with three patients with increased values of the indicator (3/17; 17.65%). In women, the indicator varies from 1.7 to 4.7 U/mL (3.15 ± 0.88), with three patients with increased values (3/13; 23.08%). There are no significant gender differences ($p > 0.05$).

Table 4. Descriptive statistics of patients with irritable bowel syndrome – control group 2 (N3=30)

Променлива	n	Mean	SD	Median
Възраст (години)	30	58.2	13.61	61
Фекален калпротектин	30	25.38	8.26	23.35
M2-PK	30	2.95	0.9	2.95
CRP	29	3.09	1.02	3.0
Левкоцити	30	6.03	1.43	5.95
Хемоглобин	30	139.6	11.94	141.5

SD – standard deviation

4. Comparative study of laboratory parameters in patients with colorectal carcinoma (target group; N1 = 30) with patients from both control groups (N2 = 30; N3 = 30)

1. KRUSKAL-WALLIS test (3-group comparison) (Table 5)

Table 5. Comparative study of laboratory parameters in the target and control groups

Променлива	H статистика	p-value	Значимост	Размер на ефекта (η^2)	Ефект
Възраст (години)	11.3844	0.003372	**	0.1079	Среден
Фекален калпротектин	79.1228	0.000000	***	0.8865	Голям
M2-PK	70.9764	0.000000	***	0.8021	Голям
CRP	59.9538	0.000000	***	0.6739	Голям
Левкоцити	24.5945	0.000005	***	0.2597	Голям
Хемоглобин	59.2004	0.000000	***	0.6575	Голям

*** p <0.0001; ** p <0.01

2. Post-hoc comparison of two groups (Mann-Whitney U test, Bonferroni corrected)(Table 6)

Group 1 (Ca): Colorectal carcinoma, n=30

Group 2 (IBD): Crohn's disease + ulcerative colitis, n=30

Group 3 (IBS): Colon Irritable (Irritable Bowel Syndrome), n=30

Significance levels: *** p<0.001, ** p<0.01, * p<0.05, ns = not significant

Kruskal-Wallis test used as primary test (non-parametric, robust to non-normal distribution)

Post-hoc: Mann-Whitney U with Bonferroni correction for multiple comparisons ($\alpha/3$)

Effect size: η^2 by Kruskal-Wallis; Small <0.06, Medium 0.06-0.14, Large ≥ 0.14

Table 6. Post-hoc comparison of two groups

Променлива	Сравняване	U статистика	p (raw)	p (Bonferroni)	Значимост
Възраст	Ca vs IBD	599	0.027971	0.083914	ns
Възраст	Ca vs IBS	680	0.000685	0.002055	**
Възраст	IBD vs IBS	502,5	0.441701	1.000000	ns
Фекален калпротектин	Ca vs IBD	900	0.000000	0.000000	***
Фекален калпротектин	Ca vs IBS	900	0.000000	0.000000	***
Фекален калпротектин	IBD vs IBS	900	0.000000	0.000000	***
M2-PK	Ca vs IBD	870	0.000000	0.000000	***
M2-PK	Ca vs IBS	870	0.000000	0.000000	***
M2-PK	IBD vs IBS	810	0.000000	0.000000	***
CRP	Ca vs IBD	900	0.000000	0.000000	***
CRP	Ca vs IBS	870	0.000000	0.000000	***
CRP	IBD vs IBS	529	0.155641	0.466923	ns
Левкоцити	Ca vs IBD	706,5	0.000151	0.000454	***
Левкоцити	Ca vs IBS	755	0.000007	0.000020	***
Левкоцити	IBD vs IBS	548	0.148951	0.446853	ns
Хемоглобин	Ca vs IBD	2,5	0.000000	0.000000	***
Хемоглобин	Ca vs IBS	5,5	0.000000	0.000000	***
Хемоглобин	IBD vs IBS	542	0,175520	0.526561	ns

KEY FINDINGS:

- All biomarkers showed highly significant levels of differences between the three groups ($p < 0.001$)
- **Fecal calprotectin:** Ca (589.8) >> IBD (166.9) >> IBS (25.4) — all comparisons were with significantly significant differences
- **M2-PK:** Ca (52.3) >> IBD (6.0) >> IBS (3.0) — all comparisons were with significantly significant differences
- **CRP:** Ca (38.4) >> IBD (3.5) $\approx$ IBS (3.1) — values in CRC differed from those in the two control groups, but values in the control groups were close (IBD $\approx$ IBS)
- **Leukocytes:** Ca (8.7) > IBD (6.5) $\approx$ IBS (6.0) — values in CRC differed from those in the two control groups, but values in the control groups are close (IBD $\approx$ IBS)
- **Hemoglobin:** Ca (101.0) << IBD (144.3) $\approx$ IBS (139.6) — values in CRC differ from those in both control groups, but values in the control groups are close (IBD $\approx$ IBS)
- **Age:** patients with CRC are significantly older than those with UC (Crohn's disease and ulcerative colitis) ($p=0.002$), there are no significant differences in age between the control groups

Chapter IV. Clinicomorphological, laboratory and imaging correlations with prognostic value in different localization of colorectal carcinoma

1. In order to establish correlations between different biomarkers in patients with CRC, a correlation heatmap (Pearson) was prepared (Figure 28). The correlation heatmap (Pearson Heatmap) shows the strength and direction of linear relationships between multiple variables at once.

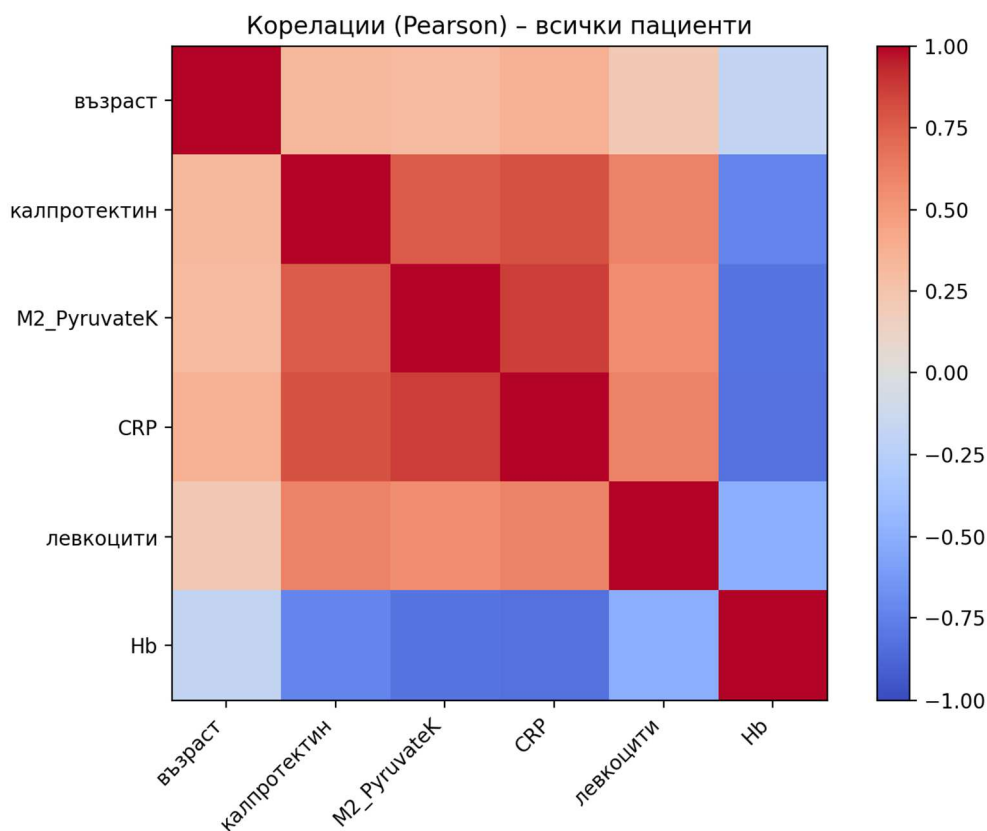


Figure 28. Correlation heatmap (Pearson)

Range: Values range between -1 and +1.

Positive relationship (+1): Both variables increase simultaneously.

Negative relationship (-1): One variable increases while the other decreases.

Zero relationship (0): There is no linear relationship at all.

The conclusion based on the above figure is that **calprotectin, M2-PK and CRP are strongly positively correlated, hemoglobin is negatively correlated and leukocytes are moderately positively correlated with inflammatory markers.**

2. Boxplot by groups (Figure 29).

A boxplot is a standardized way of showing the distribution of data based on a summary of five numbers: minimum, first quartile (Q1), median, third quartile (Q3), and maximum. The elements of a boxplot divide the data into four groups of equal size, with approximately 25% of the data falling into each section. The median (Q2) is the line drawn inside the box—it represents the average value of the data set, where half of the data are larger and the other half are smaller. The box (interquartile range—IQR) extends from Q1 (25th percentile) to Q3 (75th percentile). It represents the middle 50% of the data. The lines extending from the box to the minimum and maximum values show the variability of the lower (25%) and upper (25%) data values.

