MEDICAL UNIVERSITY
"PROF. DR. PARASKEV STOYANOV" – VARNA

SECOND DEPARTMENT OF INTERNAL DISEASES
ES OF GASTROENTEROLOGY, HEPATOLOGY AND NUTRITION

Dr. ASIYANA HRISTOFOROVA PETROVA, MD

COMPREHENSIVE PERFORMANCE ASSESSMENT OF BIOLOGICAL THERAPY IN PATIENTS WITH ACTIVE ULCERATIVE COLITIS

ABSTRACT

OF A DISSERTATION FOR THE AWARD OF THE EDUCATIONAL AND SCIENTIFIC DEGREE **"DOCTOR OF PHILOSOPHY"**

Varna

2023

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EDUCATIONAL AND SCIENTIFIC DEGREE "PHD"
Scientific specialty "Gastroenterology"- 03.01.14

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Varna, 2023

The dissertation work is in the field of specialty "Gastroenterology" - code 03.01.14.

The dissertation consists of 180 pages and is illustrated with 32 figures and 71 tables. The cited literature includes 229 sources, of which 17 in Cyrillic and 212 in Latin. The appendices are 11 and represent 26 pages.

The dissertation was discussed and referred for public defense by the Departmental Council of the Second Department of Internal Medicine of the Medical University, Varna. Faculty of Internal Medicine, Varna.

The public defense of the dissertation will take place on November 8, 2023 from .....hours at an open meeting of the Scientific Jury in WEBEX platform.

The defense materials are available at the Department of Scientific Activity and Career Development, Medical University - Varna.

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Abbreviations used

Cyrillic:

YK Ulcerative colitis

BC Crohn's disease

IBD Inflammatory bowel disease

ESU Erythrocyte sedimentation rate

FCP Fecal calprotectin

QoL Quality of life

EEA Extraintestinal manifestations

PSH Primary sclerosing cholangitis

GDA Iron deficiency anaemia

BMI Body mass index

FCS Fibrocolonoscopy

ALT Alanine aminotransferase

ASAT Aspartate aminotransferase

GGT Gammaglutamyltranspeptidase

GCS Glucocorticosteroids

LMW Low molecular weight heparin

BTE Pulmonary thromboembolism

In Latin:

IBD Inflammatory bowel disease

TNFα Tumour necrosis factor alpha

IL Interleukin

UCDAI Ulcerative Colitis Disease Activity Index

ANCA Antineutrophil cytoplasmic antibodies

TDM Therapeutic drug monitoring

ADA Antidrug antibodies

5-ASA 5-aminosalicylic acid

FDA Food and Drug Administration

ELISA Enzyme-linked immunosorbent assay

IBDQ Inflammatory Bowel Disease Questionnaire

DFIS Daily Fatigue Impact Scale

JAK 1 Janus kinase type 1

TYK 2 Tyrosine kinase 2

PCR-RT Polymerase chain reaction

MRCP Magnetic resonance cholangiopancreatography

1. INTRODUCTION

Inflammatory bowel diseases (IBD), namely ulcerative colitis (UC) and Crohn's disease (CD), are chronic immune-mediated conditions with a high prevalence in developed countries and a rapidly increasing incidence in highly industrialized countries. Their global prevalence is expected to affect nearly 30 million people by 2025. The pathogenesis of UC is complex and still unclear. The disease often progresses over time, occurring in frequent spurts, which necessitates that clinical evaluation and treatment follow its dynamic nature.

Ulcerative colitis is an idiopathic bowel disease that results in the appearance of continuous inflammation of the mucosa, from the rectum to the proximal part of the colon. Genetic factors, environmental influences, autoimmunity and the gut microbiome are the main contributing factors.

Ulcerative colitis occurs in relatively young people, requiring long-term and continuous drug therapy that allows the disease to enter remission. A significant number of patients fail to adhere strictly to the prescribed drug therapy, increasing the risk of relapse.

The choice of therapy is determined by the course, extent, severity of disease, lack of response to treatment and safety.

The main goals of UC therapy are to induce and maintain clinical and endoscopic remission of the disease, reduce the risk of complications and improve quality of life.

In patients with IBD, the effectiveness of drug therapy is usually assessed using clinical assessment in combination with inflammatory biomarkers. However, recorded symptoms often do not reflect the level of mucosal inflammation, and biomarkers are relative. Therefore, there is a need to improve the monitoring of HAI activity.

Blood tests including C-reactive protein (CRP) are commonly used, but they have only suboptimal sensitivity and specificity for intestinal inflammation. Fecal biomarkers are noninvasive and are used to specifically measure intestinal inflammation and to assess disease activity in UC.

An important goal in the treatment of UC is to maintain remission throughout the patient's life course. This goal is difficult to achieve for several reasons: available medications are not effective in all patients, decreased compliance (adherence to therapy), and subclinical manifestations of the disease prior to its active phase are difficult to predict. The therapeutic targets for IBD have changed with the introduction of biological therapy. These medications have demonstrated that control of mucosal inflammation is possible. Healing of the mucosa is accompanied by fewer complications and better patient parameters achieved. At present, symptom control, which was one of the main goals in treatment, appears to be insufficient. Today, the main task is mucosal healing. A number of studies have shown that mucosal healing in UC is accompanied by long-term clinical remission without corticosteroids and a reduced risk of colectomy. Maintenance treatment of ulcerative colitis does not provide prevention of exacerbations and complications over a long period of time.

The first element of treatment success is to administer effective therapy even when patients are asymptomatic. The second is to inform the patient of the importance of administering the treatment, and the third element is its maintenance and control of inflammation. Limited histological and endoscopic colonic damage is associated with a reduced risk of cancer. The fourth key element is the inclusion of immunomodulators when necessary.

The fifth and final element is achieving biological efficacy by avoiding low levels of the drug and monitoring for subclinical inflammation and reappearance of symptoms at the end of dose intervals.

Ulcerative colitis is a disease of the intestine, with periods of relapses and remission. Although the best indicator of disease intensity is endoscopic activity, it is also important to determine the clinical severity in order to plan optimal therapy. Achieving sustained remission is a major goal and challenge that finds an answer and solution in modern biologic treatment.

This determined our motivation for conducting this clinical observation on the efficacy and safety of biologic therapy for active ulcerative colitis.

2. AIM AND OBJECTIVES

2.1. Aim of the dissertation

The main aim of this thesis is to clinically evaluate the efficacy and safety profile of biological therapy in patients with active ulcerative colitis.

2.2. Objectives of the dissertation

In order to achieve the above goal, we set the following tasks:

1. To study the demographic characteristics of the patients undergoing biological therapy.

2. To perform a comprehensive clinical evaluation of the efficacy of biologic therapy in patients with active ulcerative colitis.

3. To analyze the results of biological therapy, by group according to the drug administered.

4. To describe the side effects and and evaluate the safety profile of the administered medication.

5. To analyze the quality of life and the degree of fatigue in patients with ulcerative colitis undergoing biologic therapy.

6. To design a therapeutic algorithm for biologic treatment, selecting the right patient for the appropriate regimen and predictors of treatment response.

7. Establish an active registry of patients with active ulcerative colitis on biological therapy.

2.3. Inclusion criteria:

1) Patient age between 18 and 85 years.

2) Endoscopically and morphologically proven UC.

3) Active form of UC (total Mayo score 6 to 12).

4) Different localization of the disease and incomplete response from the administered basic therapy.

5) Patients with existing complications - local, systemic and extraintestinal.

6) Patients refractory to previously administered therapy, cortico-dependent and cortico-resistant.

7) Patients with relapses after a course of biological therapy and those with insufficient response.

2.4. Exclusion criteria:

1) Presence of dysplasia and colorectal cancer.

2) Patients with a positive result (QuantiFeron, T-SPOT. TB test).

3) Pregnancy.

4) Infectious colitis.

5) Presence of concomitant severe chronic diseases.

3. MATERIAL - PATIENTS STUDIED

To achieve the objectives of the present work, 107 individuals with active form of ulcerative colitis (UC) were studied.

The clinical observation included 107 patients with active UC followed up in the Department of Gastroenterology for the period 2015 - 01.08.2023. Fifty-two women and 55 men were included, with a mean age at diagnosis of 36.2 +/- 14.3 years, a minimum of 12 years and a maximum of 71 years. A retrospective analysis of medical records was performed in all subjects. All patients completed informed consent form for participation in the clinical observation and quality of life and fatigue assessment surveys.

To perform a comparison, in all 107 patients we analyzed clinical data from two periods in the patients' treatment. The first was before the initiation of biologic treatment and the second after its initiation.

4. METHODS USED

4.1. Basic diagnostic methods

All patients were examined according to the classical clinical rules with the main diagnostic methods - history and physical examination, which included inspection, auscultation and palpation. History was taken in detail regarding subjective complaints - number of defecations, with/without pathological admixture, presence/absence of abdominal pain, febrility, extraintestinal manifestations, concomitant diseases, medications taken and bad habits - smoking. Physical examination was performed with anthropometric measurements - height (cm) and weight (kg). Body mass index (BMI) was calculated based on these. A normal weight was assumed at a BMI between 18.5 kg/m² - 24.9 kg/m² according to WHO 2000. The information obtained was entered in a table on a separate line and number for each person

examined. Patients also completed three questionnaires on paper and/or electronic format, one for quality of life and two for fatigue assessment.

4.2 Laboratory and clinical tests

The main part of the laboratory and instrumental examinations of the patients in the dissertation were performed at the "St. Marina" University Hospital, Varna. The fecal calprotectin, Igra tests - QuantiFeronTest, TB-Spot Test and drug monitoring - drug level and antibody presence, were performed in licensed clinical laboratories in the territory of the town of Marina, Bulgaria. The tests were performed in the licensed laboratories in Varna.

The following clinical laboratory tests were performed in all persons included in this monitoring: hemoglobin, leukocytes, platelets, monocytes, neutrophils, SUE, CRP, total protein, albumin, serum iron, AST, ALT, GGT, AP, triglycerides, cholesterol, fecal calprotectin.

All 107 patients were screened for opportunistic infections.

Virological markers such as HBsAg, anti-HBcTotal, anti-HCV were tested to exclude viral infection by different ELISA kits. In 13 patients, due to positive results for HBsAg and/or anti-HBcTotal, HBV-DNA testing was performed by PCR-RT (polymerase chain reaction).

Igra T-SPOT.TB/ QuantiFeron Test was performed in all patients to detect blood cells (T effector lymphocytes) responding to stimulation with Mycobacterium tuberculosis antigens. The test is based on the ELISPOT method, significantly more reliable than conventional ELISA methods.

In 5 subjects, due to persistently elevated cholestatic enzymes, immunological studies were performed - autoantibodies - p-ANCA, c-ANCA and ANA by indirect immunofluorescence and Westernblot (immunoblot), to exclude/confirm primary sclerosing cholangitis.

To exclude the presence of superimposed bacterial infection, all patients were tested for Salmonella, Shigella, Escherichia coli, and Clostridium difficile before initiation of biologic therapy.

Therapeutic drug monitoring to detect the presence of ADA and concentration of the biological agent in blood by ELISA method was analyzed in 17 subjects.

4.3. Ultrasound examination of abdominal organs

We performed abdominal ultrasonography with Aloka Prosound alpha 7 ultrasound machine with convex and linear transducer, with the presence of color and power Doppler in all 107 patients.

The patient's preliminary preparation included fasting between 4 and 6 hours before the study.

Analysis of colonic wall thickness in mm, presence/loss of haustration, normal/absent peristalsis was performed.

4.4. Fibrocolonoscopy

All subjects underwent a comprehensive fibrocolonoscopy (FCS) prior to the initiation of biologic treatment and every 12 months after its initiation at the Clinic of Gastroenterology

of St. Marina University Hospital, St. Marina, Bulgaria. In the first and second months of the treatment.

Prior to the FCS, patients signed an Informed Consent for the procedure, which described all possible risks associated with the manipulation.

The FCS was performed with the colonoscope model Olympus CF-H 180 AL Exera II and FUJIFILM.

Peristalsis and haustration of the colon, presence and/or absence of edema, erosions, ulcerative defects, contact bleeding, pseudopolyposis were evaluated and a test for vessel fragility was performed with possible positive or negative results. The endoscopic Mayo subscore was used to analyze the severity of the MR.

4.5 Histomorphological examination

In all patients, 3 to 4 biopsies were taken from each column segment during the course of FCS. The specimens were stained classically with hematoxylin-eosin and/or Giemsa.

4.6. Montreal classification for the extent and severity of ulcerative colitis

To assess the extent and severity of ulcerative colitis in all patients, we used the Montreal classification described in Tables 1 and 2.

Table 1. Assessment of the extent of UC (after J Satsangi, M S Silverberg, S Vermeire, J-F Colombel, Gut, 2006).

Extent		Anatomy
E1	Ulcerative proctitis	The inflammatory process involves only the rectum
E2	Left-sided/distal colitis	The inflammatory process involves the colorectum, distal to the flexure lenalis
E3	Pancolitis (extensive/diffuse UC)	The inflammatory process is proximal to the flexure lenalis

Table 2. Assessment of the severity of UC (after J Satsangi, M S Silverberg, S Vermeire, J-F Colombel, Gut, 2006)

Severity		Definition
S0	Clinical remission	Asymptomatic
S1	Mild form of JC	History of 4 or fewer defecations/day (with or without blood), no systemic disease, normal inflammatory markers
S2	Moderate form of UC	History of >4 defecations/day with minimal signs of systemic disease
S3	Severe form of UC	History of at least 6 defecations/day, pulse rate >90 bpm, temperature >37.5, hemoglobin below 10.5 g/100 ml, and SUE ≥30 mm/h

4.7. Mayo score

The Mayo score was used to assess the severity of UC in the study patients and was recorded in paper and/or electronic form for each patient prior to initiation of biological treatment and during maintenance therapy.

4.8. Quality of life questionnaire and fatigue assessment indices

Quality of life and fatigue scores were assessed in 85 patients with active UC. Three questionnaires were used. The first of these was the Inflammatory Bowel Disease Questionnaire - IBDQ, which consists of 32 questions. The second and third questionnaires included the Daily Fatigue Impact Scale (DFIS) and the Fatigue in Inflammatory Bowel Disease Scale (Inflammatory bowel disease fatigue scale - IBD-F Scale). All participants signed a pre-formed Informed Consent Form. One part of the subjects completed the questionnaires on paper and the rest on electronic media. The obtained data were tabulated with a corresponding serial number for each patient.

4.9. Statistical methods

Appropriate statistical methods have been consistently applied to process the empirical data from the studies conducted in this thesis. The selection of methods was made according to the aims and objectives of the study and the type of data obtained.

For the analysis of laboratory data, a comparative analysis (start-end) using Mann Whitney test statistic was used. Statistically significant differences in the compared means for start-end were assumed at significance level values below 0.05 ($p \leq 0.05$).

The Mann Whitney method was used to compare numerical measures. For indicators designated as yes/no, i.e. non-numeric, qualitative data, Chi-square test (X2) was used to

compare by number and percentage the differences in the start-end periods of the numeric (yes/no) indicators. Statistically significant results were those with $p \leq 0.05$.

5. RESULTS AND DISCUSSION

5.1. Demographic characteristics of the studied patients

107 patients with active ulcerative colitis were included in the study. The mean age of the studied patients at the onset of the first complaints pointing to the diagnosis of UC was 36.2±14.6 years, with a minimum age of 12 years and a maximum age of 71 years. Of these, 55 (51.4%) were male and 52 (48.6%) were female (Table 3).

5.1.1. Demographic and baseline characteristics of the study group of patients with UC

Table 3. Gender distribution (n=107)

Gender	N	%
Male	55	51.4 %
Woman	52	48.6 %

Table 4. Main characteristics of patients in the study group

	Patients with UC (N=107)
Age (in years) at diagnosis	Mean value - 36,20±14,6
Sex male/female	Under 25 years - 30(28%)
Montreal classification	26-65 years - 74(69.2%)
Extraintestinal manifestations (EIM)	Over 65 years - 3(2.8%)
Patients undergoing treatment with Imuran	55 (51,4%)/52(48,6%)
Patients on treatment with Adalimumab	E1 (proctitis) - 1(0.9%)
Patients on treatment with Infliximab	E2 (left colitis)- 34(31.8%)
Patients on treatment with Golimumab	E3 (pancolitis) - 72(67.3%)
Patients on treatment with Vedolizumab	With EIP- 79(73,8%)

Patients on treatment with Tofacitinib	Without EIP- 28(26.2%)
Patients on treatment with Upacitinib	Yes - 38(35.5%)
Adverse drug events	No - 69(64.5%)
SUE	Yes - 56(52.3%)
CRP	No - 51(47.7%)
FCP	Yes - 45(42.1%)
Mucosal healing	No - 62(57.9%)

Of all 52 women included in the study, 63.5% had ulcerative pancolitis and 36.5% had left colitis (Table 5).