The analysis of the figures provides grounds for the following conclusions:

1) There are clear differences between the target group (patients with CRC) and the control groups (group 2 – patients with Crohn's disease and ulcerative colitis and group 3 – patients with irritable bowel syndrome) regarding fecal calprotectin, M2-PC, C-reactive protein (significantly higher in the target group) and hemoglobin (lower in the target group).

2) Leukocytes are higher in the target group compared to the control groups, but the differences are moderate.

2) Age is higher in the target group compared to the control groups, but the differences are more moderate.

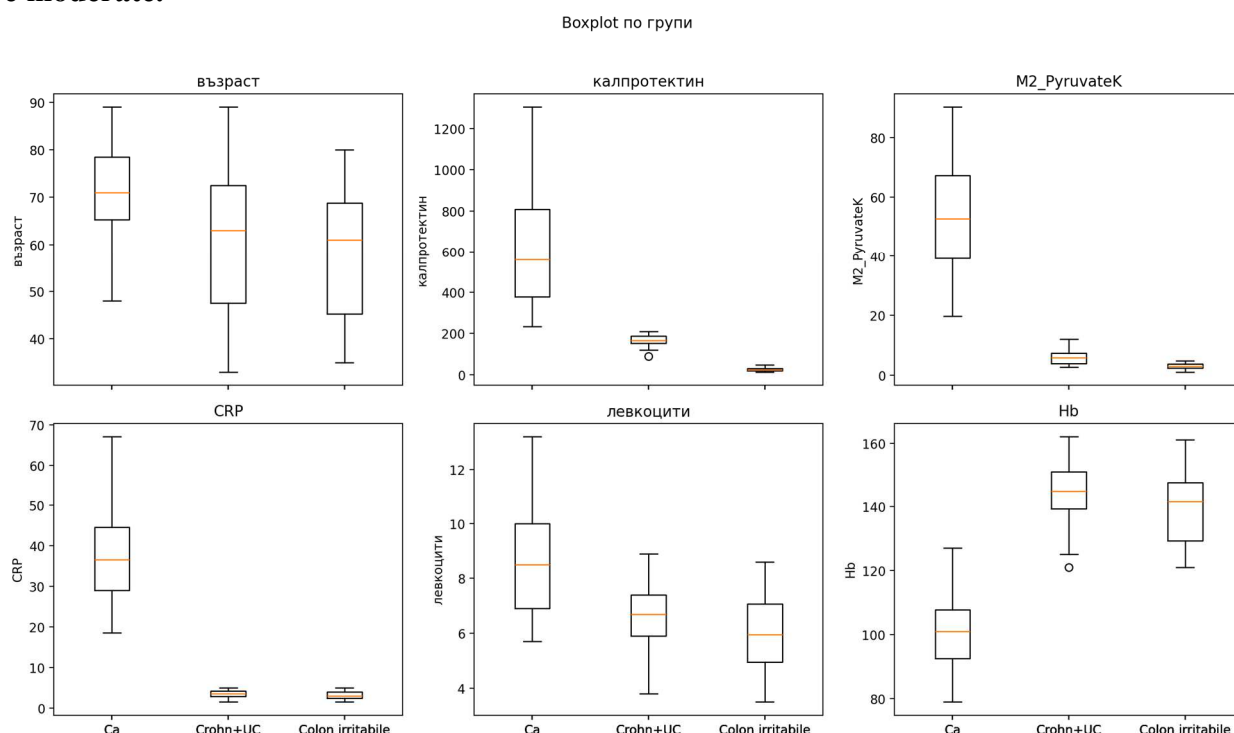


Figure 29. Boxplot by groups

3. Violin plots by groups (Figure 30).

A violin plot is a data plot that shows how numbers are distributed. It combines a box plot (for quickly summarizing numbers) and a density plot (for the shape of the data). It shows where data is common and where it is sparse.

Wide sections show where many data points are clustered. Narrow sections show where there are fewer data points. The inner box plot shows the median, quartiles, and range. The symmetrical shape makes it easier to see the overall pattern of the data.

Although a box plot shows exactly the same summarized numbers, it hides the shape of the data. A box plot can look exactly the same for a bell curve (one peak) and a U-shaped curve (two peaks). A violin plot reveals hidden peaks, called multimodality, so that the underlying patterns can be clearly seen.

We use this type of chart to compare distributions between different groups, to find multiple peaks or clusters in data sets, and to visualize the probability of certain values occurring.

The conclusion is that right-skewed distributions justify the use of Kruskal–Wallis & Dunn statistical tests.

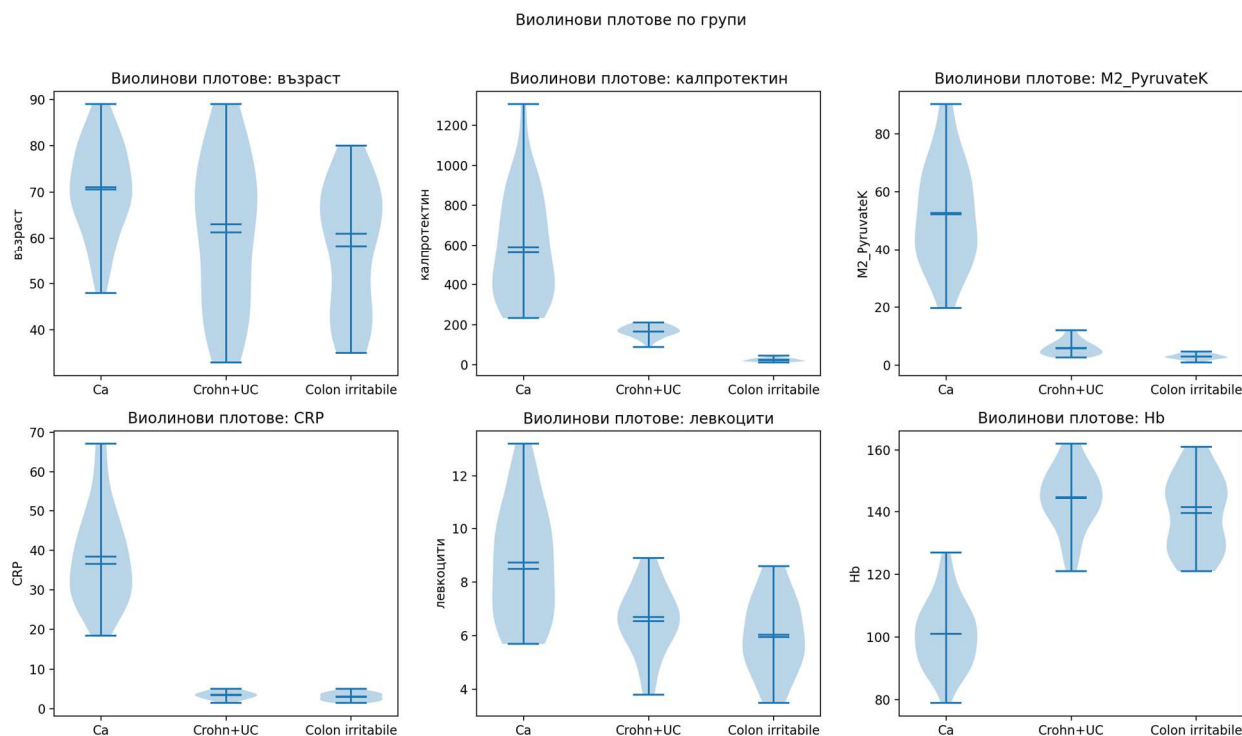


Figure 30. Violin plots by groups

4. Bland-Altman test (Figures 31, 32, 33).

The test is a statistical method used to compare two different measurement techniques or instruments. It assesses whether two methods agree well enough to be used interchangeably. The test produces a scatter plot – the Bland-Altman plot – where the difference between the paired measurements is plotted on the Y-axis and the mean of the two measurements is plotted on the X-axis.

Mean difference (deviation): A horizontal line representing the average of all the differences between the two methods. It indicates whether one method consistently overestimates or underestimates the other.

Limits of agreement: Two horizontal lines drawn at ± 1.96 times the standard deviation of the differences. These lines represent the range within which 95% of the differences between the two methods are expected to fall in future measurements.

Tolerance limits: Predetermined thresholds of acceptable error. The statistical method only displays the data; the user must decide whether the limits are practically acceptable.

How to interpret:

Good agreement: Most data points fall between the agreement limit lines, are randomly scattered, and cluster tightly around the zero difference line.

Systematic deviation: The mean difference line is far from zero, indicating that one method consistently measures higher or lower values than the other.

The Bland-Altman test directly analyzes how far apart the two measurements are, not just whether they follow the same trend.

The conclusion of the analysis is that the systematic deviation and wide agreement limits indicate that fecal calprotectin and C-reactive protein are not interchangeable.

4.1. Comparison calprotectin with CRP (log10)

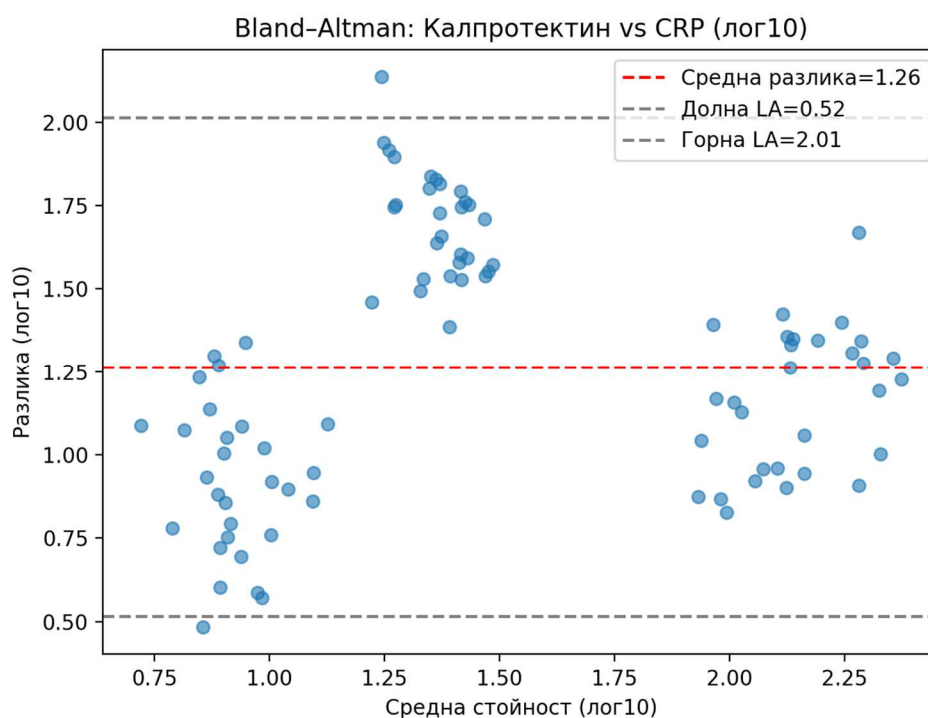


Fig. 31. Comparison of calprotectin and C-reactive protein

4.2. Comparison of M2-PK with CRP (log10)

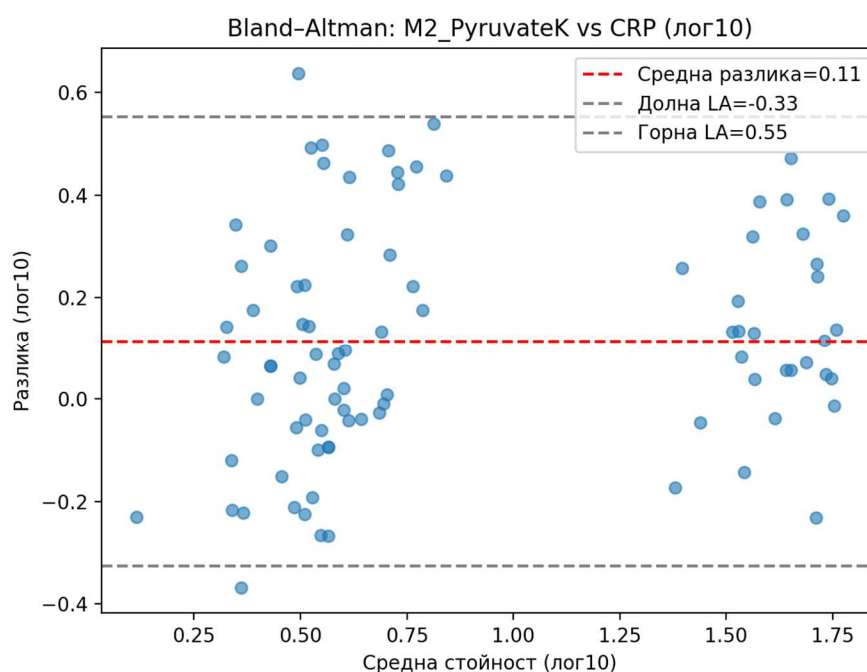


Fig. 32. Comparison of M2-PK and C-reactive protein

The conclusion from the analysis is that the systematic deviation is smaller, but the dispersion remains significant.

4.3. Comparison of fecal calprotectin with M2-PK (log10)

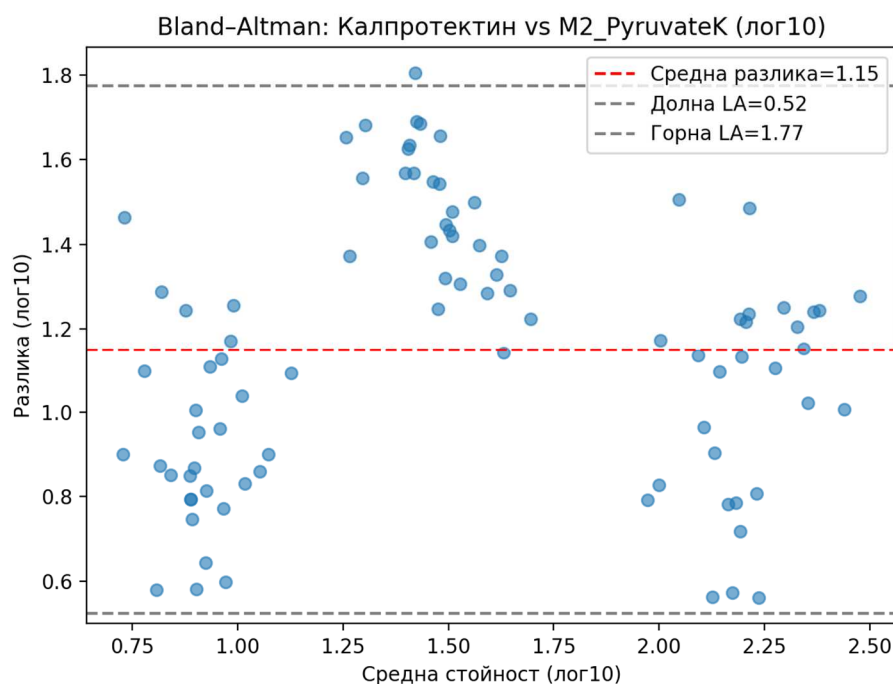


Fig. 33. Comparison of fecal calprotectin and M2-PK

The conclusion from the analysis is that there is systematic group bias and limited compliance.

5. Summary of correlation analyses in patients with CRC

5.1. Pearson correlation coefficient (Pearson r , p) (*Tables 7, 8*) – measures the strength and direction of the linear relationship between two quantitative (continuous) variables. It is a dimensionless index that ranges from -1 to +1. The value of the coefficient indicates the following:

- **+1:** Perfect straight positive relationship. Both variables increase simultaneously.
- **0:** There is no linear relationship between them. The variables are independent of each other.
- **-1:** Perfect straight negative relationship. When one increases, the other decreases.

The test is only for linear relationships – it works well for straight lines, but may miss complex curvilinear patterns.

Robustness to deviations (anomalies): One very large or very small result (outlier) can drastically change the entire coefficient.

Dependence on normal distribution: The data must follow at least an approximate normal distribution for the test to be accurate.

Table 7. Pearson r coefficient

показател	възраст	калпротектин	M2-□K	□R□	левкоцити	Hb
възраст	1.0	0.324	0.309	0.373	0.206	-0.173
калпротектин	0.324	1.0	0.764	0.8	0.607	-0.734
M2-□K	0.309	0.764	1.0	0.866	0.547	-0.806
□R□	0.373	0.8	0.866	1.0	0.595	-0.825
левкоцити	0.206	0.607	0.547	0.595	1.0	-0.507
Hb	-0.173	-0.734	-0.806	-0.825	-0.507	1.0

Table 8. Pearson_p coefficient

показател	възраст	калпротектин	M2-PK	CRP	левкоцити	Hb
възраст	0.0	0.0018	0.0032	0.0003	0.0513	0.1025
калпротектин	0.0018	0.0	0.0	0.0	0.0	0.0
M2-PK	0.0032	0.0	0.0	0.0	0.0	0.0
CRP	0.0003	0.0	0.0	0.0	0.0	0.0
левкоцити	0.0513	0.0	0.0	0.0	0.0	0.0
Hb	0.1025	0.0	0.0	0.0	0.0	0.0

5.2. Spearman's rank correlation coefficient

The Spearman's coefficient (*Tables 9, 10*) is a statistical measure of the strength and direction of the relationship between two variables. The test does not require that the data be normally distributed and evaluates monotonic relationships (whether the variables increase or decrease together, even if not at a constant rate).