The distribution of men included in the study (n=55) - 70.9% had ulcerative pancolitis, 27.3% had left colitis and only 1.8% had proctitis (Table 5).

Table 5. Sex distribution and extent of patients with UC

			Diagnosis			
			Left colitis	Pancolitis	Proctitis	All
Gender	Male	Бр.	15	39	1	55
		%	27.3%	70.9%	1.8%	100.0%
	Female	Бр.	19	33	0	52
		%	36.5%	63.5%	0.0%	100.0%
Total		Бр.	34	72	1	107
		%	31.8%	67.3%	0.9%	100.0%

The mean age of the patients studied at the time of diagnosis of UC was 36.2±14.3, with a minimum age of 12 years and a maximum age of 71 years (Table 6).

Table 6. Distribution of patients with UC in the study by minimum and maximum age at diagnosis.

	N	Min.	Max.	Sr.arrhythm.	Std.
Age_diagnosis	107	12.00	71.00	36.1963	14.28315

The results of the analysis showed that by the age of 25 years, 28% of the subjects were diagnosed with UC. For persons between 26 and 65 years this percentage was the highest - 69,2%. Above 65 years, the symptoms of the disease were manifested in only 2.8% (Table 7).

Table 7. Distribution of patients with UC by age

Age		Number	%
	<25	30	28.0
	26-65	74	69.2
	>65	3	2.8
	Total	107	100.0

Most often, the first manifestation of the disease is between 25 and 65 years of age, with later manifestations possible after 65 years.

In patients diagnosed before the age of 25 years, 31.9% of individuals had ulcerative pancolitis. In the group between 26-65 years, 73.5% were patients with left-sided colitis. In the study subjects aged 65 years and older, the highest percentage, 5.9%, were patients with left-sided colitis (Table 8). Lakatos et al. found that left-sided colitis was prevalent in elderly patients. In the multicentre study by Ismail Hakki Kalkan et al. conducted from 1995-2011 in Ankara, Turkey, 12.3% of patients aged over 60 years had pancolitis compared with 26.5% of patients aged under 60 years. In contrast to these results, Triantafillidis et al. found no significant differences in disease severity between younger and older patients.

Table 8. Distribution of UC patients by age and extent of disease at study entry

χ^2=3.33, p=0.504			Age			Total
			<25	26-65	>65	
Diagnosis	Left colitis	number	7	25	2	34
		%	20.6%	73.5%	5.9%	100.0%
	Pancolitis	number	23	48	1	72
		%	31.9%	66.7%	1.4%	100.0%
	Proctitis	number	0	1	0	1
		%	0.0%	100.0%	0.0%	100.0%
Total		number	30	74	3	107
		%	28.0%	69.2%	2.8%	100.0%

5.2 Clinical characteristics of patients

5.2.1. Comorbidities

In the covered group of 107 patients, about 55% of the patients were free of concomitant diseases. With known chronic gastritis were 4.7% of patients, 2.8% reported hypertensive disease, and less than 2% were patients with proven malabsorption syndrome and chronic viral hepatitis "B". Similar data have been reported by other authors (Antonio López San Román, Fernando Muñoz, et al.), and the presence of comorbidities implies consideration of possible consequences of their treatment, both through the possibility of interaction and by facilitating

potential adverse effects. Prognosis also changes, especially in the presence of cardiovascular comorbidity. The coexistence of several diseases requires collaboration and coordination between specialists from different specialties, making joint decisions and implementing appropriate clinical regimens to facilitate patient care.

Table 9. Summary data on comorbidities of patients with UC at initiation of biological treatment

Comorbidities of patients with UC	N	%
CD	3	2.8 %
Malabsorption syndrome	2	1.9 %
Chronic gastritis	5	4.7 %
Hashimoto's thyroiditis	2	1.9 %
Without concomitant diseases	59	55.1 %
Asthma	1	0.9 %
Bronchial asthma	1	0.9 %
Deep vein thrombosis	1	0.9 %
Splenomegaly	1	0.9 %
Left-sided hemicolectomy	1	0.9 %
Absolute arrhythmia in atrial fibrillation; Post-BTE state	1	0.9 %
PSH	1	0.9 %
Colonic diverticulosis	1	0.9 %
Echinococcosis of the liver	1	0.9 %
Bronchial asthma	1	0.9 %
Uterine myoma	1	0.9 %
Ankylosing spondylitis	1	0.9 %
DM type 2	1	0.9 %

Comorbidities of patients with UC	N	%
Psoriasis	1	0.9 %
Coxarthrosis	1	0.9 %
Obesitas	1	0.9 %
Condition after AMI	1	0.9 %
Ischaemic heart disease	1	0.9 %
Ischaemic heart disease; Unstable angina pectoris	1	0.9 %
Ischaemic heart disease; Angina pectoris on exertion	1	0.9 %
Right-sided nephrolithiasis with grade 2 hydronephrosis	1	0.9 %
TBC	1	0.9 %
Left-sided heart failure	1	0.9 %
Gout	1	0.9 %
Glaucoma	1	0.9 %
Supraventricular tachycardia	1	0.9 %
Carcinoma of mammary gland	1	0.9 %
Diabetic polyneuropathy	1	0.9 %
Right hemicolectomy	1	0.9 %

5.2.2 Extraintestinal manifestations in patients with UC

Extraintestinal manifestations in patients with UC are articular - IBD-associated arthropathy, cutaneous - erythema nodosum, ocular - uveitis, hematological - anaemic syndrome and biliary - PSH. Of the total number of patients included in the study, 79 (73.8%) individuals had proven EIM of UC (Table 10).

Table 10. Distribution of UC patients included in the study with/without EIMs

	Number	%

	With EIM	28	26.2
	Without EIM	79	73.8
	Total	107	100.0

Of all 107 patients with UC studied, 28 (26.2%) had proven IBD arthropathy, 4 (3.7%) had manifestations of erythema nodosum, 3 (2.8%) had uveitis, and 5 (4.7%) had PSH. The most common extraintestinal manifestation of the patients was anaemic syndrome in 69 (65.1%) of the subjects. Inflammatory bowel diseases are associated with various EIPs that may manifest before diagnosis. The reported incidence of EIPs by other authors (Bernstein, Vadstrup, Alulis, et al.) ranges from 14% to 47%. The distribution is presented in Table 11, 12, 13, 14 and 15.

Table 11. Distribution of patients according to the presence of IBD-associated arthropathy (n=107)

IBD- associated arthropathy	N	%
yes	28	26.2 %
no	79	73.8 %

The small joints of the upper and lower limbs were most commonly affected in the group of patients studied. In four patients, imaging evidence of sacroiliitis was described.

Table 12. Distribution of patients according to the presence of skin manifestations (n=107)

Skin manifestations	N	%
Yes	4	3.7 %
no	103	96.3 %

The cutaneous manifestation of UC in four of the patients was erythema nodosum.

Table 13. Distribution of patients according to the presence of ocular manifestations (n=107)

Ocular manifestations	N	%
Yes	3	2.8 %
No	104	97.2 %

Ocular manifestations of the disease were proven by an ophthalmologist. Uveitis was described in two patients and iridocyclitis in one patient. Over the course of the study and after inclusion of biologic treatment, there was a positive trend of ocular manifestations in all three individuals.

Table 14. Distribution of patients according to the presence of anemia (n=107)

Anemia	N	%
Yes	69	65.1 %
No	38	34.9 %

Iron deficiency anaemia was present in 69 (65.1%) individuals included in the study. In a proportion of patients, blood stransfusion was required during the hospital stay. In the majority, iron replacement therapy was performed.

Table 15. Distribution of patients according to the presence of PSH (n=107)

PSC	N	%
yes	5	4.7 %
no	102	95.3 %

In five of the patients with UC included in the described study, the diagnosis of PSC was accepted after MRCP and positive immunological markers (4.7%). Ursodeoxycholic acid treatment was performed in all patients. In a retrospective study by Heba Adam et al. conducted

in 2019 in patients with UC, patients with PSC accounted for 6.8% of the EIP, and its appearance significantly increased the cumulative risk of colorectal cancer in patients with UC.

Table 16. Distribution of UC patients included in the study with/without EIP by sex

χ^2=6.08, p=0.014			Sex		
			Male	Female	Total
With/Without EIM	Without EIM	бр	20	8	28
		%	71.4%	28.6%	100.0%
	With EIM	бр	35	44	79
		%	44.3%	55.7%	100.0%
Total		бр	55	52	107
		%	51.4%	48.6%	100.0%

In men included in the follow-up, 71.4% were without proven EIP. Conversely, in women, nearly 55.7% had EIPs (Table 16). Similar to the results obtained in our study, a 25-year Hungarian study by Laszlo Lakatos et al. described a higher prevalence of EIPs in females compared with males.

5.2.3. Results for body weight and height

Body mass index was calculated in 107 patients, with a mean value of 25.4±7.54 kg/m2 (Table 17). This value is within the WHO norm. Over the last decade, various authors (Hass DJ, Brensinger CM, Lewis JD, et al.) have described the increasing prevalence of obesity in patients with IBD. Although there is no evidence that obesity alters the course of the disease, it is associated with adverse outcomes, such as colonic adenomas, increased cardiovascular mortality and risk of thrombotic disease.

Table 17. Body mass index in patients with UC

	Height/cm/	Weight/kg/	BMI
Sr.arrhythm.	169	72.3	25.4
Median	168	70.0	23.7
Standard deviation	7.07	19.7	7.54
Minimum value	152	43.0	17.1
Maximum value	192	180	84.0

	Height/cm/	Weight/kg/	BMI

5.3. Determination of the clinical characteristic of MR

5.3.1. Distribution of patients according to the Montreal classification of the extent of UC at the start of biological treatment

In the study population of patients with UC, the highest percentage of individuals with pancolitis was 72 (67.3%), followed by left-sided colitis - 34 (31.8%) and proctitis 1 (0.9%), respectively (Table 18).

Table 18. Distribution of patients in relation to coverage

Diagnosis	N	%
Left colitis	34	31.8 %
Pancolitis	72	67.3 %
Proctitis	1	0.9 %

5.3.2. Analysis of clinical symptoms

The subjective complaints of the patients included in the study are presented in Table 19, 20, 21, 22, 23, 24, 25, 26. Abdominal pain was the most common complaint prior to inclusion of biologic treatment in 102 (95.3%) subjects, hematochezia in 106 (99.1%) and astheno-dynamia in 76 (71%). Patients also reported rectorrhagia in 64 (59.8%) and joint pain in 30 (28%). Significant weight reduction of more than 10 kg was reported by 62 (57.9%) patients, with loss of appetite in 65 (60.7%). Objective examination revealed febrility in 39 (36.4%) of the subjects.

Table 19. Abdominal pain in patients with UC (n=107)

Abdominal pain	N	%
Yes	102	95.3 %
No	5	4.7 %

Nearly 100% of patients participating in the study reported abdominal pain. In the majority of them, abdominal pain was constant, unrelated to defecation, and increased in the course of dietary error.

Table 20. Hematohesia in patients with UC (n=107)

Hematohesia	N	%
Yes	106	99.1 %
No	1	0.9 %

Table 21. Astheno-adynamia in patients with UC (n=107)

Astheno-adynamia	N	%
Yes	76	71.0 %
No	31	29.0 %

Table 22. Rectorrhagia in patients with UC (n=107)

Rectorrhagia	N	%
Yes	64	59.8 %
No	43	40.2 %

Table 23. Weight reduction in patients with UC (n=107)

Weight reduction	N	%
Yes	62	57.9 %

Weight reduction	N	%
No	45	42.1 %

Table 24. Loss of appetite in patients with UC (n=107)

Loss of appetite	N	%
Yes	65	60.7 %
no	42	39.3 %

Table 25. Joint pain in patients with UC (n=107)

Joint pain	N	%
Yes	30	28.0 %
no	77	72.0 %

Table 26. Febrility in patients with UC (n=107)

Febrility	N	%
Yes	39	36.4 %
No	68	63.6 %

5.3.3. Comparative analysis of laboratory parameters of individuals relevant to the clinical characteristics of patients with UC

The Mann Whitney statistical test was used for the comparative analysis. In terms of comparisons of qualitative (non-numeric) indicators, Chi-square analysis was used with statistical significance in the start-end comparison made ($p \leq 0.05$).

6.3.3.1. Comparative analysis of hematological parameters of persons with JC

Hemoglobin

There was an increasing trend in the mean hemoglobin values of patients (n=107) during the follow-up period (Fig. 1, Table 27). For individuals with UC, the mean hemoglobin level before initiation of biologic treatment was 119.77±23.38 g/L. These values were closer to the lower limit of normal. At the end of the study, the levels are close to the reference values, respectively 133,96±17,89 g/L. The favorable dynamics of this indicator in the course of treatment we associate with the control of the inflammatory process, as well as with the drug substitution when indicated.

Table 27. Comparative analysis of hemoglobin values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
Hemoglobin	start	107	119.766	120.000	23.384	U=3622, p=0.001
	end	107	133.96	133.000	17.891	

Comparing the values at the beginning of the study at the start of biological treatment and at the end of the study recorded an increase in mean hemoglobin values (Fig. 1). The difference in mean levels found was statistically significant.

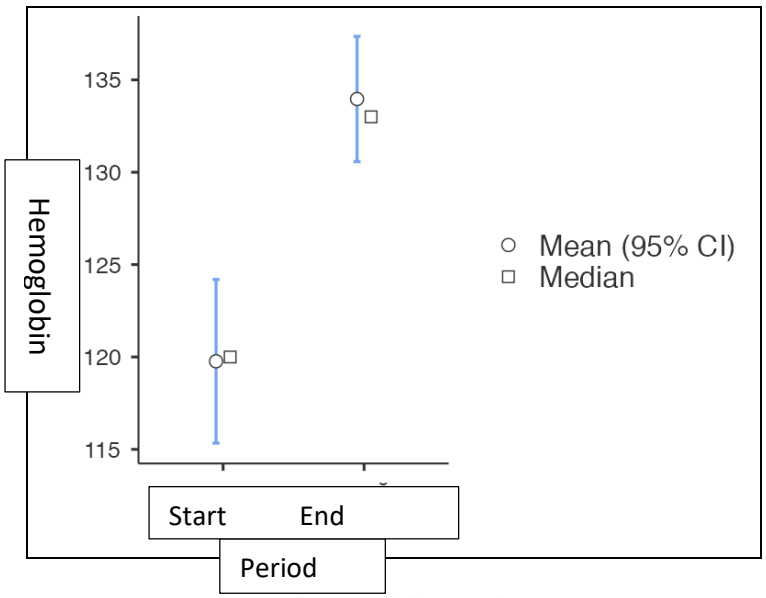


Fig. 1. Dynamics in hemoglobin values

Leukocytes

The mean leukocyte values of the patients (n=107) showed a slight decline, tending towards the reference range of normal at the end of the study. Accordingly, the mean value before starting biologic treatment was 9.29±8.82 G/L and at the end of the study it was 7.88±7.30 G/L. Other reasons for the elevated leukocyte count in the patients, besides the boost of UC, were - GCS treatment or infection. A 2016 study by Jost Langhorst and James Boone in 91 patients with UC described that leukocytes were not a marker that would distinguish between endoscopically active disease and patients with mucosal healing.