Range of values: The coefficient varies from -1 to +1.

Value +1: Indicates a perfect positive relationship (both variables increase simultaneously).

Value -1: Indicates a perfect negative relationship (as one variable increases, the other decreases).

Value 0: Indicates no relationship between the variables.

The test is used for:

- **Non-normally distributed data:** When the source data does not follow a normal distribution.
- **Rank data:** When ordinal numbers/ranks are available.
- **Ordinal scales:** For survey data.
- **Nonlinear relationships:** When the relationship between variables is not a straight line, but the direction is maintained (monotonic).

Table 9. Spearman_r coefficient

показател	възраст	калпротектин	M2-PK	CRP	левкоцити	Hb
възраст	1.0	0.312	0.247	0.288	0.149	-0.145
калпротектин	0.312	1.0	0.853	0.706	0.492	-0.652
M2-PeK	0.247	0.853	1.0	0.72	0.475	-0.677
CRP	0.288	0.706	0.72	1.0	0.449	-0.689
левкоцити	0.149	0.492	0.475	0.449	1.0	-0.427
Hb	-0.145	-0.652	-0.677	-0.689	-0.427	1.0

Table 10. Spearman_p coefficient

показател	възраст	калпротектин	M2-PK	CRP	левкоцити	Hb
възраст	0.0	0.0027	0.0196	0.0062	0.1624	0.1728
калпротектин	0.0027	0.0	0.0	0.0	0.0	0.0
M2-PK	0.0196	0.0	0.0	0.0	0.0	0.0
CRP	0.0062	0.0	0.0	0.0	0.0	0.0
левкоцити	0.1624	0.0	0.0	0.0	0.0	0.0

Нб	0.1728	0.0	0.0	0.0	0.0	0.0
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5.3. ANOVA

ANOVA (analysis of variance) is a statistical method used to test for significant differences between the means of three or more independent groups. It determines whether the differences are real or simply due to chance by comparing the variance between groups to the variance within groups.

ANOVA evaluates the ratio of between-group variance to within-group variance, resulting in an F-statistic. If the between-group variance is significantly greater, this indicates that the group means are truly different from each other. If the variance is similar, the differences are likely due to random variation.

One-way ANOVA – compares the means of one continuous dependent variable based on one categorical independent variable (e.g., test scores from three different research methods) (*Table 11*).

Two-way ANOVA – examines the influence of two independent categorical variables on a continuous dependent variable (e.g., test scores from three different testing methods and two different age groups), allowing us to see whether the two variables interact.

To ensure the accuracy of the results, the data should generally meet the following criteria: 1) Normality – the dependent variable should be approximately normally distributed in each group; 2) Homogeneity – the variance between groups should be approximately equal; 3) Independence – observations within and between groups should be independent of each other.

Table 11. ANOVA

променлива	F	p_ANOVA	eta2_appr	n_total
възраст	6.72	0.0019	0.134	90
калпротектин	106.862	0.0	0.711	90
M2-PK	214.807	0.0	0.833	89
CRP	239.089	0.0	0.848	89
левкоцити	22.567	0.0	0.342	90
Нб	139.051	0.0	0.762	90

5.4. Kruskal–Wallis Test (*Table 12*)

The Kruskal–Wallis test (also known as the H-test) is a nonparametric statistical method used to compare three or more independent groups. It is the nonparametric alternative to the one-way analysis of variance (ANOVA) and is used when the data do not meet the requirements of a normal distribution. Instead of comparing means, the test ranks all the data from smallest to largest and checks whether the ranks are evenly distributed across the groups.

The test is nonparametric – it does not require the data to follow a normal distribution. The variables must be at least ordinal or continuous, converted to ranks. It is used to assess whether the medians of the groups are the same. It is only applicable to designs with 3 or more independent groups.

Table 12. Kruskal–Wallis test

променлива	H	p_Kruskal	n_total
възраст	11.384	0.0034	90
калпротектин	79.123	0.0	90
M2-PK	70.976	0.0	89
CRP	59.954	0.0	89
левкоцити	24.594	0.0	90

Hb	59.2	0.0	90
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5.5. Dunn post-hoc test (*Tables 13-18*)

After the initial ANOVA test has found a significant difference in three or more means, Dunn's test can be used to determine which specific means are significant relative to the others. Dunn's multiple comparison test is a post hoc (i.e., performed after the ANOVA) nonparametric test ("distribution-free test" that does not assume that the data come from a particular distribution). It is one of the weakest multiple comparison tests and can be a very conservative test, especially for a large number of comparisons. For example, if you have 10 comparisons with an alpha level of 0.05, then the formula results in a very high error rate per comparison of 0.005. The null hypothesis for the test is that there is no difference between the groups (the groups can be of equal or unequal size). The alternative hypothesis for the test is that there is a difference between the groups.

Table 13. Dunn post-hoc: age

група 1	група 2	z	p_raw	p_holm
Ca	Colon irritable	3.2713	0.0011	0.0032
Ca	Crohn + UC	2.3515	0.0187	0.0374
Crohn + UC	Colon irritable	0.9198	0.3577	0.3577

Table 14. Dunn post-hoc: fecal calprotectin

група 1	група 2	z	p_raw	p_holm
Ca	Colon irritable	8.8951	0.0	0.0
Ca	Crohn + UC	4.4476	0.0	0.0
Crohn + UC	Colon irritable	4.4476	0.0	0.0

Table 15. Dunn post-hoc: M2-PK

група 1	група 2	z	p_raw	p_holm
Ca	Colon irritable	8.3996	0.0	0.0
Ca	Crohn + UC	4.8316	0.0	0.0
Crohn + UC	Colon irritable	3.5986	0.0003	0.0003

Table 16. Dunn post-hoc: CRP

група 1	група 2	z	p_raw	p_holm
Ca	Colon irritable	7.0992	0.0	0.0
Ca	Crohn + UC	6.204	0.0	0.0
Crohn + UC	Colon irritable	0.9479	0.3432	0.3432

Table 17. Dunn post-hoc: leukocytes

група 1	група 2	z	p_raw	p_holm
Ca	Colon irritable	4.7698	0.0	0.0

Ca	Crohn + UC	3.5607	0.0004	0.0007
Crohn + UC	Colon irritable	1.2091	0.2266	0.2266

Table 18. Dunn post-hoc: Hb

rpyna 1	rpyna 2	z	p_raw	p_holm
Ca	Crohn + UC	-7.0773	0.0	0.0
Ca	Colon irritable	-6.1528	0.0	0.0
Crohn + UC	Colon irritable	0.9245	0.3552	0.3552

DISCUSSION

Colorectal carcinoma (CRC) is one of the most serious and socially significant problems facing modern public health. Globally, the disease ranks second in terms of cancer mortality (cause of 9.4% of cases) and third in terms of frequency of diagnosis among all oncological localizations. Forecasts for the coming decades outline a critical trend – by 2040, the global burden of CRC is expected to increase to 3.2 million new cases (+63%) and 1.6 million deaths per year (+73%), with the steepest escalation predicted for less industrialized countries and countries in economic transition.

In the Republic of Bulgaria, the statistical picture is particularly alarming and unfavorable. Nearly 5,000 new cases are diagnosed annually in our country, which constitutes 15.5% of all oncological morbidity, giving CRC the second place in frequency both in men (after lung cancer) and in women (after breast cancer). Unlike most countries in the European Union, where mass population screening has led to stabilization and a decline in mortality, Bulgaria is among the few exceptions with a continuously increasing cancer mortality rate – between 2,000 and 2,400 deaths annually. The main reason lies in the critically low coverage of prevention (barely 10% of the risk population between 50-74 years old) and the subsequent late diagnosis: over 60% of patients are detected in clinical stages III or IV, when the five-year survival rate drops below 15-20%.

The development of CRC is a slow, multi-step process, often lasting over ten years, which opens a wide window for early detection through non-invasive biomarkers and imaging methods. Modern scientific research proves that CRC is not a homogeneous nosological entity, but is divided into right-sided (R-S) and left-sided (L-S) carcinomas based on their different embryonic origin, anatomical blood supply, histological and genetic profile (MSI versus CIN pathways). The disclosure of precise clinicomorphological, laboratory and imaging correlations, as well as the differentiation of cancer inflammation from chronic autoimmune and functional intestinal diseases, is the key to an individualized diagnostic and therapeutic approach and the improvement of the unfavorable prognostic framework in our country.

1. Discussion of the importance of age as a risk factor for the development of CRC.

The incidence of colorectal cancer is closely related to age, and in developing countries the average age at diagnosis is 68 years for men and 72 years for women. In our study, the average age of patients with CRC was 67 ± 5.7 years, with the youngest being 39 years old and the oldest 89 years old. Worldwide, there is a trend towards a “rejuvenation” of the disease, which some authors define as the “under 50 phenomenon”. Increasingly, in countries with high standards, the disease is diagnosed in younger individuals in the age range of 40-49 years, which is probably due not only to a literal increase in the number of CRC patients at a younger age, but also to a well-developed CRC screening program. This justifies the need to reduce the universal age for screening from 50 to 45 years.

There is no debate about the logical concept that screening is necessary to detect precancerous changes and early forms of cancer and to reduce mortality from CRC. However, there are difficulties in the practical implementation of effective CRC screening. The use of CRC screening and local recommendations vary in different regions of the world and depend on the incidence of the disease, economic resources, health care structure and infrastructure involved in screening. For example, in the United States, screening is recommended to begin at age 45 for patients at average risk. European guidelines consider it appropriate to begin screening at age 50. In Japan, screening for CRC is

recommended to begin at age 40. In our study, a total of 16.66% of the target group was under the age of 60. Later inclusion of patients in a CRC screening program carries the risk of detecting the disease at a more advanced stage, which worsens the individual prognosis. For these reasons, we tend to agree with the American Cancer Society's recommendation that screening for CRC begin at age 45 for patients at average risk.

The situation is different for Lynch syndrome (the most common familial cancer syndrome) – a hereditary predisposition to CRC, which accounts for 3-5% of all CRC. Due to the high cumulative risk of CRC (up to 48% in patients with Lynch syndrome), there are international recommendations that it is appropriate to conduct genetic testing in all patients with CRC up to the age of 70. The prevailing opinion is that regular colonoscopies are the optimal method for preventing CRC in individuals with Lynch syndrome and should be performed depending on the genotype, starting at age 20-25 or 35 every 2 years. The recommendations are identical for Peutz-Jeghers syndrome (PJS) and MYH-associated polyposis (MAP).

2. Discussion of the importance of gender as a risk factor for the development of CRC.

A large number of studies have found that men are more often diagnosed with CRC than women. This was also found in our study - in the general group, men are the more frequently affected gender with CRC - men with proven CRC are 118 (57.3%), and women – 88 (42.7%) ($p < 0.05$). In the target group of patients with CRC, men also predominate (56.67%); women are 43.33% ($p < 0.05$).

3. Discussion of the localization of CRC as a prognostic criterion (alone and in combination with demographic characteristics – gender, age).

Men are more often diagnosed with CRC, but the female gender is predominant in right-sided CRC localizations, and men - in left-sided ones. In our study, in the general group of patients with CRC, men are the most frequently affected sex with CRC and are more prevalent than women in the localizations in the left colon and rectum ($p < 0.01$). In terms of the gender distribution of CRC in the right colon, both sexes are equally represented in terms of the number of cases. Analogously to the statistics presented for CRC in GOBOCAN 2023, our data confirm that the more frequent localization of CRC is in the left parts of the colon, compared to the right localizations. Analysis of the localization of CRC and gender from our data for the general group reveals that females prevail over males only in the group with cancer of the cecum and splenic flexure, but the results do not have a statistically significant value ($p > 0.05$). In the target group with CRC, the relative proportion of patients with CRC in the sigmoid colon (50%) is the highest ($p < 0.05$). The distribution of the remaining localizations is as follows: colon ascendens – 10%, colon transversum – 10%, colon descendens – 6.67%, rectum – 23.33%.

Studies from the 1980s and 1990s confirm that there are significant differences in the biology, symptoms and clinical course of CRC at different localization in the colon, with the location of the tumor affecting postoperative survival mainly in the groups of patients diagnosed in stages III and IV of the disease.

The analysis of patients from the target group with CRC regarding the stage of the disease established the largest relative proportion of patients in stage II of the disease (63.33%) ($p < 0.05$). The summarized distribution of stages by age and gender is as follows: stage I ($n = 10$) – seven men aged 56 to 89 years (median age 75 years); three women aged 59 to 65 years (median age 62 years); stage II ($n = 19$) – ten men aged 56 to 87 years (median age 75 years); nine women aged 48 to 81 years (median age 68 years); stage III – one woman aged 71 years. A limitation of the study is the impossibility of precise statistical analysis of the above data due to the “dispersion” of patients into very small subgroups.

According to a study on a cohort of 950 patients with CRC, 431 (45.3%) were right-sided, the rest were localized in the left colon and rectum, and 19.3% of patients with CRC were detected as a result of a screening program that included FCS and a test for occult bleeding. The significance of FCS both in screening and in the diagnosis of CRC gives the method a place as the “Gold Standard”. The study may have diagnostic and therapeutic possibilities with regard to early premalignant and initial malignant lesions and provides an opportunity to take histological material and to carry out endoscopic treatment. In the spirit of modern technologies, attention is now being paid to the benefits of artificial intelligence in screening endoscopies in order to optimize the accuracy and efficiency of detection and characterization of polypoid structures during endoscopic examination. In our study, there are no patients who were

identified through screening, and all underwent diagnostic FCS due to already manifested symptoms that became the reason for hospitalization.

The topographic localization of CRC along the colon is associated with 5-year survival and has been shown to be statistically significant in multivariate analysis in a number of studies. Along with patient age, ethnicity, TNM status, and histological type of tumor, tumor localization is also evaluated as an important prognostic factor. In a study of 33,789 patients with proven CRC (stage II), the following tumor localization was found – 46.8% right colon, 37.5% left colon, and 15.7% rectum. The team evaluated tumor localization along the colon as a new parameter for high risk in patients with stage II CRC, with the worst prognosis being patients with proven rectal carcinoma, with intermediate risk for patients with CRC in the left colon, and with the lowest risk being patients with CRC in the right colon. However, another study found that the combination of right-sided CRC with male gender, age over 75 years and some of the following histological variants of the tumor – mucinous or mucin-producing adenocarcinoma, or “stone ring” type carcinoma, poorly or moderately differentiated variants; CRC in stage III and IV of the disease had a worse prognosis. In right-sided CRC, a genetic link of tumor expression was established, leading to poorly differentiated adenocarcinomas – they have weakly expressed chromosomal instability and high microsatellite instability; a high number of polyps and a pronounced tendency for familial manifestation, as in Lynch syndrome. The genetic features of these tumors determine the right-sided localization of CRC as a higher risk for early metastasis, manifestation of poorly differentiated variants and those with a mucinous component. In left-sided CRC localizations, significant mutations in entire chromosomes have been identified, which leads to the development of polyps in the intestine, late manifestation of the disease, and it debuted with an advanced stage or manifestation of multiple primary CRC on the left. These features and differences in the course and manifestation of CRC and division of tumors into right, left colon and rectum can be explained by differences in embryogenesis - the right colon originates from the midgut, and the left colon and rectum from the embryonic hindgut. Fluid resorption increases in the distal sections and there the thickened fecal matter has more concentrated ingredients and is in closer contact with the intestinal mucosa. In the proximal sections of the colon, the incoming bile salts are of higher concentration, and their importance for tumorigenesis has already been confirmed.

4. Discussion of symptoms as a prognostic criterion.

Common symptoms of colorectal carcinoma (change in bowel physiology, rectal bleeding, weight loss) present late in the course of the disease. However, these symptoms are not specific for CRC and therefore have a rather low positive predictive value (3-4%), thus precluding their usefulness in establishing a diagnosis of CRC. However, the most important prognostic factor is the stage of the disease at diagnosis, which is why screening programs for CRC have been established in many countries for individuals over 60 years of age.

The most common specific symptoms in right-sided CRC locations in our study were change in stool consistency and changes in bowel interval and frequency. These are the most frequently reported symptoms in the right-sided CRC ($p < 0.02$), and in the left-sided CRC – colicky pain and blood in the stool ($p < 0.01$), which is consistent with the results of other studies. In our study, the most common laboratory abnormality in the right-sided CRC was anemic syndrome, followed by elevated CRP levels and, to a lesser extent, leukocytosis.

The relative proportions of specific symptoms for CRC in the target group of patients with CRC were as follows: abdominal pain in all patients in the group (100%), blood in the feces in 73.33%, impaired defecation rhythm in 70%, weight loss – in 26.67%.