Table 28. Comparative analysis of leukocyte values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
Leukocyte	start	107	9.299	8.820	3.227	U=4136, p=0.001
	end	107	7.88	7.300	2.587	

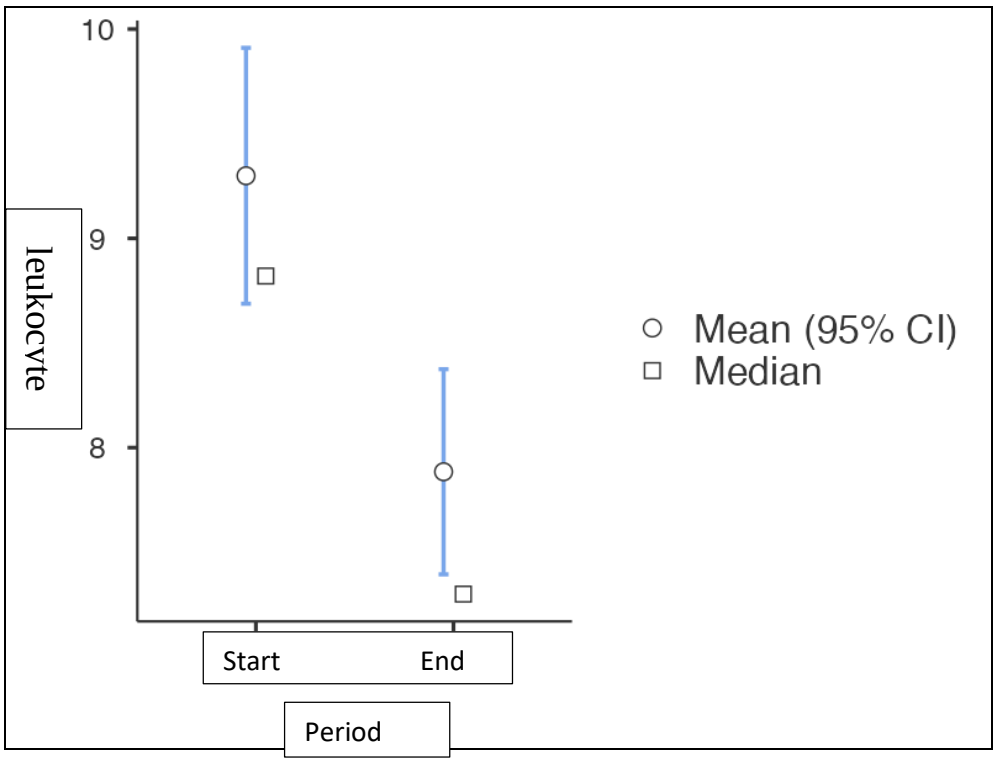


Fig. 2. Dynamics in leukocyte values

Platelets

The mean platelet values in subjects with UC were within the reference range both at the start of biologic treatment (377.65±132.75 G/L) and at the end of the study (311.71±95.28 G/L). The difference was statistically significant at significance level of 0.001 (Table 29). In contrast to the described possible thrombocytosis in patients with UC (Asuka Nakarai, Jun Kato, Sakiko Hiraoka et al.), no such trend was found in our group of patients. Reports from studies by Kapsoritakis and Kayahan tracking hematologic parameters in patients with UC described that the mean platelet count was higher in patients with active disease than in patients with inactive disease or healthy controls.

Table 29. Comparative analysis of platelet values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
Platelet	start	107	377.645	342.000	132.748	U=3901, p=0.001
	end	107	311.71	292.000	95.280	

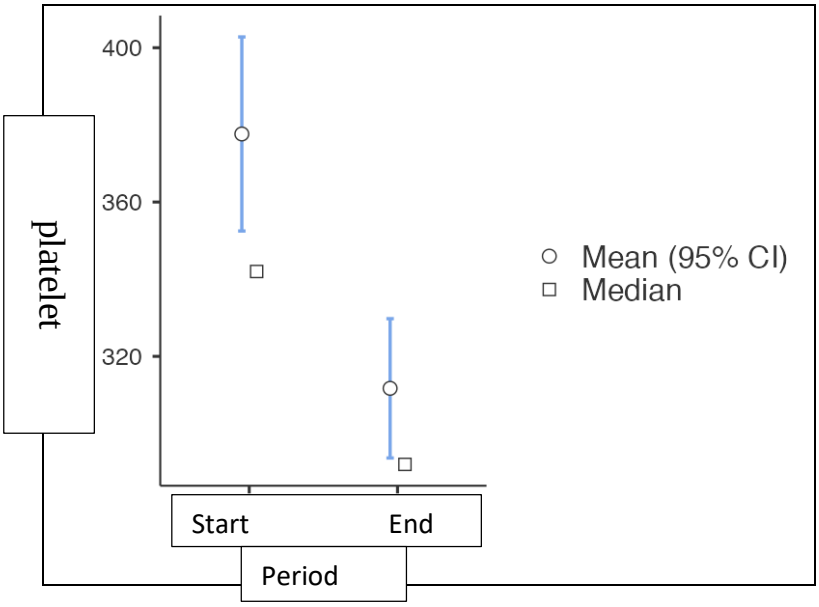


Fig. 3. Dynamics in platelet values

Erythrocyte sedimentation rate (ESR)

Mean ESR levels during the study period showed a decreasing trend due to the inclusion of biological treatment and reduction of inflammation in the column (Table 30).

Table 30. Comparative analysis of ESR values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
ESR	start	106	47.425	43.000	29.646	U=3777, p=0.001
	end	107	31.06	26.000	21.373	

For individuals with UC, mean ESR levels at initiation of biologic treatment were 47.43±29.65 mm/h. At the end of the study, ESR levels were 31.06±21.37 mm/h (Figure 4).

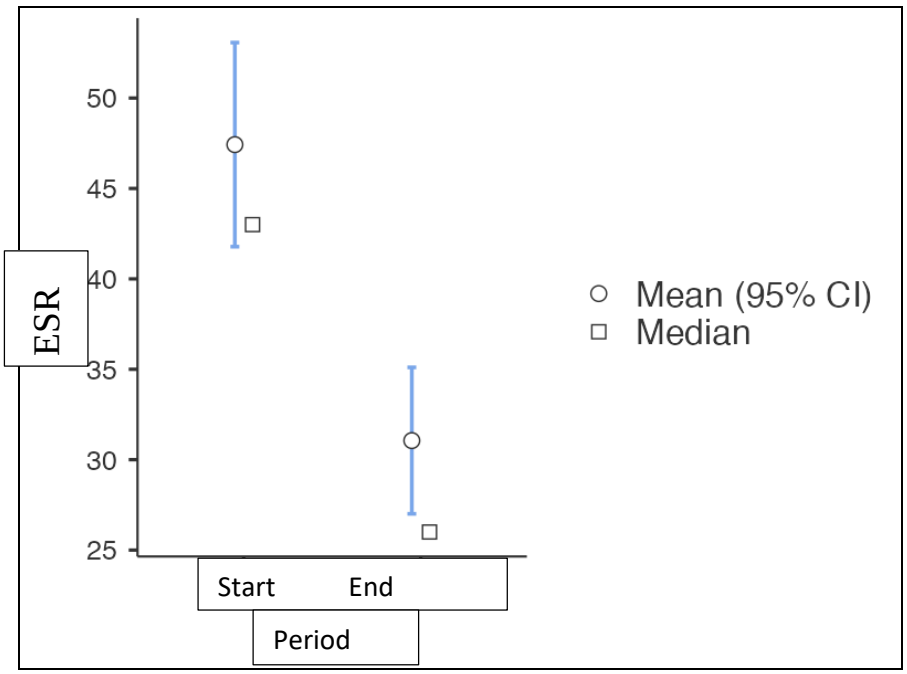


Fig. 4. Dynamics in the ESR values

5.3.3.2. Comparative analysis of biochemical parameters of individuals with UC

Total protein and albumin

The mean value of total protein at the start of the biological treatment was 67.24 g/L, with a deviation of ±10.83, while at the end the mean level was 70.75±6.17 g/L (Table 31).

Table 31. Comparative analysis of total protein and albumin values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
Total protein	start	107	67.241	69.000	10.830	U=4641, p=0.017
	end	107	70.75	72.000	6.169	
Albumin	start	107	39.368	40.100	7.383	U=3140 p=0.001
	end	107	44.19	44.200	5.880	

Results of statistical hypothesis testing in correlated samples indicate that this apparent difference between total protein values is statistically significant (Fig. 5).

Patients in whom albumin values <35 g/l were observed had a more severe disease course, requiring more frequent hospitalizations and the administration of human albumin replacement therapy and parenteral nutrition. After consultation with both a specialist dietician and improved nutrition, patients overcame the malabsorption present.

Similar findings were reported by other authors (Nabeel Khan, Dhruvan Patel et al.) who assumed that lower albumin level was associated with higher inflammatory activity in UC and thus may be associated with worse clinical outcomes in patients. Data from previous studies (Kumar S, Ghoshal et al.) also suggest that a lower serum albumin level during an exacerbation of UC predicts treatment failure and colectomy.

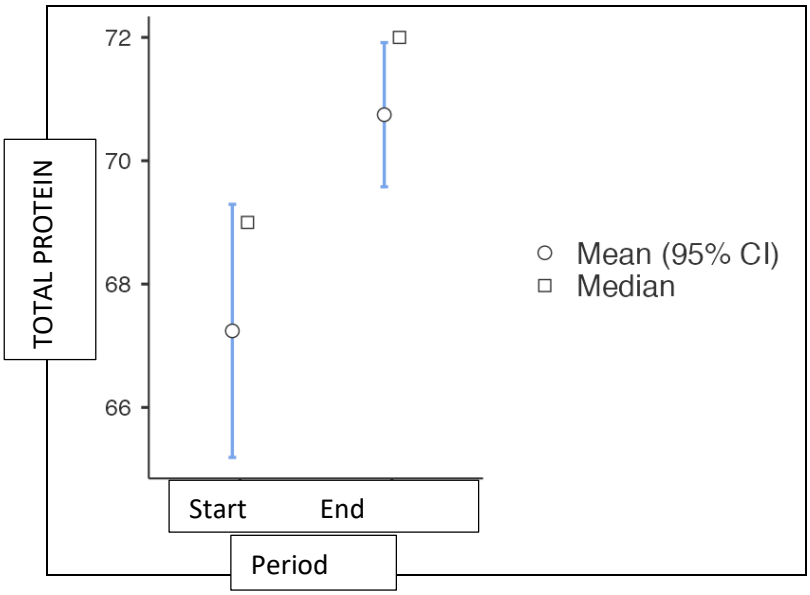


Figure 5. Dynamics of total protein values

For individuals with UC, the mean albumin level at initiation of biologic treatment was 39.37±7.38 g/L. This mean value is within the reference values of this indicator. At the end of the study, albumin levels were higher at 44.19±5.89 g/L (Fig. 6, Table 31).

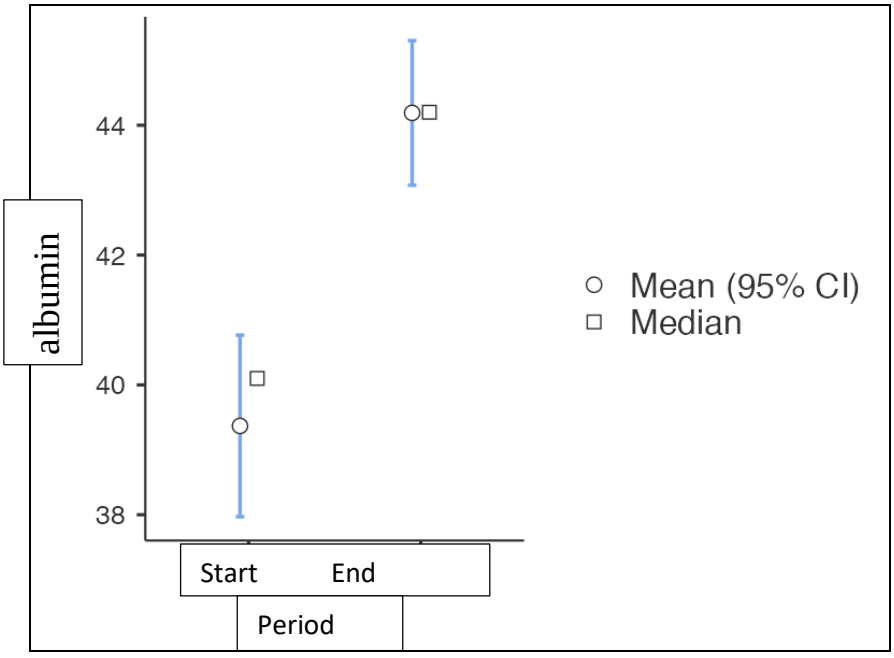


Fig. 6. Dynamics in albumin values

Serum iron

The mean serum iron level in the subjects at the initiation of biological treatment was 8.43±6.02 µmol/L (Table 32). These values tended to be low or at the lower limit compared to

the reference values of this indicator. This is consistent with the evidence of hematochezia in nearly 99.1% of the subjects, which is a significant cause of iron loss.

Table 32. Comparative analysis of serum iron values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
Serum iron	start	107	8.426	6.200	6.019	U=3077, p=0.001
	end	107	14.26	13.400	7.759	

At the end of the study, serum iron levels were significantly higher and fell within the reference range of the indicator. The mean value in the subjects was 14.26 μmol/L with a standard deviation of 7.76. The difference in mean levels found was statistically significant at 0.001 level of significance (Fig. 7).

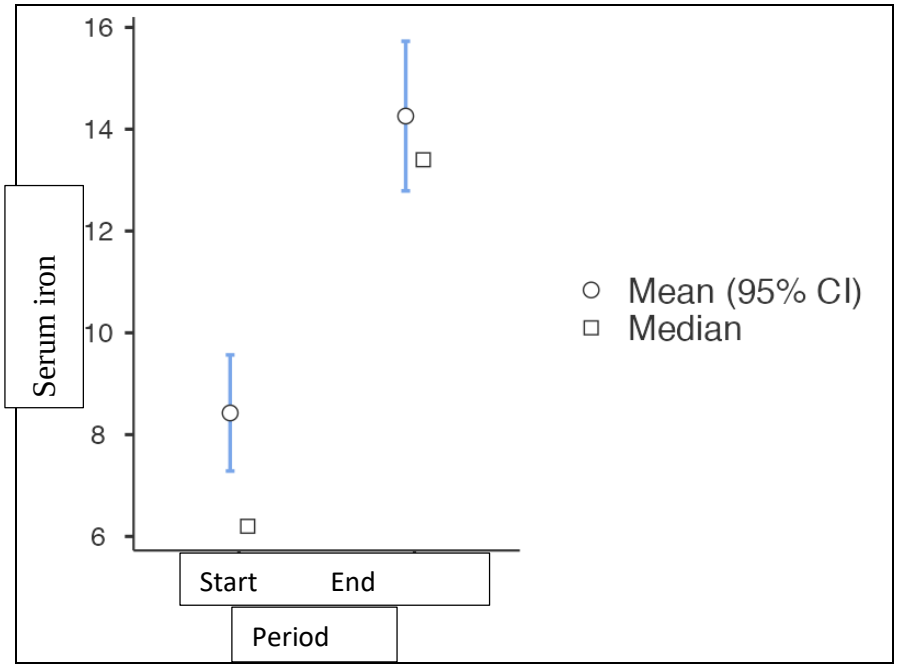


Fig. 7. Dynamics in serum iron values

C-reactive protein (CRP)

All subjects (n=107) showed a significant decrease in mean CRP values during biologic treatment and at the end of the study. The mean value of CRP at the beginning of treatment was 28.89±47.32 mg/L, which as a value was significantly higher than the reference limits for this indicator (Table 33). Comparative analysis of CRP values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
CRP	start	107	28.885	10.600	47.323	U=3495, p=0.001
	end	107	8.60	3.000	13.384	

In the course of biological treatment, there was a significant decrease in mean CRP, 8.6±13.38 mg/L, respectively (Fig. 8). These data are consistent with those reported by a number of authors (Prantera, Davoli, Loftus, Tremaine, Harmsen, Zinsmeister, Sandborn, Chouhan, and others), according to whom measurement of CRP levels is a simple method of assessing disease activity and severity. According to these studies, a CRP level >12 mg/L is indicative of severe and extensive disease; accordingly, a decrease in CRP in response to therapy is objective evidence that the drug has a beneficial effect on intestinal inflammation. The positive dynamics during treatment proves the importance of this indicator for the evaluation of the healing process.