Regarding the frequency of specific symptoms, the following was found: abdominal pain was present in all patients in the target group, regardless of the stage and localization of the tumor process. Blood in the stool was present in 73.33% of the group; in all patients in stage I of CRC, in 11 patients in stage II and in the only patient in stage III ($p < 0.05$). Regarding the localization of CRC, blood in the stool was present in all patients with CRC of the sigmoid colon and in all patients with CRC of the rectum. The symptom was absent in CRC of the colon ascendens, colon transversum and colon descendens ($p < 0.05$). Disturbed defecation rhythm was present in 70% of the patients in the group; in half of the patients in stage I, in 78.95% of the patients in stage II and in the only patient in stage III ($p < 0.05$). Regarding the localization of CRC, a disturbed rhythm of defecation was present in all three

patients with CRC of the colon transversum, in two patients with CRC of the colon descendens, in fourteen patients with sigmoid cancer and in two patients with CRC of the rectum. The symptom was absent in CRC of the colon ascendens ($p < 0.05$). Weight loss was present in 26.67% of the patients in the target group – in one patient in stage I of CRC and in seven patients in stage II. One of these patients had a tumor localization in the sigmoid colon and seven – in the rectum. The symptom was absent in the proximal parts of the colon ($p < 0.05$).

5. Discussion of tumor histology as a prognostic criterion.

Regarding histological verification, the results in our study support the global data and the most common variant was proven to be adenocarcinoma, and according to the degree of differentiation, the highly differentiated variants G1 (42.2%) prevailed, followed by the moderately differentiated G2 (32.0%) and the poorly differentiated ones G3 (24.3%). Regarding tumor localization in the right colon, the moderately differentiated and highly differentiated histological variants G2+G1 with the highest relative share were ($p < 0.01$). In the tumors of the left colon, those with a high degree of differentiation G1 predominated, followed by those with a low G3, and all cases of rectal carcinoma were moderately differentiated variants G2, but this result was based on only four cases, which may obscure the interpretation of the results. These results are comparable to results from other studies.

It has been shown that tumors of the right colon are histologically distinguished from those of the left colon by hyperexpression of the markers CD44s, CD44v6, β -catenin and CD68+. Rectal tumors, unlike those in the left colon, are characterized by a lower T-status, respectively size, and have a higher level of e-cadherin expression. When comparing the histological differences between the right colon and rectum, it was found that tumors with right localization have a larger diameter, a more advanced pT status and much more often have microsatellite instability in genetic analysis.

6. Discussion of biomarkers as prognostic criteria.

The gold standard for the diagnosis of colorectal cancer is colonoscopy, but the disadvantages of this method are that it is invasive, resource-intensive, and expensive. Instead, the screening method for CRC used in most countries is the fecal hemoglobin (F-Hb) test, followed by instrumental examination of the colon in subjects with a positive test. The accuracy of the F-Hb test depends on the method used and the cutoff value for defining a positive test. Fecal immunochemical tests (FIT) perform better than the F-Hb test – they have a specificity of 94% in detecting colorectal cancer, but only a moderate sensitivity (74%) and fail to detect precancerous conditions (polyps and adenomas). The low sensitivity of FIT for detecting early stages of colorectal cancer highlights the need to find new biomarkers that would improve the detection rate in CRC screening.

Having discussed the above-mentioned fecal occult blood tests, we decided to shift the screening narrative from looking only for microscopic blood traces to detecting the metabolic and inflammatory tumor microenvironment. Inflammation of the colon can lead to carcinogenesis. The presence of an inflammatory microenvironment is a key hallmark of cancer. In addition, inflammatory responses at the local and systemic levels play an important role in disease progression and survival in colorectal cancer. Current methods for assessing local inflammation rely on tissue sampling, which is not suitable for population screening.

We decided to take a comprehensive approach, starting from key stages in carcinogenesis and selecting biomarkers that encompass three different biological pathways of early colorectal oncogenesis:

6.1. Metabolic switch (M2-PK): M2-PK is a key dimeric glycolytic enzyme overexpressed by proliferating tumor cells. It reflects altered tumor metabolism (aerobic glycolysis – the so-called “Warburg effect”), not bleeding. M2-PK allows the panel to detect non-bleeding adenomas and early-stage tumors that standard fecal immunochemical tests (FIT) often miss.

6.2. Local inflammatory response (fecal calprotectin): Calprotectin is a neutrophil-derived protein that indicates inflammation of the intestinal mucosa. Although highly sensitive to colorectal changes, it lacks specificity, as it is also elevated in inflammatory bowel disease (IBD). We discussed that combining it with M2-PK would help differentiate malignant inflammation from benign organic intestinal diseases.

6.3. Systemic cascade (CRP, WBC and Hb): As a localized tumor progresses, it triggers an undetectable systemic inflammatory response (elevation of CRP and WBC) and microbleeding (decrease in systemic hemoglobin over time).

By using fecal calprotectin (FCP), M2-pyruvate kinase (M2-PK), C-reactive protein (CRP), hemoglobin (Hb) and white blood cell count (WBC), we decided that the project would combine fecal markers (local) with blood indicators (systemic).

Fecal calprotectin is a sensitive marker of colonic inflammation, primarily used in the clinical evaluation of inflammatory bowel disease. It is used to differentiate between organic and functional disorders of the colon. It is a calcium-binding protein and is found primarily in the cytoplasm of neutrophils and the membrane of monocytes. FCP is released on neutrophils upon cell death or injury and is thought to have regulatory roles on components of the inflammatory process, including other myeloid cells (e.g., CD11b+ cells). FCP enters the intestinal lumen by migration and is resistant to enzymatic degradation and can therefore be readily detected in body materials such as feces. Elevations in FCP occur in a wide variety of gastrointestinal conditions, including colitis and malignancy, and although it is a sensitive measure of inflammation, it is not specific for any condition. However, given the importance of inflammation in the development and progression of cancer, the presence of elevated PCP may provide additional assessment of a patient's risk of colorectal neoplasia and progression. PCP is resistant to enzymatic degradation during transit through the gastrointestinal tract and remains stable for up to 7 days at room temperature. It is also easily measurable. The final result can be available after 15 minutes of processing. A value below 50 µg/g is considered normal.

Pyruvate kinase isoenzyme type M2 (M2-PK) is an isoenzyme of pyruvate kinase, a key enzyme within glycolysis, controlling the metabolism of cells with a high rate of proliferation, such as tumor cells. It detects specific changes in intestinal cells in polyps and colorectal carcinoma, as well as patients with acute or chronic intestinal inflammation (e.g. ulcerative colitis, Crohn's disease). This isoenzyme catalyzes the conversion of phosphoenolpyruvate to adenosine triphosphate, producing pyruvate. Depending on the metabolic functions of the tissues, different isoenzymes of pyruvate kinase are prominent. The isoenzyme M2-pyruvate kinase (M2-PK) can occur in a highly active tetrameric form, as well as in a dimeric form with low activity.

The dimeric form is almost inactive and favors the channeling of glucose carbons into synthetic processes, such as nucleic acid, amino acid and fatty acid synthesis. The tetrameric form is highly active and favors the energy-reducing conversion of phosphoenolpyruvate to pyruvate and lactate. In tumor cells, M2-PK is mainly found in a dimeric form and is therefore called “tumor M2-PK”. M2-PK dimerization is induced by interaction with various oncoproteins, including pp60v-src-kinase, oncogenic fibroblast growth factor 1, and human papillomavirus 16 E7. The dimeric form of M2-PK is released from tumors into the blood and can be quantified by sandwich enzyme-linked immunosorbent assay (ELISA; ScheBo Biotech AG, Giessen, Germany). There have been about 40 published studies on M2-PK concentrations in blood since 1997. They have shown a significant increase in M2-PK and a correlation with the staging of the following tumors: melanoma, thyroid, breast, lung, kidney, esophageal, gastric, pancreatic, colorectal, ovarian, cervical and renal cell carcinoma. Long-term determination of M2-PK in EDTA plasma is used as a follow-up tool to monitor failure, relapse or success during therapy. In colorectal carcinoma and adenoma, M2-PK is released in the feces.

M2-PK is essential for CRC tumor growth by regulating cell proliferation and migration of colon cancer cells. Higher amounts of this isomer are found in advanced tumor stages and are associated with lymphatic metastasis in patients with colorectal cancer. Tumor M2-PK is detected in the feces of patients with colorectal carcinoma and colorectal polyps. The amount of M2-PK in feces can be quantified by enzyme-linked immunosorbent assay (ELISA), and thus fecal M2-PK has been proposed as a tool for the detection of colorectal carcinoma.

The rapid fecal M2-PK test showed sensitivity, specificity, positive predictive value, and negative predictive value of 58.8%, 85.5%, 11.2%, and 98.5%, respectively, in the detection of colorectal adenocarcinoma. For the detection of colorectal adenoma, this test gave sensitivity, specificity, positive predictive value, and negative predictive value of 27.3%, 86.3%, 27.0%, and 86.5%, respectively.

C-reactive protein (CRP) – this marker is not used alone for the diagnosis of CRC, but is a key biomarker for prognosis, complications and treatment monitoring. The key role of CRP in colon cancer is

as a prognostic marker: 1) Persistently high CRP levels before treatment are often associated with a more advanced stage of the tumor; 2) Metastasis indicator – elevated values may signal the spread of cancer, especially to the liver; 3) Survival assessment – patients with low CRP levels usually have a better prognosis and longer survival; 4) Postoperative monitoring – a sharp rise in CRP days after surgical removal of the tumor alerts for infection or a leak in the anastomosis (the connection of the intestines); 5) Relapse control – a chronic rise during remission may be an early sign of tumor recurrence.

Why is CRP elevated in oncological diseases? The answer to this question lies in the so-called tumor microworld: colon cancer causes constant chronic inflammation in the surrounding tissues. An immune response follows – the body produces cytokines (IL-6), which stimulate the liver to secrete large amounts of CRP into the blood. There is also tissue destruction – tumor growth damages healthy cells and causes micronecrosis, which activates the inflammatory marker. An important limitation of the use of CRP is its low specificity – CRP is a general marker of inflammation. It increases in bacterial and viral infections, dental abscesses or arthritis. It does not replace tumor markers. For colon cancer, the main specific marker is CEA (carcinoembryonic antigen), not CRP.

7.1. Correlation of local with systemic biomarkers

Relationship between local and systemic inflammation: There is a direct positive correlation between markers in stool and blood markers. Patients with the highest fecal calprotectin (local inflammation) showed the highest CRP and increased leukocyte count. **All patients in the target group had elevated C-reactive protein (100%) ($p < 0.0001$) and elevated fecal calprotectin ($p < 0.0001$).**

Bleeding vs. anemia: All 30 patients with CRC (target group) presented with systemic anemia (low hemoglobin) ($p < 0.0001$), showing increased levels of inflammatory markers. This gives reason to assume that tumor bleeding is related to the local inflammatory microenvironment.

7.2. Metabolic profile of confirmed malignancy (M2-PK)

Validation of the “Warburg effect”: Since all 30 patients had confirmed colorectal cancer, we were able to evaluate M2-PK as a definitive metabolic marker. **All patients in the target group had elevated values of the marker (100%), in contrast to the two control groups.**

7.3. Study limitations

The selected target group of 30 CRC patients ($N_1 = 30$) limits broad epidemiological claims, but provides a well-characterized, pathologically verified baseline to demonstrate that this panel of five markers corresponds to real oncogenesis.

A limitation of the study is the “dispersion” of patients into small subgroups regarding stages and locations, which does not allow for statistically reliable conclusions in these directions.

7.4. The advantage of the study is the possibility of comparing the target group with the two control groups and the proven significant statistical differences of the studied biomarkers in patients with CRC, compared separately with each of the two control groups. This conclusion is confirmed by all applied statistical methods. **By comparing 30 patients with colorectal cancer (CRC) with 30 patients with inflammatory bowel disease (IBD: Crohn's disease and ulcerative colitis) and 30 patients with irritable bowel syndrome (IBS), we have a very good basis for solving a major clinical problem: diagnostic differentiation.** In primary care, CRC, IBD and IBS often present with overlapping gastrointestinal symptoms (abdominal pain, altered bowel habits). The studied 5-biomarker panel acts as a biological filter.

At the same time, the panel studied outlines different biological profiles for each group, proving that **this panel does not simply detect a disease, but identifies the type of disease. It helps outline three biological profiles:**

Profile 1: Malignant/Metabolic Profile (CRC)

The expected constellation is high M2-PK, moderately high fecal calprotectin, normal to high CRP/WBC and progressive decline in hemoglobin (chronic microbleeding). In this profile, M2-PK is the main tool for specificity. While both CRC and IBD patients will show increased local inflammation, M2-PK (the metabolic marker for the Warburg effect) should be significantly higher in the CRC group than in the SCD and IBD groups. This allows us to differentiate tumor growth from standard mucosal inflammation.

Profile 2: Purely inflammatory profile (UCD – Crohn's disease/Ulcerative colitis)

The expected constellation is elevated FCP, high systemic CRP, elevated leukocytes, variable hemoglobin and moderate to low M2-PK. It is debatable whether a massive fecal calprotectin peak, combined with a normal or slightly elevated M2-PK, can safely rule out IBS in favor of an active flare-up of IBS.

Profile 3: Functional Baseline (FB)

The expected constellation is a normal FB, normal M2-PK, normal CRP, normal WBCs, and normal hemoglobin.

IBS is a functional bowel disorder, meaning that there is no structural or biochemical pathology. Ideally, the data should show that the panel yields a very high rate of true negatives (high specificity) in the IBS group. This demonstrates that the test panel studied in our study can be used in primary care to quickly reassure patients with irritable bowel syndrome (IBS) and prevent unnecessary, invasive colonoscopies.

8. The following issues are further subject to discussion:

8.1. Clarifying the “calprotectin confound”

Fecal calprotectin is highly sensitive to intestinal problems, but is not good enough to distinguish cancer from colitis. The solution to this problem is to study blood markers (Hb, WBC, CRP) and a metabolic marker (M2-PK), which act as “modulators”. Simultaneous study of these markers with fecal calprotectin creates a multidimensional view that separates autoimmune tissue damage (ATD) from neoplastic cell mutation (NCM).

8.2. Systemic vs. Local Inflammation

By comparing the ratio of local inflammation (FP) to systemic inflammation (CRP) in all three groups, the logical conclusion is the following: exacerbations of inflammatory bowel disease (IBD) often cause massive, parallel peaks in both FP and CRP, while early IBD may present with elevated local markers but relatively quiescent systemic blood markers.

8.3. Clinical triage and health economics.

The specific three-group data set demonstrated in our study justifies the use of the investigated 5-marker panel as a cost-effective triage tool in primary care. Instead of referring all patients for colonoscopy, the general practitioner can use the present panel to expedite the diagnosis of patients suspected of having CRC, refer the group with IBD to a gastroenterologist for treatment of inflammation, and manage the group with SRC noninvasively.

At the end of the dissertation work, we propose a model algorithm for early screening for CRC (**Fig. 34**), suitable for detecting patients with low/intermediate/high risk of CRC, suitable for primary care, which will reduce unnecessary and expensive examinations by narrow specialists or will assign such examinations with justification and will reduce overall health care costs.

CONCLUSION

Our study confirms that there are clinicomorphological and laboratory correlations with different localization of CRC along the colon and they should be taken into account in the assessment of the treatment strategy of the individual patient, as well as in the prognosis for 5-year survival after complex surgical and oncological treatment. The lack of mandatory screening leaves many patients with early stage CRC undetected. This predisposes to slow development and progression of the disease stage, until it manifests itself with some of its complications. This indicates the need for a national strategy regarding mandatory screening for CRC.