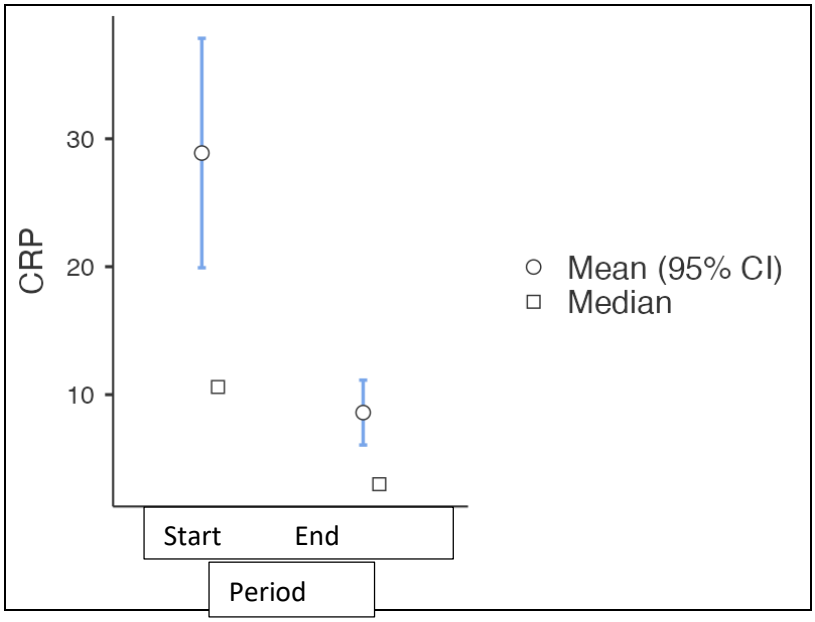


Fig. 8. Dynamics in CRP values

AST, ALT, GGT, AF, Cholesterol and triglycerides

The differences in mean levels of AST, ALT, GGT, cholesterol and triglycerides were statistically insignificant between the periods compared (Table 34).

Table 34. Comparative analysis of ASAT, ALT, GGT, AF, cholesterol and triglyceride levels during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
AST	Start	107	19.150	17.700	7.389	U=4399, p=0.003
	End	107	22.00	20.000	8.146	
ALT	Start	107	19.356	17.000	11.378	U=4757, p=0.033
	end	107	22.31	20.000	12.472	
GGT	Start	106	27.181	20.500	25.229	U=5082, p=0.190
	end	107	23.91	19.000	20.302	
AF	start	106	76.028	68.500	31.742	U=5641, p=0.948
	end	107	72.69	69.000	23.392	
Triglyceride	start	107	0.994	0.880	0.490	U=5329, p=0.382
	end	107	1.18	0.950	0.854	
Cholesterol	start	107	4.191	4.070	1.062	

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
	end	107	4.36	4.220	1.282	U=5221, p=0.266

5.3.3.3. Comparative analysis of fecal markers of persons with UC

Fecal calprotectin (FCP)

The largest decrease in the mean value in the study group of patients (n=107) between the periods compared was observed for FCP. The mean value of the parameter at baseline before initiation of biological treatment was 1110.644±806.53 mg/kg, which was nearly 20 times higher than its reference value (Table 35).

Table 35. Comparative analysis of FCP values during the follow-up period in patients with UC.

		N	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level	Arrhythm Median Standard deviation	Mann Whitney U-test/ p-level
FCP	start	107	1110.644	1000.000	806.529	U=848, p=0.001
	end	107	162.99	70.000	241.838	

Over the course of the study, the mean FCP value was 162.99±241.84 mg/kg, which was again above the upper reference limit of the index, but with a significant decrease from the values at the beginning of the study (Fig. 9).

Our results confirm the generally accepted facts about the dynamics of this parameter during follow-up of patients. The 2009 study by Schoepfer et al. reaffirmed that fecal

calprotectin allows to distinguish inactive disease from mild and severe disease, highlighting its importance for activity monitoring.

In a control study including 163 patients (89 with CD, 74 with UC) over 12 months, Gisbert et al. evaluated the role of FCP in recurrence of IBD (89). In the presence of marked disease activity, an increase in FCP was also described. The sensitivity and specificity of fecal calprotectin (>150 μg/g) for predicting relapse described in this study were 69% and 69%, respectively.

Data from another study conducted by Alain M. Schoepfer and Christoph Beglinger, at the University Hospital of Bern and Basel, in 152 patients with JC showed that FCP strongly correlated with endoscopic disease activity.

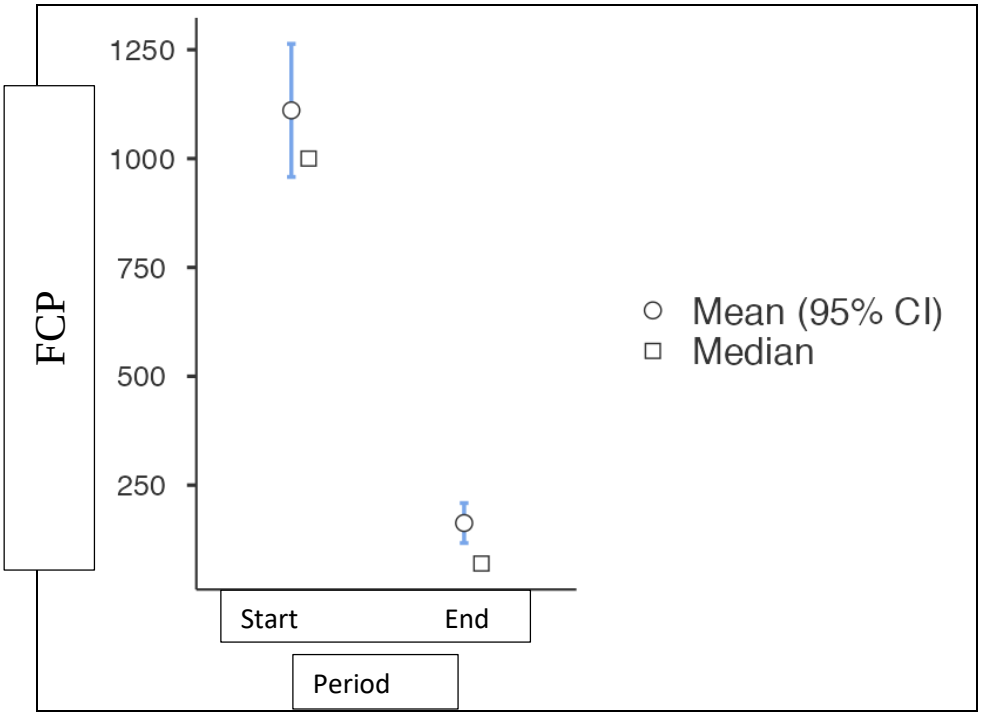


Fig. 9. Dynamics of FCP values

5.3.3.4. Comparative analysis of therapeutic drug monitoring of individuals with UC

Therapeutic drug monitoring included testing drug level and determining the presence of antibodies. Medication level was investigated in 18 individuals and antibody presence in 7 patients with UC. The reason for the low number of subjects tested is most likely the high monetary value of these indicators and their payment by patients.

Adalimumab, Infliximab and Vedolizumab levels were investigated in 9 men and 9 women with ulcerative pancolitis due to disease boost (against a background of ongoing biological treatment). High ADAs were found in 7 subjects (6 males and 1 female). Serum total protein and albumin levels were within reference ranges. Due to the low drug level and high ADA titer found in these seven patients, a drug switch was performed.

A total of 35 patients in the JC study group required switching to another biologic medication. The most common reasons for this were - allergic reaction and/or other adverse drug events, patient's compliance or presence of antibodies. A proportion of patients also

underwent intensification - increasing the dose or shortening the infusion period of the medication.

6.3.3.5 Comparative analysis for opportunistic infections of persons with JC

Igra test

A positive Igra test was detected in 3 (1.4%) of the individuals tested. Two of the patients discontinued their biological treatment at different stages due to an active form of tuberculosis proven by CT scan. In one of the patients, a latent form of TB was proven and the patient underwent tuberculostatic treatment after consultation with a pulmonologist, following T-SPOT negation. TB test, biological treatment was reinstated.

HBV infection

HBV infection was detected in 5 (2.3%) of the subjects. In these patients, the level of viral replication was strictly monitored. Antiviral treatment with nucleoside analogue was performed in all HBV positive patients.

Clostridium difficile

Prior to initiation of biologic treatment, 15 (7%) of the subjects were found to have Clostridium difficile infection. Oral treatment with Vancomycin, Metronidazole - intravenous and probiotic was given. After negative sample, biological treatment was started. During the course of treatment, some of the patients' samples became positive for Clostridium difficile, which necessitated temporary discontinuation of biological therapy until the results were negative.

6.3.3.6. Comparative analysis of immunological parameters of persons with UC

Positive markers for c-ANCA, p-ANCA, AMA and ANA were demonstrated in 3 (1.4%), 2 (0.9%) and 1 (0.5%) of the study patients, respectively. A total of 5 subjects with UC, were proven to have PSC. A proportion of these patients due to persistence of mildly elevated cholestatic enzymes remained on maintenance therapy with ursodeoxycholic acid at a dose calculated per kg body weight. In a study by Banski, Fleming and Chapman from the University of Oxford, UK, a higher ANCA titer was described in patients with JAK+PSC compared to those with JAK (ANCA diagnostic sensitivity 54% vs 25% at 1:50, p=0.0006).

The increasing use of biologic therapy for chronic immuno-inflammatory diseases such as IBD and rheumatoid arthritis has proven to have a role, but has so far shown little effect on PSH. The biologic drugs used in PSH (for induction of UC) are the anti-TNFα drugs Adalimumab and Infliximab, and the anti-integrin drug Vedolizumab. Results of the largest report in the literature on anti-TNF therapy in PSH was recently reported in a North American study by the Mayo Clinic. There were no differences in mean AF over time in patients on Infliximab, but there was a statistically significant decline in mean AF in patients on Adalimumab.

6.3.4. Place of abdominal ultrasonography in the diagnosis and follow-up of patients with UC

Abdominal ultrasonography was performed in all 107 patients involved in the follow-up. The most frequently observed changes were colonic wall thickness measured in mm, the presence of normal and/or absent peristalsis and normal and/or absent column haustration. The presence/absence of freely mobile fluid in the abdominal cavity and subileus/ileus imaging were also assessed during the course of the study. Fig. 12 A and B show ultrasonographic images of a thickened colonic wall in patients with active UC.

Active inflammation was characterized by thickening of the intestinal wall, intramural hemorrhage on Doppler imaging, and changes in the layered structure of the intestinal wall.

Chi-square test (X2), which compares the number and percentage differences in the start-end periods of count (yes/no) indicators, was used to statistically process indicators labeled as yes/no, i.e., non-numeric, qualitative data. Statistically significant results were those with $p \leq 0.05$.

6.3.4.1. Analysis of colonic wall thickness data

At the beginning of the study, before starting biological treatment, 73 patients were found to have a thickened colonic wall with a mean value of 8.84±3.50 mm. In the course of the study, only 22 subjects were found to have a thickened colonic wall with a mean value of 7.05±2.33 mm (Table 36, Fig. 10). According to the results of the TRUST&UC study, only the measurement of colonic wall thickness has the potential to predict therapeutic response.

Table 36. Comparative analysis of colonic wall thickness (mm) over the follow-up period in patients with UC.

		N	Median arrhythm.	Mann Whitley U/ p=level	Median arrhythm.	Mann Whitley U/ p=level
Colonic wall thickness	Start	73	8.84	8.00	3.50	U=507, p=0.009
	end	22	7.05	6.00	2.33	

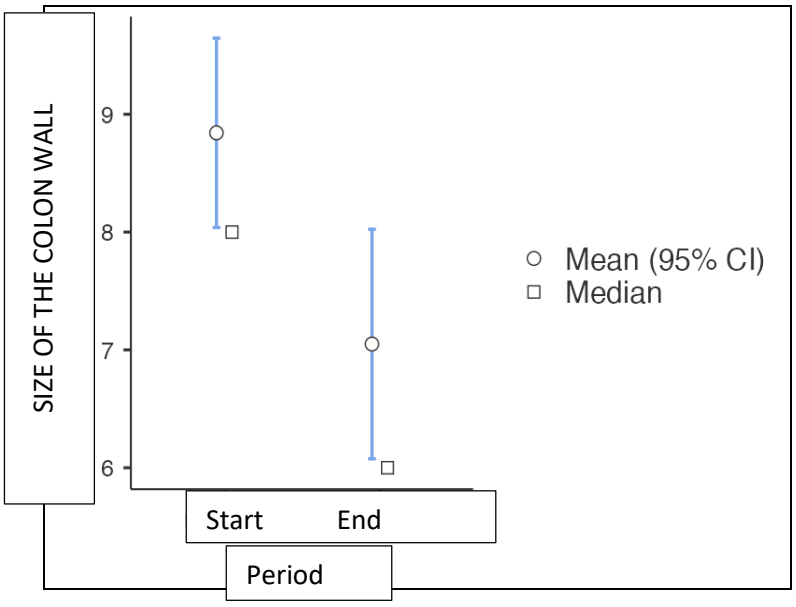


Fig. 10. Dynamics in the size (mm) of the colonic wall

6.3.4.2 Analysis of normal column loss of haustration data

In 50 (46.7%) of the subjects at the beginning of the study, loss of normal haustration (haustris 3 to 5 cm apart) was found by abdominal ultrasonography. Over the course of the study, after inclusion of biologic treatment, loss of haustration was observed in only 9 (8.5%) of the patients (Fig.11). Abdominal ultrasonography represents a noninvasive method to evaluate the activity of the UC and to determine the effect of the applied therapeutic strategies. In the TRUST&UC study, conducted in Germany in 2019 by Christian Maaser et al. in 224 patients with UC, an improvement in loss of haustration on abdominal ultrasonography was described, 56.7% before initiation of treatment and 32.6% at week 12.

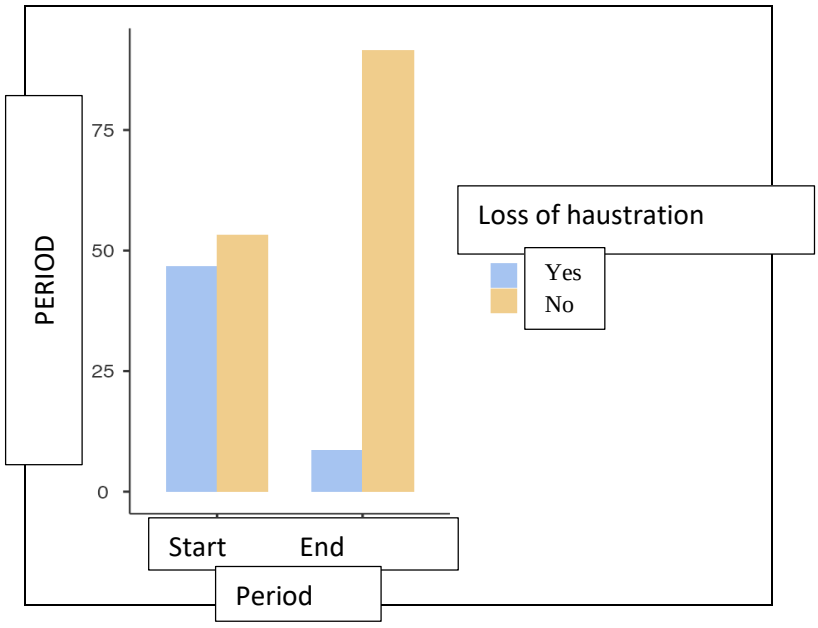


Fig. 11. Comparative analysis of the loss of haustration in the studied patients with UC

6.3.4.3. Analysis of missing column peristalsis data

Out of the entire study group (n=107), only 15 (14%) individuals had an ultrasonographically described missing peristalsis at the beginning of the study and only 4 (3.8%) at the end (Table 37).

Table 37. Comparative analysis of missing colonic peristalsis during the follow-up period in patients with UC.

χ^2= 6.88 p=0.009		**Missing colonic peristalsis**		
period		**yes**	**no**	**total**
start	бр	15	92	107
	%	14.0 %	86.0 %	100.0 %
end	бр	4	102	106
	%	3.8 %	96.2 %	100.0 %
total	бр	19	194	213
	%	8.9 %	91.1 %	100.0 %

χ^2= 6.88 p=0.009	Missing colonic peristalsis		
period	yes	no	total

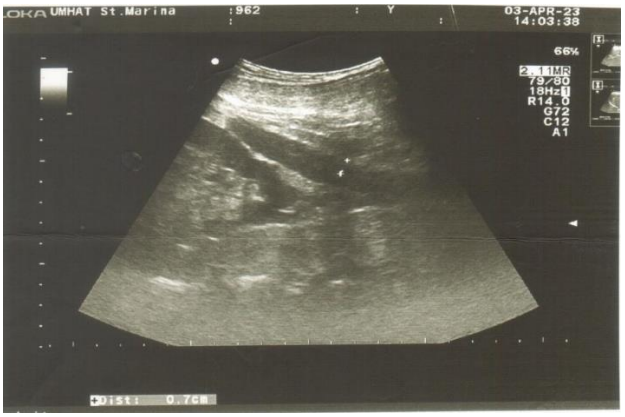


Fig. 12. A. Abdominal ultrasonography of a patient with UC - K.D.K, male, 62 years old, with left-sided colitis, thickened colonic wall up to 0.7 cm.

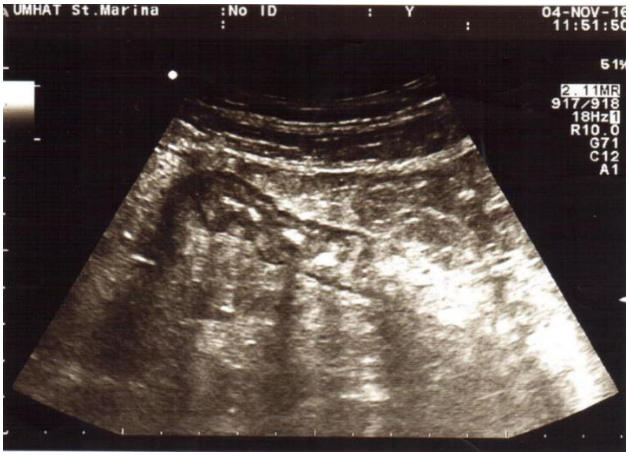


Fig. 12. B. Abdominal ultrasonography of a patient with UC - M.M.T., male, 42 years old, with ulcerative pancolitis, thickened colonic wall.

5.3.5 The place of fibrocolonoscopy (FCS) in the diagnosis and follow-up of patients with UC

5.3.5.1. Analysis of coverage in patients with UC at the start of the study

Comprehensive FCS was performed in all 107 patients in the study group. The study was conducted before initiation of biologic treatment as well as during therapy at an interval of 12 months. The extent of UC was assessed by the Montreal scale at the time of endoscopic examination.

Table 38. Comparative analysis of the Montreal classification coverage at the beginning of the study in patients with UC.

Extent	бр	%
to flexure lienalis (E3)	35	32,5 %
whole column (E3)	71	66,3 %
sigma, rectum (E1)	1	0.9 %

There was 1 (0.9%) patient with rectum and sigmoid involvement at the beginning of the study. The disease involved the colon to flexure lenalis in 35 (32.5%) of the patients. The entire colon was involved in 71 (66.3%) of patients with JC (Table 38).

6.3.5.2. Montreal classification coverage analysis in patients with UC over the course of the study

In the course of the study, only 5 (4.6%) of the patients described pancolitis. In 44 (41.1%) of the subjects, the inflammation reached the lienal flexure, or descendind colon. The largest group of patients, 58 (54%), had involvement of the rectum and/or sigmoid colon (Table 39).

Table 39. Comparative analysis of coverage over the course of the study in patients with UC.

Extent	бр	%
sigma, rectum (E1)	58	54 %
to flexure lenalis (E2)	44	41,1 %
entire colon (E3)	5	4.6 %

5.3.5.3. Analysis of endoscopic changes described in patients with UC at baseline and during the course of the study

Haustration

At the beginning of the study, haustration was absent in 50 (46.7%) of the subjects. During the course of the study, this percentage was significantly reduced to 10 (9.3%) of the patients, respectively.

Table 40. Comparative analysis between loss of colonic haustration before initiation of biologic therapy and during the course of therapy.

χ^2=37.1, p=0.001		Loss of colonic haustration		
period		**yes**	**no**	**total**
start	бр	50	57	107
	%	46.7 %	53.3 %	100.0 %
end	бр	10	97	107
	%	9.3 %	90.7 %	100.0 %

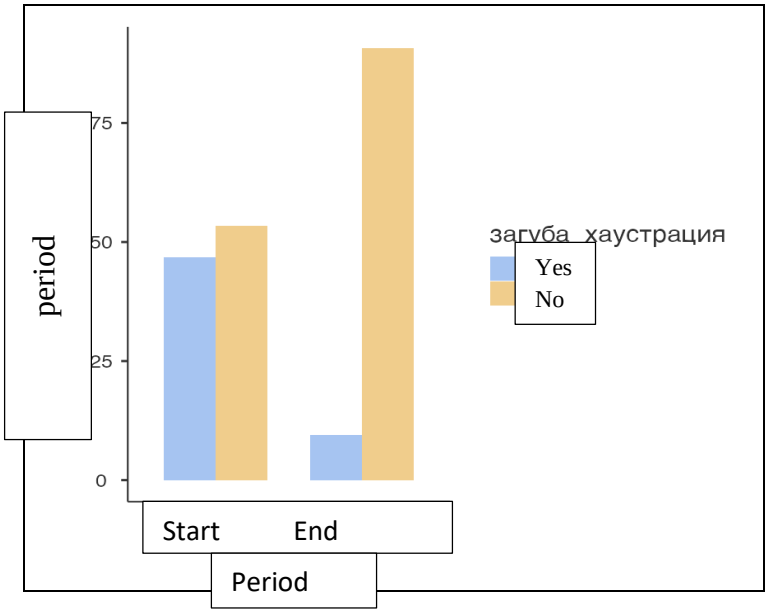


Fig. 13. Comparative analysis of the loss of haustration in the studied patients with UC

Mucosal edema

Mucosal edema was observed in all the subjects at the beginning of the study, 107 (100%) underwent FCS. This percentage remained almost the same during the course of the study - 103 (97.2%) (Table 41).

Table 41. Comparative analysis between the presence/absence of edema before initiation of biological treatment and during the course of therapy.

χ^2=3.07, p=0.08		Edema		
period		**yes**	**no**	**total**
start	бр	107	0	107
	%	100.0 %	0.0 %	100.0 %
end	бр	103	3	106
	%	97.2 %	2.8 %	100.0 %
	%	98.6 %	1.4 %	100.0 %

Erosion

The incidence of erosions described during the FCS in patients before initiation of biologic treatment and those during the course of the study declined. Accordingly, from 106 (99.1%) at baseline to 69 (64.5%) during the course of the study. With 38 (35.5%) of the subjects showing no erosions during FCS after inclusion of biologic treatment (Table 42, Fig. 14).

Table 42. Comparative analysis between the presence/absence of erosions before initiation of biological treatment and during the course of therapy.

χ^2=42.9, p=0.001		Erosions		
period		**yes**	**no**	**total**
start	бр	106	1	107
	%	99.1 %	0.9 %	100.0 %
end	бр	69	38	107
	%	64.5 %	35.5 %	100.0 %

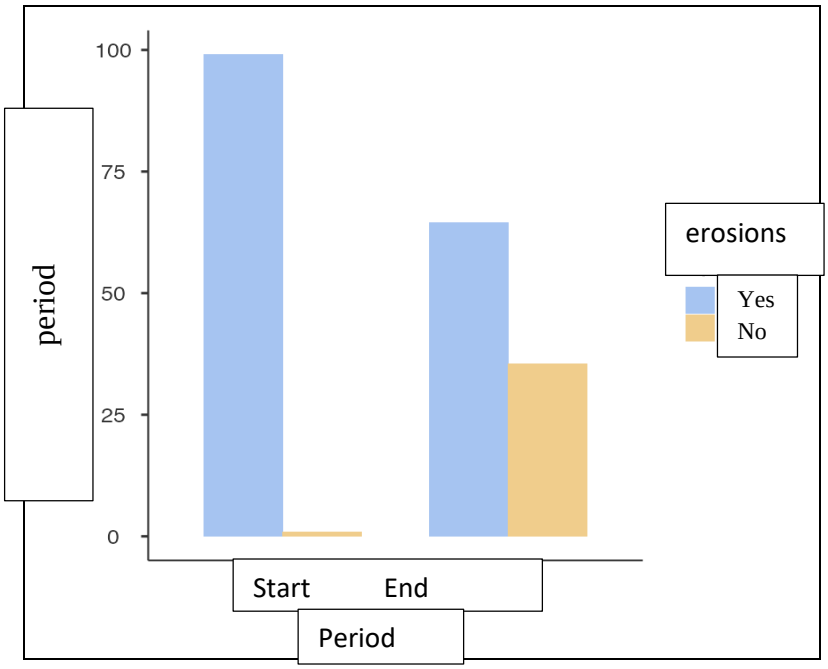


Fig. 14. Comparative analysis of the presence/absence of erosions in the studied patients with UC

Ulcerative defects

The presence of ulcerative defects also determines the greater severity of UC. They are one of the causes of clinical manifestation of hematochezia in these patients. In the group studied in this thesis, there was a significant decrease in the number of patients who were found to have ulcers in FCS before the initiation of biological treatment compared to the number during the course of treatment (Fig. 15 and Table 43).

In 98.1% of the patients, ulcer defects were found prior to inclusion of biologic treatment during FCS. Over the course of the study, this percentage was 40.2% (Table 43).

Table 43. Comparative analysis between the presence/absence of ulcers before the initiation of biological treatment and in the course of therapy

χ^2=83.6 p=0.001		**Ulcers**		
period		**yes**	**no**	**total**
start	бр	104	2	106
	%	98.1 %	1.9 %	100.0 %
end	бр	43	64	107

χ^2=83.6 p=0.001	Ulcers		
period	**yes**	**no**	**total**
%	40.2 %	59.8 %	100.0 %

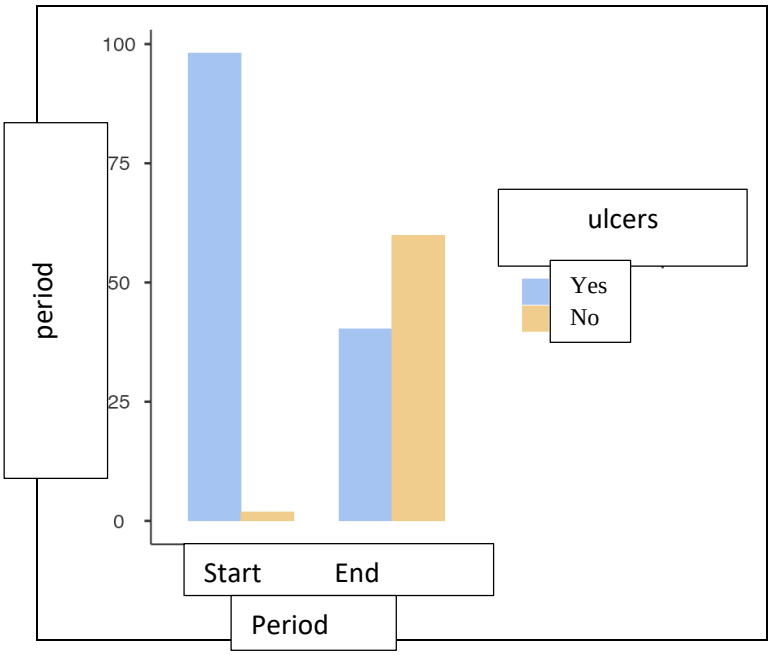


Fig. 15. Comparative analysis of presence/absence of ulcers during FCS in the studied patients with UC.

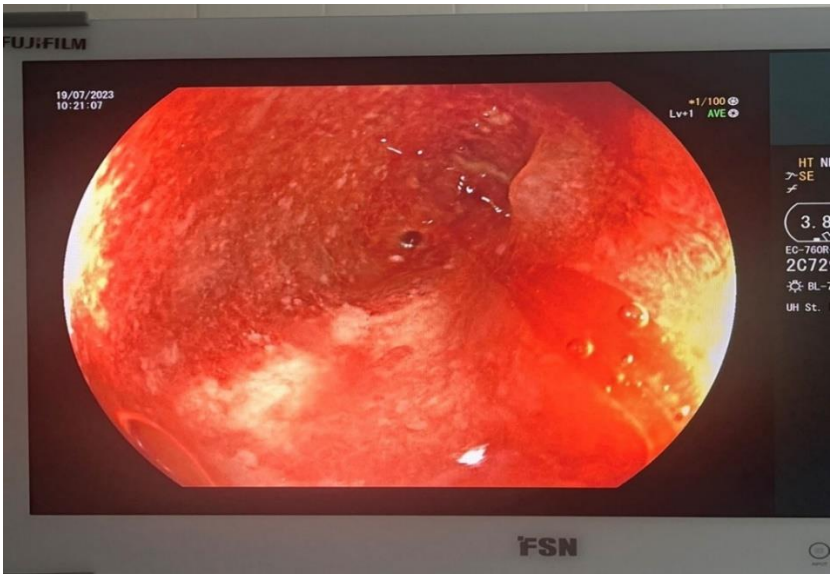


Fig. 16. Endoscopic image of severe UC, M.A.K., 61-year-old woman with ulcerative pancolitis - absent peristalsis, mucosal edema, erosions, multiple ulcerative defects covered with fibrin, spontaneous bleeding.

Vessel fragility and contact bleeding

Significant reduction in the manifestation of contact bleeding in patients with UC. Prior to initiation of biologic therapy, 75 (70.1%) of patients had FCS evidence of contact bleeding. Correspondingly, the number after inclusion of biological treatment was only 13 (12.1%) (Table 44, Fig. 17).

Table 44. Comparative analysis between the presence/absence of contact bleeding before initiation of biological treatment and during the course of therapy.

χ^2=72.4 p=0.001		Contact bleeding		
period		yes	no	total
start	бр	75	32	107
	%	70.1 %	29.9 %	100.0 %
end	бр	13	94	107
	%	12.1 %	87.9 %	100.0 %

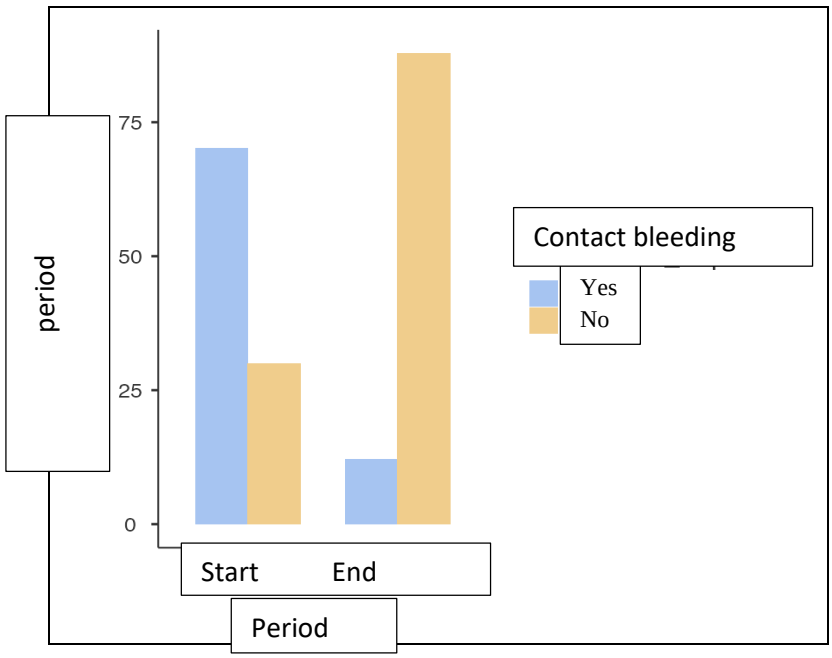


Fig. 17. Comparative analysis of presence/absence of contact bleeding during FCS in the studied patients with UC.

For the indicator of vessel fragility test, there was also a significant decrease in the percentage of patients with a positive test over the course of the study (Table 45, Fig. 18).

Table 45. Comparative analysis between positive/negative test for vessel fragility before initiation of biological treatment and during the course of therapy.