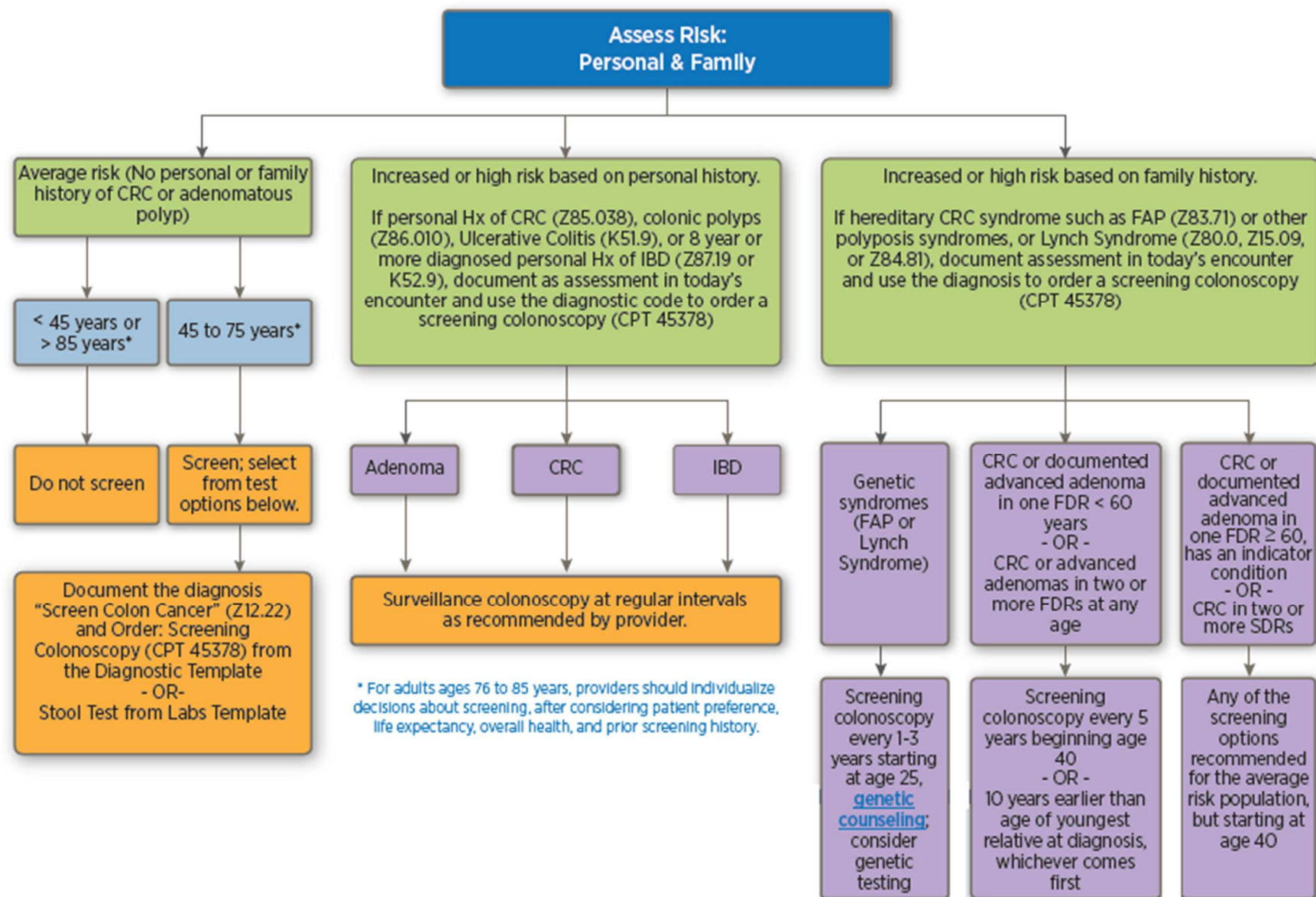


Fig. 34. Algorithm for early screening for colorectal cancer

CONCLUSIONS

1. The demographic and epidemiological characteristics of the studied group of patients with CRC are identical to those of other populations globally.

- Age is a key factor for carcinogenesis.
- Male gender is at higher risk for developing colorectal carcinoma.
- The presence of a family history of genetic predisposition to colorectal carcinoma requires early and more intensive follow-up of the relevant patients.
- The presence of chronic bowel diseases (Crohn's disease, ulcerative colitis) also requires more intensive follow-up of the relevant patients.

2. The risky behavioral characteristics of the modern population mediate impaired control of chronic conditions with a risk of carcinogenesis and a generally impaired quality of life.

3. There is a correlation between the localization and detectability of colorectal carcinoma, which gives rise to the need for a personalized diagnostic approach in patients.

4. Clinical symptoms (specific and non-specific) are not decisive for the diagnosis of colorectal carcinoma, but their presence is a mandatory requirement for more precise screening with a wider range of diagnostic methods.

5. There is a reliable dynamics in the values of some basic laboratory indicators in the course of carcinogenesis.

- Hemoglobin – categorically below reference limits for both sexes in patients with colorectal carcinoma, significantly different compared to control groups.
- Leukocytes – average values within the reference range in patients with colorectal carcinoma, but the presence of an increased number requires expansion of laboratory and instrumental screening.

6. The established correlations between biomarkers of chronic inflammation (CRP, fecal calprotectin, M2-pyruvate kinase) and clinical and laboratory indicators confirm their potential as an alternative tool to invasive methods for the diagnosis of colorectal carcinoma and a reliable tool for a more precise prognostic assessment of a patient with colorectal carcinoma.

- M2-pyruvate kinase (M2-PK) – the rapid test for fecal M2-PK shows sensitivity, specificity, positive predictive value and negative predictive value of 58.8%, 85.5%, 11.2% and 98.5%, respectively, in the detection of colorectal adenocarcinoma.

- Fecal calprotectin – a sensitive marker of colon inflammation. It is used to distinguish between organic and functional disorders of the colon. Increased FC can provide an additional assessment of the patient's risk of colorectal neoplasia and progression. Easily measurable.

- C-reactive protein (CRP) – is not used for colon cancer diagnosis, but is a key biomarker for prognosis, complications and treatment monitoring. It requires careful interpretation due to its low specificity.

- Differential diagnostic capacity of biomarkers: through the comparative study between the target group with CRC and the control groups (CVD and irritable bowel syndrome), the high diagnostic value of the combined study of systemic inflammatory markers (CRP) and specific fecal indicators (fecal calprotectin, M2-PK) for distinguishing cancer-related persistent inflammation from functional disorders is proven.

CONTRIBUTIONS:

ORIGINAL CONTRIBUTIONS

Original scientific contributions for Bulgaria:

1. The standard diagnostic panel in colorectal cancer monitoring is discussed, with a view to determining key clinical and laboratory indicators and including more sensitive biomarkers for chronic inflammation.
2. Integrated correlation model – for the first time on a regional scale, a complex model is being built, linking the anatomical localization of the tumor (left-sided versus right-sided CRC) with its specific clinicomorphological, laboratory and imaging characteristics with direct prognostic value.
3. The role of early screening for colorectal cancer and the selection of alternative methods to colonoscopy in order to prevent delayed diagnosis are studied.
4. The correlation of age with the process of carcinogenesis and its importance as a prognostic factor are studied.
5. Correlations of specific clinical laboratory parameters (hemoglobin, leukocytes) with biomarkers (C-reactive protein, fecal calprotectin and M2-pyruvate kinase) and their potential role in early screening for colorectal carcinoma were studied.
6. An algorithm for early screening for colorectal carcinoma (convenient for clinical practice) was proposed, aiming to improve the prognosis of the disease.

CONTRIBUTIONS WITH A SCIENTIFICALLY CONFIRMATIVE AND AN APPLICABLE CHARACTER

1. Global trends of increasing incidence of colorectal carcinoma and gradual decrease in life expectancy in patients with this disease are confirmed.
2. Higher risk of developing colorectal carcinoma in men is confirmed.
3. The importance of the stage and localization of colorectal carcinoma for the overall prognosis and specifically for the 5-year survival after personalized therapy is confirmed.
4. Early diagnosis of colorectal carcinoma and a personalized treatment approach based on evidence are key to improving the prognosis.
5. Algorithm for early screening – a clinically applicable approach for prevention and early screening of CRC has been developed, consistent with the specifics of the Bulgarian healthcare system and modern European guidelines.
6. Optimization of differential diagnosis – a three-component diagnostic panel (target group CRC, CBC, irritable bowel syndrome) is proposed for implementation in practice, which will assist the physician in quickly distinguishing malignant from benign intestinal conditions.

PUBLICATIONS RELATED TO THE DISSERTATION TOPIC
with the participation of Dr Dilyana Stefanova, MD

1. **Petrova D**, Stoykov D, Gorcheva Z. *Non-invasive Screening Methods for Early Detection of Colorectal Cancer*. J of IMAB. 2026 Jul-Sep;32(3):7076-7080. <https://doi.org/10.5272/jimab.2026323.7076> ISSN: **1312-773X** (Online)
2. **Dilyana Stefanova**, Dimitar Stoykov. *CLINICO-MORPHOLOGICAL CORRELATIONS BETWEEN COLORECTAL CARCINOMAS ACCORDING TO LOCATION*. Surgery, VOL. 89, Issue 1 Online ISSN:3033-151X, Print ISSN:0450-2167.
3. **Стефанова Д.**, Д. Стойков. *Синдром на дразнимото черво (колон иритабиле) – често срещано заболяване след КОВИД-инфекция*. Сп. ХИРУРГИЯ, 2025; 89(1):1-9. Online ISSN: 3033-151X, Print ISSN: 0450-2167

PARTICIPATIONS IN SCIENTIFIC FORUMS RELATED TO THE DISSERTATION TOPIC:

- 1) Participation in the National Conference on Gastroenterology – 2022, 2023, 2024
- 2) Participation in the I,II,III,VII,VIII Conference of a Young Gastroenterologist.

PARTICIPATION IN SCIENTIFIC AND RESEARCH PROJECT, FINANCED BY MEDICAL UNIVERSITY – PLEVEN

Scientific and research project (D2/2024) topic: “*Colorectal carcinoma – clinicomorphological, laboratory and imaging correlations with prognostic value in different localizations*”

Research team: Dr Dilyana Stefanova Petrova, MD, Prof. Dr. Dimitar Zhivkov Stoykov, MD, DScs; Prof. Dr. Adelaide Lazarova Ruseva, MD, PhD; Assoc. Prof. Dr. Zornitsa Veselinova Gorcheva, MD, PhD.