χ^2=68.7 p=0.001		Vessel fragility		
period		**yes**	**no**	**total**
start	бр	106	1	107
	%	99.1 %	0.9 %	100.0 %
end	бр	53	54	107
	%	49.5 %	50.5 %	100.0 %

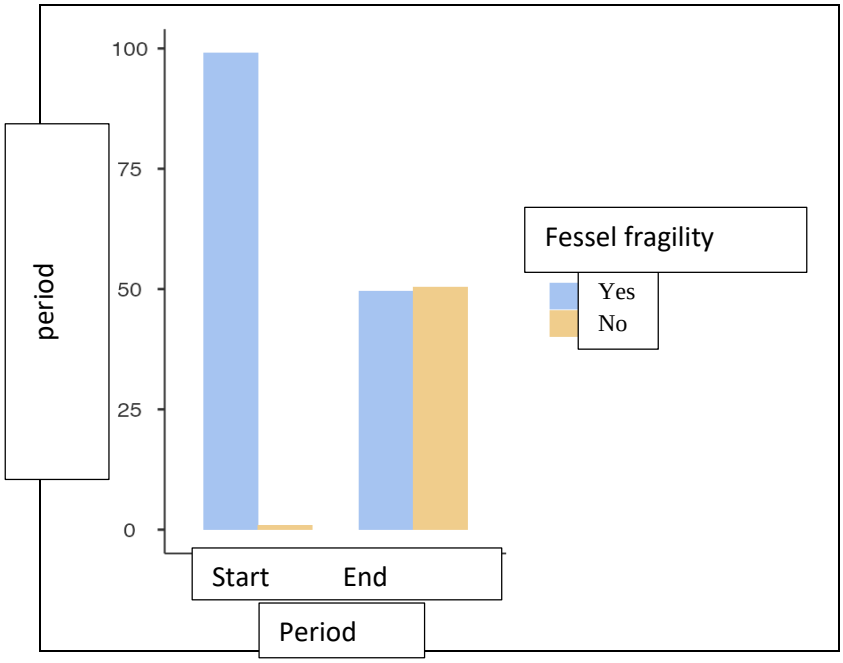


Fig. 18. Comparative analysis of positive/negative test for vessel fragility during FCS in the studied patients with UC.

Pseudopolyposis

In the present study, the percentage of patients with JC who had pseudopolyposis remained relatively the same at baseline and during the course of the study, 37.4% versus 35.5% (Fig. 19).

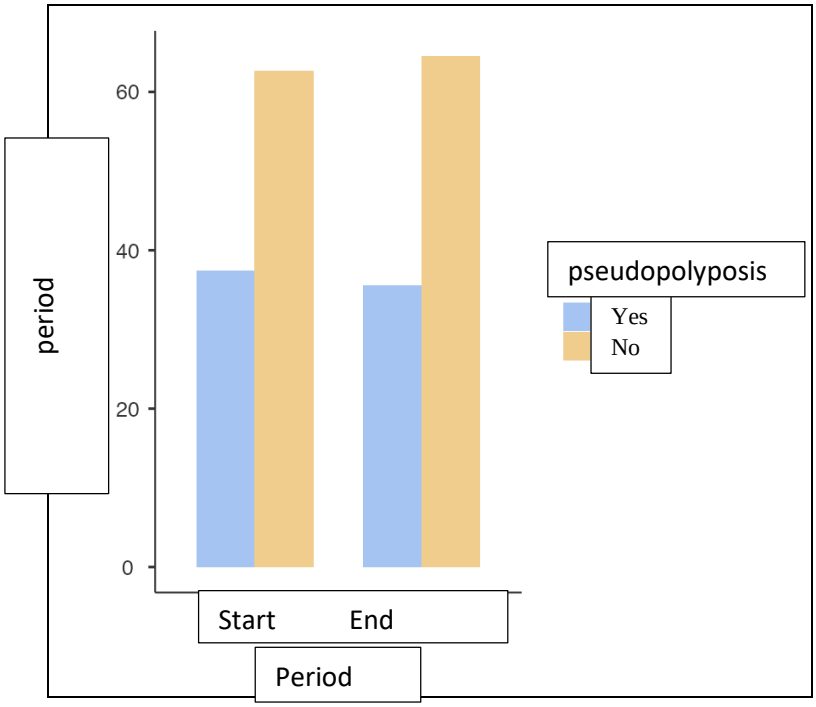


Fig. 19. Comparative analysis of the presence/absence of pseudopolyposis during FCS in the studied patients with UC.

6.3.5.4. Analysis of endoscopic Mayo scoring in patients with UC at baseline and during the course of the study

At the beginning of the study in the studied patients, 31.8% had moderate activity and 68.2% had severe activity. Over the course of the study, there was a significant decrease in the number of patients with severe activity, 6.8% and 6.8%, respectively (Table 46).

In a study by Klaudia Farkas, Péter László Lakatos et al. patients with active UC at one year of biologic treatment showed a significant improvement in endoscopic subscore values, 3 (interquartile range: 2-3) versus 1 (interquartile range: 0-2), respectively, $p < 0.001$. Clinical remission was achieved in 71% of patients with UC.

Table 46. Comparative analysis between endoscopic Mayo subscore before initiation of biologic therapy and during the course of therapy.

χ^2=108, p=0.001		Mayo subscore				
period		**remission**	**mild**	**moderate**	**severe**	**total**
start	бр	0	0	34	73	107

χ^2=108, p=0.001		Mayo subscore				
period		**remission**	**mild**	**moderate**	**severe**	**total**
	%	0.0 %	0.0 %	31.8 %	68.2 %	100.0 %
end	бр	27	25	44	7	103
	%	26.2 %	24.3 %	42.7 %	6.8 %	100.0 %
total	бр	27	25	78	80	210
	%	12.9 %	11.9 %	37.1 %	38.1 %	100.0 %

Figure 20 also presents the percentage of patients in remission after inclusion of the biological treatment 27 (26.2%) individuals.

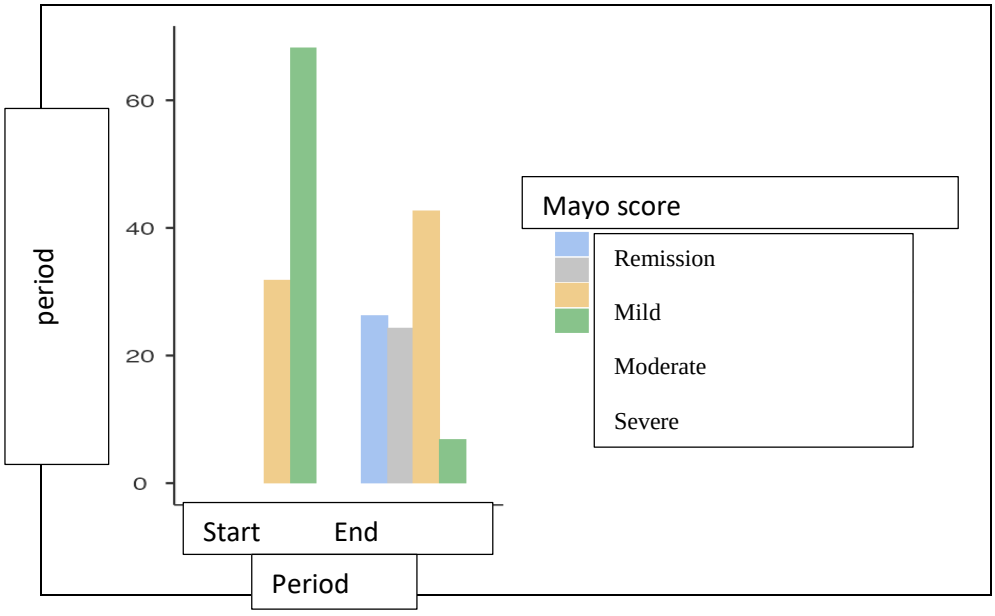


Fig. 20. Comparative analysis of endoscopic Mayo subscore in the studied patients with UC.

5.3.5.5. Analysis of mucosal healing in patients with UC over the course of the study

During the course of the study, the number of patients who achieved mucosal healing was 36 (33.6%) with n=107 (Table 47 and Figure 22).

Table 47. Analysis of mucosal healing in the course of biological therapy.

χ^2=43.3, p=0.001		Mucosal healing		
period		**yes**	**no**	**total**
start	number	0	107	107
	%	0.0 %	100.0 %	100.0 %
end	number	36	71	107
	%	33.6 %	66.4 %	100.0 %

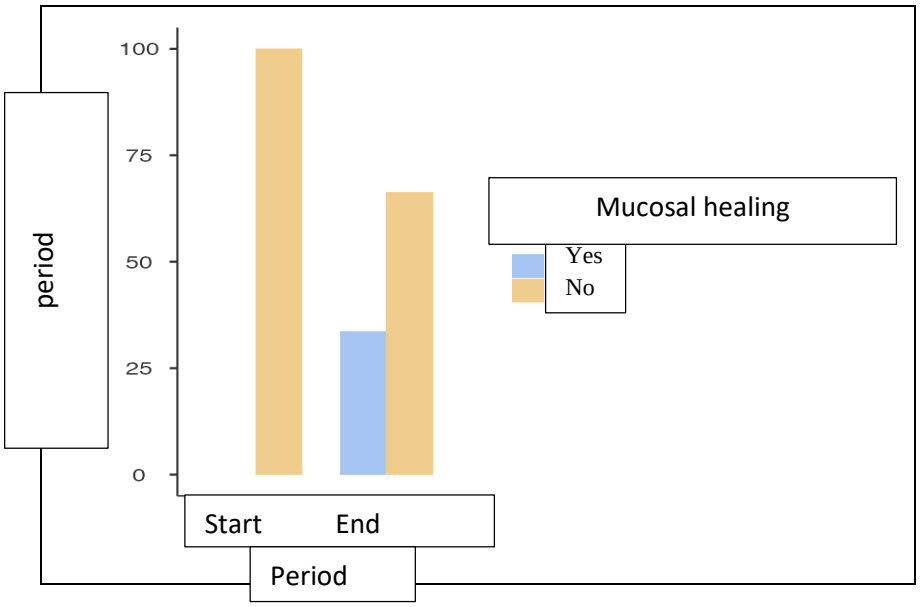


Fig. 21. Analysis of mucosal healing

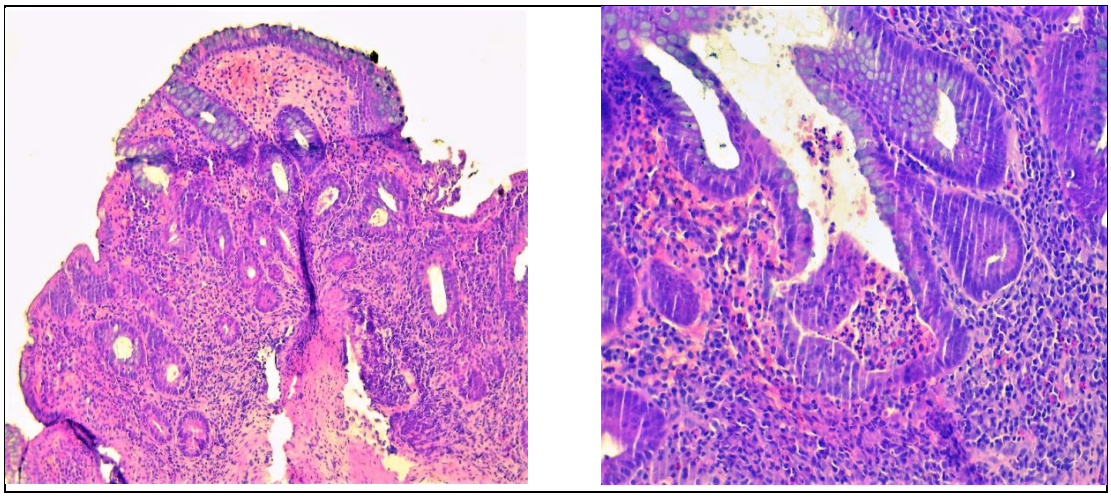


Fig. 22. Histological preparation in a patient with UC - Z.M.B, a 29-year-old woman with ulcerative pancolitis - presence of colonic mucosa with edematous changes in the chorion and inflammatory infiltrate with predominance of plasma cells, erosions, crypt and crypt abscesses. Hematoxylin-eosin staining.

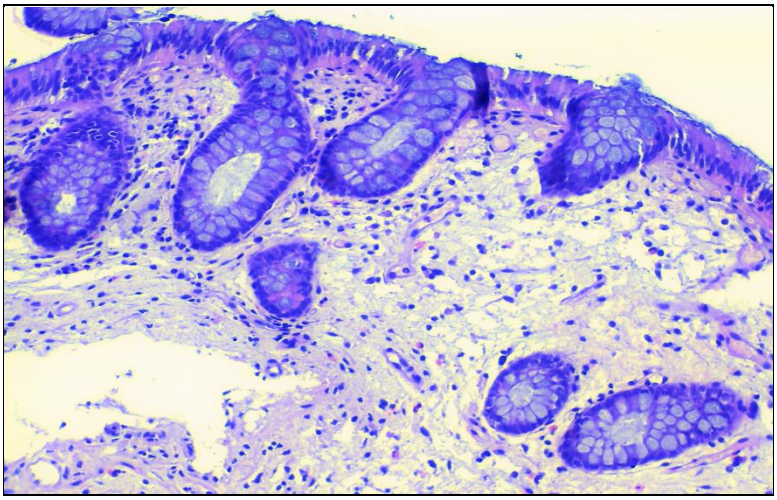


Fig. 23. Histological specimen in a patient with UC - Z.M.B., a 34-year-old woman with UC in remission on the background of biological treatment - colonic mucosa material with mild to moderate lymphoplasmacytic infiltration in the chorion, mixed with eosinophilic leukocytes. Hematoxylin-eosin staining, at ×200 magnification.

5.3.5.6. Analysis of total Meio scoring in patients with UC during the course of the study.

Table 48 shows the mean value of total Meyo scoring at baseline and during the course of the study. There was a significant decrease in the index - 11.7±0.51 compared to 3.79±0.87.

Table 48. Analysis of total Mayo scoring at the beginning and in the course of biological therapy.

		N	Median arrhythm.	mediana	Stand.deviat.	Mann Whitney U test/p-level
Общ Мейо скор	начало	106	11.7	12.0	0.511	U=84, p=0.001
	край	106	3.79	4.00	0.870	

In the present study, we observed high efficacy of the biological treatment in patients with active UC. We observed a significant decrease in a number of inflammatory markers - leucocytes, CRP, SUE and FCP, as well as a significant increase in hemoglobin and serum iron. On the background of treatment in patients by abdominal ultrasonography we found restoration of normal haustration and colonic wall size. Contact bleeding persisted in only 12.1% of individuals, and 33.6% of patients in our group achieved mucosal healing. This dynamic is related to adequate and effective treatment with modern biological agents and small molecules. Similar data have been widely discussed and validated in a number of clinical trials and observational studies in real clinical practice, and our data are in complete agreement with them.

5.4. Treatment of patients with UC during the course of the study

5.4.1. Analysis of patients treated with glucocorticosteroids (GCs) prior to initiation of biological treatment

Table 49 shows the distribution of patients in percentage, by group, according to the duration of GCS intake. Of all patients (n=107), the group with the highest percentage was the group of patients with 3 months of treatment with GCS, 78 (72.9%) of the study subjects, respectively. Only 5 individuals or 4.7% had 6 months of GCS use. 18 (16.8%) of the study subjects developed cortico-dependence and only 1 (0.9%) developed cortico-resistance, which were the reasons for inclusion of biological treatment. All patients in the study group with pancolitis - 72 (67.3%) and left colitis - 34 (31.8%) were treated with systemic GCS, and it was started during the stay in the clinic (intravenous) and continued in decreasing dose (oral) after dehospitalization. Only 1 patient with ulcerative proctitis was treated with a topical corticosteroid. According to a study by Curtis, Westfall, Allison, et al. >90% of patients receiving GCS for the treatment of chronic inflammatory disease will exhibit at least one adverse drug event. This is one of the reasons why systemic GCs are not recommended for long-term treatment and maintenance of remission in patients with UC. In our group of patients, some of the manifested adverse events were - acne, stretch marks, myopathy and osteoporosis.

Table 49. Analysis of patients at the beginning of the study undergoing treatment with GCS

GCS_duration	N	%
cortico-dependence	18	16.8 %
more than 6 months	4	3.7 %
3 months	78	72.9 %
corticoresistance	1	0.9 %
cortico-dependence and resistance	1	0.9 %
6 months	5	4.7 %

5.4.2 Analysis of patients treated with 5-ASA and immune in the course of biological treatment

All patients in the study group were on oral 5-ASA treatment before and during the biological treatment. Of all study subjects (n=107), 38 (35.5%) were on treatment with a second immunosuppressant, Azathioprine at a dose of 1.5-2.5 mg/kg body weight taken orally. The dose in all patients was titrated to the minimally effective dose due to the prevention of multiple possible adverse drug events associated with the use of the medication. When the dose of Azathioprine was increased, strict monitoring of complete blood count and liver parameters was performed weekly in the first months and monthly thereafter.

5.4.3. Analysis of patients treated with probiotic and human albumin at the beginning and course of biological treatment

Only 2 (1.9%) of the patients included in the study had not been treated with probiotic prior to starting biological treatment. The aim of probiotic therapy is to improve the intestinal microflora, intestinal barrier integrity and maintain a balanced immune response. Alterations of the gut microbiota in patients with active UC before and during biologic therapy may influence the remission period. The use of conventional probiotics in the treatment of UC has been investigated in a number of meta-analyses (Kaur, Derwa, Jana Štofilová et al.), all of which summarise the prophylactic and therapeutic potential of probiotic preparations.

Seventeen patients or 15.9% required replacement therapy with human albumin during follow-up due to marked hypoalbuminemia. Treatment was given intravenously. The albumin level was inversely correlated with the extent of the inflammatory response, which was attributed to a decrease in albumin synthesis in the liver due to the hypercatabolic state associated with the inflammatory process and downregulation of synthesis by cytokines. Many studies (García-Bosch, Hindryckx et al.) have found that hypoalbuminemia is associated with

disease activity, lack of response to treatment and increased risk of colectomy in patients with acute severe UC.

5.4.4. Analysis of patients treated with LMWH (low molecular weight heparin) and an iron-containing agent at baseline and during the course of biological treatment

Iron administration is recommended in all patients with HF and iron deficiency anaemia. In this regard, randomized controlled trials by Kulnigg and Evstatiev and real-world studies by Befrits between 2008 and 2013 showed that intravenous iron carboxy-maltose is effective for the treatment of anemia in UC. However, intravenous preparations are not well accepted by all patients and are accompanied by higher prices.

Administration of intravenous iron was necessary in 69 (64.5%) of the subjects before initiation and during the course of biological treatment (Table 50).

Table 50. Analysis of patients undergoing treatment with an iron-containing preparation

Iron treatment	бр	%
yes	69	64.5 %
no	38	35.5 %

The drugs that were used were - Idafer 20 mg/ml (Iron III in the form of ferrous saccharate); Cosmofer 50 mg/ml (Iron III - hydroxide dextran complex); Monofer 100 mg/ml (iron derisomaltosis) and Ferinject 50 mg/ml (iron carboxymaltose).

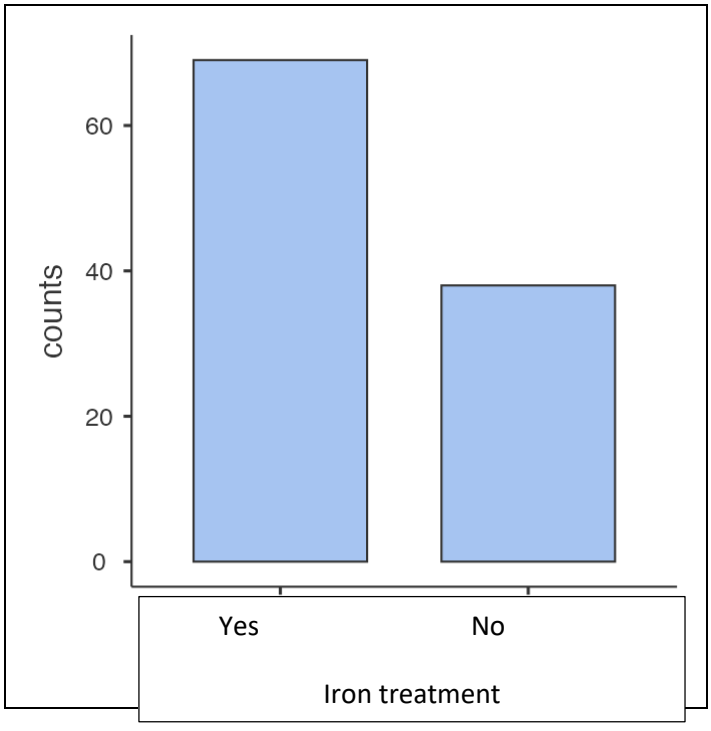


Fig. 24. Analysis of patients treated with iron preparation

Prophylaxis of thrombotic complications with NMH was performed in 24.3% of the observed subjects (n=107).

5.4.5. Analysis of patients undergoing biological treatment during the course of the study

A total of 56 (52.3%) patients underwent treatment with Adalimumab - Humira® and Hulio with subcutaneous administration at an induction dose of 160 mg at week 0, 80 mg at week 2 and maintenance therapy of 40 mg weekly.

Infliximab - Remicade® and the biosimilar - Inflectra were administered to 45 (42.1%) of the subjects. The regimen administered to them was induction with 5 mg/kg body weight in week 0, 2nd and 6th and maintenance therapy of 5 mg/kg body weight in 8 weeks.

Only 6 patients or 5.7% were treated with Golimumab - Simponi®, subcutaneously. The induction dose was 200 mg at week 0, 100 mg at week 2, 50/100 mg at week 6 and maintenance therapy of 50/100 mg every 4 weeks.

With Vedolizumab - Entyvio®, 27 patients, or 25.7%, had intravenous therapy at an induction dose of 300 mg at week 0, week 2 and week 6 and maintenance therapy of 300 mg every 8 weeks.

In the group of patients treated with JAK inhibitors, Tofacitinib (Xeljanz®) - 11 (10.4%) and Upadacitinib (Rinvoq®) - 1 (0.9%) were co-advised. The induction dose in Tofacitinib patients was 20 mg oral for 8 weeks followed by maintenance therapy of 10 mg/day.

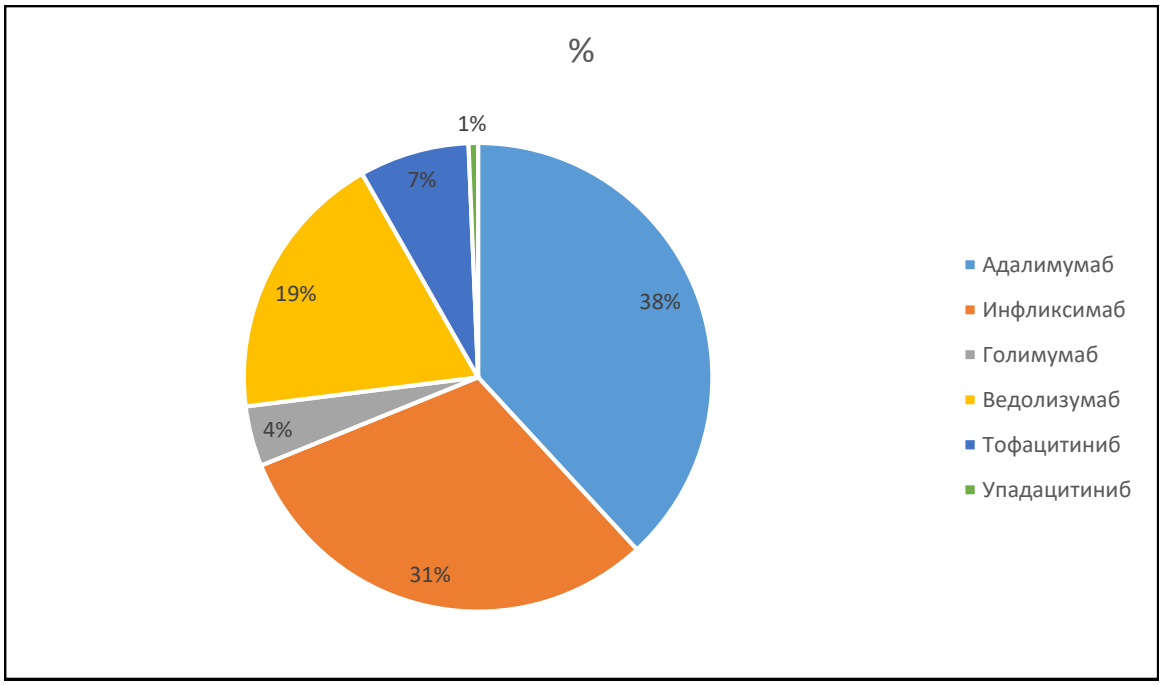


Fig. 25. Distribution of drugs (%) used to treat patients with UC during the course of the study

In some of the patients, 37 (34.6%), a change of biologic medication was performed (Table 51 and Table 52).

Table 51. Analysis of patients with biologic medication switch during the course of the study

Switch biologic	n	%
yes	37	34.6 %
no	70	65.4 %

The most frequent reasons that necessitated biologic drug switching were - adverse drug event, decreased patient compliance and/or spontaneous discontinuation, presence of neutralizing drug antibodies.

Table 52. Analysis of patients with switching by biologic medication group over the course of the study

Biologic treatment	бр	%
Adalimumab	32	29.9 %
Golimumab/Infliximab	2	1.9 %
Infliximab	24	22.4 %
Adalimumab/Infliximab	6	5.6 %
Prouchvane-JAK Inh./Tofacitinib	1	0.9 %
Infliximab/Infliximab/Clinic Proofreading	1	0.9 %
Infliximab/Tofacitinib	3	2.8 %
Infliximab/Adalimumab	2	1.9 %
Golimumab(trial)/Infliximab	1	0.9 %
Vedolizumab	12	11.2 %
Adalimumab/Golimumab	1	0.9 %
Adalimumab/Vedolizumab	9	8.4 %
Infliximab/Adalimumab/Vedolizumab	1	0.9 %
Vedolizumab/Infliximab	1	0.9 %

Biologic treatment	бр	%
Golimumab/Vedolizumab/Tofacitinib	1	0.9 %
Adalimumab/Infliximab/Vedolizumab/Infliximab	1	0.9 %
Vedolizumab(prochuvane)/Adalimumab/Tofacitinib	1	0.9 %
Infliximab/Vedolizumab/Tofacitinib	1	0.9 %
Vedolizumab/Tofacitinib	1	0.9 %
Vedolizumab/Adalimumab	1	0.9 %
Adalimumab/Tofacitinib	2	1.9 %
Golimumab	1	0.9 %
Rinvoc	1	0.9 %
Infliximab/Tofacitinib	1	0.9 %

In a small proportion of the patients included in the follow-up, 19 (26.2%), intensification of biological treatment took place.

5.4.5.1 Analysis of adverse drug events during the course of biological treatment in patients with UC included in the study

We found adverse drug events in 28 (26.2%) patients during the course of biological treatment (Table 53).

Table 53. Analysis of adverse drug events in patients in the course of biological treatment

Adverse events	n	%
yes	28	26.2 %
no	79	73.8 %

The most common adverse events were - astheno-dynamia, nausea, skin erythema, urticaria, pruritus, hair loss and tenderness at the site of subcutaneous application.

Tuberculostatic treatment was administered in 6 patients due to clinical, laboratory and imaging evidence of latent/active TB. These patients were strictly followed up by a pulmonologist. In 4 of them, biological therapy was resumed after successful cure.

In one patient, a 46-year-old man with left-sided colitis, E2S3 on a background of treatment with Azathioprine 100 mg/day and maintenance therapy with Vedolizumab 300 mg intravenous infusion every 56 days showed laboratory evidence of neutropenia and anemia as well as ultrasonographic evidence of progressive splenomegaly. The patient consulted with a hematologist and underwent a trepanobiopsy with morphology for hypoplastic bone brain, without infiltration by non-Hodgkin's lymphoma. Flow cytometric examination was performed in the same patient and revealed no pathological B-cell population.

In two of the patients followed, a lethal outcome occurred. The first patient was a 52-year-old woman with ulcerative pancolitis, and BTE (pulmonary thromboembolism) was assumed as the cause of death. The second patient was a 55-year-old man, also with ulcerative pancolitis, who died after a severe Covid 19 pneumonia and probable BTE.

5.5 Correlation between the total Mayo score at baseline and the course of biological treatment in the studied patients with UC

Table 54. Analysis of total Mayo scoring at baseline and during the course of biological treatment

	Period	N	Mean	Std. Deviation	Independentt -test	Confidence interval
Total Mayo score	Start	106	11.6698	.51124	t=80.37, p=0.0001	[7.68;8.07]
	End	106	3.7925	.86978		

The mean value of the total Mayo score in patients (n=106) was > 8, which corresponds to a severe form of UC, approaching the maximum reference limit for this indicator - 11.67. On inclusion of biologic treatment and over the course of patient follow-up, we observed a significant decrease (mean of 8 points) in the total Mayo score value of 3.79, and this fell into the group of patients 1-6, or mild form of UC (Table 54). This result is consistent with the literature data. The analysis in a study by Su C, Lewis JD, Goldberg B et al. reported that a decrease of at least 3 points in the total Mayo score is required to determine clinically significant improvement in the course of the disease, which has a sensitivity of 88% and specificity of 80%.

5.6. Correlation between total Meyo scoring in patients with/without treatment with Imuran and biologic drug

Table 55. Analysis of total Mayo score in patients with/without Imuran treatment

	Imuran	N	Mediana	Stand.dev.	Independent t test
Total Mayo score	Yes	37	11.6757	.52989	t=0.86, p=0.963
	No	69	11.6667	.50488	

**. Correlation is significant at the 0.01 level (2-tailed).

a. period = start

In our follow-up group of patients with active UC (n=107), 37-seven individuals were treated simultaneously with Imuran and the corresponding biologic. In the correlation performed in this study, we did not find a statistically significant difference in the form of UC, as assessed using the total Mayo score, compared with patients who did not take Imuran during the course of their treatment (Table 55).

5.7. Correlation between total Mayo scoring and leukocyte count in patients with UC enrolled in the study

Table 56. Analysis of total Mayo score to leukocyte count over the course of the study

Correlations					
			Diagnosis	Total Mayo score	Leucocyte
Spearman's rho	Diagnosis	Correlation Coefficient	1.000	.337**	.143
		p	.	.000	.141
	Total Mayo score	Correlation Coefficient	.337**	1.000	.213*
		Sip	.000	.	.028
	Leucocyte	Correlation Coefficient	.143	.213*	1.000
		p	.141	.028	.

a. period = start

			Total Mayo score	Leucocyte
Spearman's rho	Diagnosis	Correlation Coefficient	.	.
		p	.	.
	Total Mayo score	Correlation Coefficient	1.000	.329**
		p	.	.001

	Leucocyte	Correlation Coefficient	.329**	1.000
		p	.001	.

The correlation analysis showed a positive association between the severity of UC, with an increase in the value of total Mayo scoring and an increase in leukocyte values in the group of subjects (Table 56).

Table 57. Analysis of total Mayo scoring to platelet count in the course of the study

			Diagnosis	Total Mayo score	Platelate
Spearman's rho	Diagnosis	Correlation Coefficient	1.000	.337**	.306**
		p	.	.000	.001
	Total Mayo score	Correlation Coefficient	.337**	1.000	.336**
		p	.000	.	.000
	Platelate	Correlation Coefficient	.306**	.336**	1.000
		p	.001	.000	.
**. Correlation is significant at the 0.01 level (2-tailed).					
a. period = start					

Table 57 shows the correlation between the total Mayo score and the platelet count, with no statistical significance observed for these two parameters.

5.8. Correlation of CRP, FCP and Total Mayo Score at baseline and during the course of biological treatment in patients with UC

The paired t-test was used for the statistical processing of the examined indicators - start-end values in 3 indicators. The difference in CRP, FCP and Total Mayo Score values at the beginning of inclusion of biological treatment and during the course of therapy was statistically significant.

The mean value of CRP at the beginning of the study was 28.83±47.55 mg/L, and a significant decrease of the indicator was observed when the biological treatment was included - 8.68±13.42 mg/L.

In terms of FCP, a decrease in values was described in the patients with UC included in the study after initiation and during the course of biological treatment, 1110.64±806.53mg/kg and 162.99±241.84 mg/kg, respectively.

In the analysis of the Total Mayo Score at the beginning of the study, the mean value was 11.67±0.51, which corresponded to a severe form of UC. We again observed a statistically significant difference in this parameter after inclusion of biological treatment, with a mean value of 3.79±0.86, or mild form of UC.

Table 58. Correlation analysis of the indicators - CRP, FCP and total Mayo score

Paired Samples Statistics					
		Mean	Mediana	Stand.dev.	Paired t-tets
	CRP_start	28.8347	106	47.54515	t=4.42, p=0.0001
	CRP_end	8.6795	106	13.42224	
	FCP start	1110.6436	107	806.52900	t=12.70, p=0.0001
	FCP end	162.9945	107	241.83815	
	Total Mayos score start	11.6729	107	.50982	t=91.86, p=0.0001
	Total Mayo score end	3.7944	107	.86590	

5.9. Analysis of the Inflammatory Bowel Disease Quality of Life Questionnaire (IBDQ)-32, the Daily Fatigue Impact Scale (D-FIS) and the Inflammatory Bowel Disease-Fatigue (IBD-F) Self-assessment Scale during the course of the study.

The three questionnaires assessing quality of life and fatigue were completed by a total of 85 IBD patients on biological treatment, on paper and/or electronically during the course of the study.

The mean IBDQ-32 score in the group of patients considered (n=85) was 188 (with a minimum of -72 and a maximum of 224), which is significantly close to the maximum score for this questionnaire of 224. This determines the better quality of life of patients undergoing biological treatment (Table 59).

In section 1 of the IBD-F, the mean value in the described group of patients was 6.87±4.4, defining a low level of fatigue in patients undergoing biological treatment. In section 2 of the same scale, the mean value was 16±16.5. This result defines a low impact of fatigue on patients with active form of UC undergoing biological therapy (Table 59).

With regard to the third questionnaire, the D-FIS, assessing the daily fatigue of patients (n=85), its mean value was 9±7.6, again determining a low degree of fatigue in patients undergoing biological therapy (Table 59).

Table 59. Analysis of mean values of IBDQ)-32, D-FIS and IBD-F

	N	Rang	Min.	Max.	Mediana	Stand.dev.
IBDQ_32	**85**	**152.00**	**72.00**	**224.00**	**188.4000**	**32.19738**
D_FIS	**85**	**26.00**	**.00**	**26.00**	**8.5176**	**7.57284**
IBD_F1	**85**	**19.00**	**.00**	**19.00**	**6.8706**	**4.38012**
IBD_F2	**85**	**59.00**	**.00**	**59.00**	**15.9412**	**16.48401**

5.9.1. Correlation between IBDQ-32, D-FIS and IBD-F and gender of patients with UC during the course of the study

Spearman's correlation (rho) was used to calculate these indices, between quantitative and qualitative data.

A weak correlation, but statistically significant, existed between gender and the Quality of Life Questionnaire in patients with UC. Higher scores on the questionnaire were described in men, i.e. they had a better quality of life than women (Table 60).

Table 60. Correlation between IBDQ-32 and gender

			gender	IBDQ_32
Spearman's rho	gender	rho	1.000	-.221*
		p	.	.043
		N	107	85
	IBDQ_32	rho	-.221*	1.000
		p	.043	.
		N	85	85
*. Correlation is significant at the 0.05 level (2-tailed).				

In women, higher values of the Daily Fatigue Impact Scale were found, which also determined the greater fatigability in this group of patients. The correlation was weak but statistically significant (Table 61).

Table 61. Correlation between D-FIS and gender

			gender	D_FIS
Spearman's rho	gender	rho	1.000	.221*
		p	.	.043
		N	107	85
	D_FIS	rho	.221*	1.000
		p	.043	.
		N	85	85
*. Correlation is significant at the 0.05 level (2-tailed).				

No statistically significant correlation was observed between section 1 and section 2 of the Fatigue Self-Rating Scale in IBD patients.

5.9.2. Analysis of the correlation between the presence/absence of anaemia and daily fatigue in patients with UC during the course of the study

When a comparative analysis with t-test was performed between the presence of anaemia and daily fatigue in patients, no statistically significant correlation was found (Table 62).

Table 62. Correlation between anemia and D-FIS

	Anaemia	N	Mediana	Stand.dev.	Independent t-test/p-level of significance	Confidence Interval
D_FIS	yes	51	9.1765	8.35394	t=0.982, p=0.329	[-1.68;4.98]
	no	34	7.5294	6.20965		

5.10. Comparative analysis of CRP, FCP and Total Mayo Score for each biologic used in this study

Comparisons here were made using a Paired t-test, again discussing the mean values compared, then indicating t and statistical significance.

Adalimumab

The number of patients who received treatment with Adalimumab was 56. At the beginning of the study, the mean CRP value was 33.32±54.39 mg/L, and during the course of the study there was a statistically significant decrease in its value - 8.64±13.63 mg/L. Significant decrease was also observed in FKP: mean value at baseline-1072.32±645.89 mg/kg and mean value during the study-172.72±281.20 mg/kg. Before inclusion of biological treatment, the Total Mayo Score of the subjects was almost 12, which corresponds to a severe form of UC, but in the course of the study the Total Mayo Score was <6, which is a mild form. Similar to our data, in a study by Reinisch, Sandborn et al. conducted in North America and Europe in patients with UC, Total Mayo Score >12 and Endoscopic Mayo Score >2, with no response from conventional treatment, at week 8, 18.5% of patients in the 160/80 Adalimumab group were in remission (p=0.031) versus 9.2% of the placebo group.

Table 63. Comparative analysis in patients on Adalimumab treatment

Adalimumab					
	Mediana	N	Stand.dev.	Paired t-test/ p- level of significance	Confidence Interval

	CRP_start	33.3212	56	54.39547	t =3.51, p=0.001	[10.61;38.73]
	CRP_end	8.6425	56	13.63403		
	FCP_start	1072.3157	56	645.88716	t =11.30, p=0.0001	[740.13;1059.03]
	FCP_end	172.7273	56	281.20762		
	Total Mayo score-start	11.7143	56	.49412	t =73.7, p=0.0001	[7.74;8.18]
	Total Mayo score-end	3.7500	56	.69413		

Infliximab

There were 45 patients who underwent treatment with Infliximab. A statistically significant difference was found for all three parameters - CRP, FCP and Total Mayo Score. At the beginning of the study, the mean CRP was 22.24±32.30 mg/L and during the course of the study, the mean CRP was 9.0±14.30 mg/L. In the follow-up of FCP, a positive trend in the mean value over the course of the study was also described. In all patients with UC undergoing treatment with Infliximab, the mean Total Mayo Score was <6, corresponding to mild disease (Table 64).

Table 64. Comparative analysis in patients on Infliximab treatment

Infliximab					
	Mediana	N	Stand.dev.	Paired t-test/ p- level of significance	Confidence Interval
CRP_start	22.2493	45	32.30384	t =2.68, p=0.001	[3.28;23.10]
CRP_end	9.0541	45	14.30982		
FCP_start	1174.4002	45	964.32159	t =7.03, p=0.0001	[710.12;1280.60]
FCP_end	179.0309	45	233.94768		
Total Mayo score_start	11.5778	45	.58344	t =48.5, p=0.0001	[7.47;8.12]
Total Mayo score_end	3.7778	45	1.02000		

Golimumab

Six patients with JC had treatment with Golimumab. In these patients, there was no statistically significant difference in the mean CRP and FCP at baseline and during the course of biologic treatment. There was a statistically significant decrease in Total Mayo Score values, with a mean value at baseline of 11.66±0.51 and during the course of the study of 4.16±0.40, respectively (Table 65).

Table 65. Comparative analysis in patients on Golimumab treatment

Golimumab	

	Mediana	N	Stand.dev.	Paired t-test/ p- level of significance	Confidence Interval
CRP_start	14.1583	6	12.62768	t =1.02, p=0.351	[3.28;23.10]
CRP_end	11.5233	6	13.53516		
FCP_start	712.1183	6	829.26286	t =1.78, p=0.135	[710.12;1280.60]
FCP_end	95.7667	6	57.61801		
Total Mayo score_start	11.6667	6	.51640	t =33.5, p=0.0001	[7.47;8.12]
Total Mayo score_end	4.1667	6	.40825		

Vedolizumab

We followed 27 patients on Vedolizumab treatment. We found a statistically significant difference in both CRP and FCP values from baseline and during the course of biologic treatment. The difference in FCP values was significantly more significant. With regard to the Total Meio Score, all patients had severe UC at baseline, and in the course of treatment the mean Total Meio Score was 3.85±0.81, corresponding to mild UC (Table 66).

Table 66. Comparative analysis in patients on Vedolizumab treatment

Vedolizumab					
	Mediana	N	Stand.dev.	Paired t-test/ p- level of significance	Confidence Interval
CRP_start	35.3619	27	61.30166	t =2.15, p=0.041	[3.28;23.10]
CRP_end	9.3444	27	11.72232		
FCP_start	1127.5833	27	690.09884	t =7.13, p=0.0001	[710.12;1280.60]
FCP_end	238.6089	27	298.54580		
Total Mayo score_start	11.6667	27	.55470	t =40.54, p=0.0001	[7.47;8.12]
Total Mayo score_end	3.8519	27	.81824		

Tofacitinib

Eleven (n=11) patients were treated with Tofacitinib. Our data showed a statistically significant difference in FQP values at baseline and during the course of the study, 1503.59±615.10 mg/kg versus 244.46±285.61 mg/kg, respectively. The data analysis showed that the Total Mayo Score at the beginning of treatment reached 12 points and during the course of Tofacitinib treatment was 4±0.77. The regression analyses performed in OCTAVE 1 and 2 trials showed that the decrease in CRP at week 4 and Total Mayo Score at week 2 were predictors of achieving good clinical response, clinical and endoscopic remission.

Table 67. Comparative analysis in patients on Tofacitinib treatment

Tofacitinib					
	Mediana	N	Stand.dev.	Paired t-test/ p- level of significance	Confidence Interval
CRP_start	27.9245	11	37.07418	t =1.04, p=0.319	[-13.04;36.18]
CRP_end	16.3527	11	21.52120		
FCP_start	1503.5955	11	615.10931	t =7.36, p=0.0001	[878.07;1640.19]
FCP_end	244.4636	11	285.61257		
Total Mayo score_start	11.7273	11	.46710	t =54.86, p=0.0001	[7.41;8.04]
Total Mayo score_end	4.0000	11	.77460		

Upadacitinib

In the study described, only one patient underwent treatment with Upadacitinib. Therefore, no comparison for statistical significance can be made, only a description of the mean values of the indicators in the respective periods. Analysis of the data showed a slight increase in CRP values, but a downward trend in FCP, with a mean value before inclusion of Upadacitinib of 1000 mg/kg and during the course of treatment of 259 mg/kg. A positive trend was also observed with respect to Total Mayo Score (Table 68).

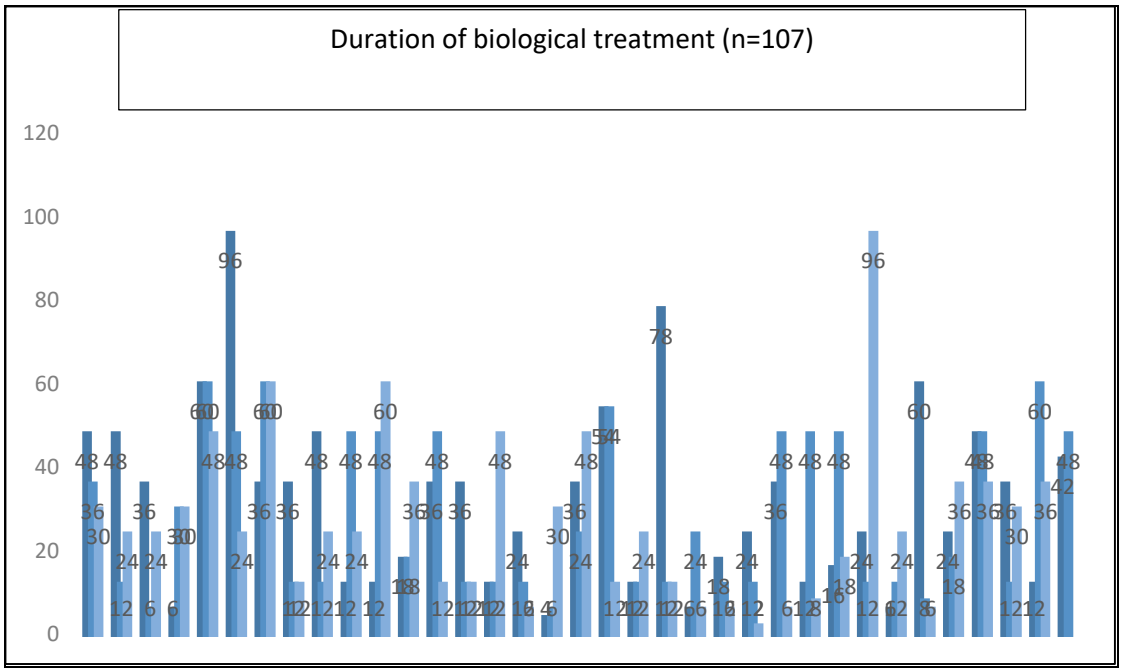
Table 68. Comparative analysis in patient on treatment with Upadacitinib

Upadacitinib	N	Min.	Max.	Mediana
CRP_start	1	4.59	4.59	4.5900
CRP_end	1	14.75	14.75	14.7500
FCP_start	1	1000.00	1000.00	1000.0000
FCP_end	1	259.40	259.40	259.4000
Total Mayo score_start	1	12.00	12.00	12.0000
Total Mayo score_end	1	5.00	5.00	5.0000

5.11. Duration of biological treatment in the group of patients with UC included in the study

The variation in treatment time in the study subjects was wide. It was found during the course of the study that the shortest duration was 2 months and the longest duration of therapy was 96 months (Table 69).

Table 69. Duration of biological treatment (in months)



5.12. Period from diagnosis of the disease to initiation of biological treatment in subjects with UC

In the studied group of patients with active UC, the time from diagnosis to initiation of biological treatment ranged from 5 months to 40 years. Our analysis showed that in 66 (61.7%) of the study subjects, biologic treatment was initiated <5 years after the diagnosis of UC.

5.13. Surgical treatment in patients with UC included in the study

Only one patient in the active UC group undergoing biological treatment underwent surgical treatment. This was a man diagnosed with ulcerative colitis at the age of 40 years. Nearly 30 years after diagnosis, due to marked activity, family history of colonic cancer and pathomorphologically proven moderately differentiated tubulovillous polyp, left-sided hemicolectomy with transversoanastomosis was performed. Repeated periods of disease impaction followed. After another hospitalization in the Gastroenterology Clinic, the decision was made to include biologic treatment. The patient did not have a good clinical response in the first year of treatment, and due to marked pelvic incontinence impairing quality of life, the patient was referred for repeat surgical intervention.

5.14. Patient follow-up - course of disease. Survival.

Biological treatment of the patients with UC (n=105) included in this study is ongoing. They were followed up every 6 and 12 months from the start of treatment in the Gastroenterology Clinic of St. John's University Hospital. The study was carried out at the University of St. Marina in Sofia. The study was carried out in the first six months of follow-up. Clinical and laboratory examinations and abdominal ultrasonography were performed every 6 months, and FCS at the 12th month. Monitoring of the patients is carried out by a multidisciplinary team of specialists - gastroenterologist, nutritionist, imaging specialist and pathologist.

Fatal outcome in the course of the disease was found in two patients, respectively at the 13th and 15th month from the start of biological treatment.

5.15. Register of patients with UC on biological therapy, treated and monitored at the Clinic of Gastroenterology, Hepatology and Nutrition, "St. Marina" University Hospital, Varna.

A registry of patients with active form of UC on biological therapy was created in the form of an Excel table. The registry contains detailed information on various indicators and invasive tests performed. Each patient has a serial number in the table, which allows upgrading the registry. The data included in the table allows a summary sample to be drawn for each patient.

5.16. Proposal of a biological treatment algorithm for patients with active UC unresponsive to conventional therapy.

Based on our experience in the biological treatment of patients with active UC, we propose the following approach:

Identify the right patient for biologic treatment - with no response from conventional therapy, proven cortico-dependence, proven cortico-resistance.

Selection of the right drug for treatment based on the patient's age, presence/absence of comorbidities, presence/absence of EIP, presence/absence of adverse drug reactions, and the patient's desire related to the route of administration of the biologic.

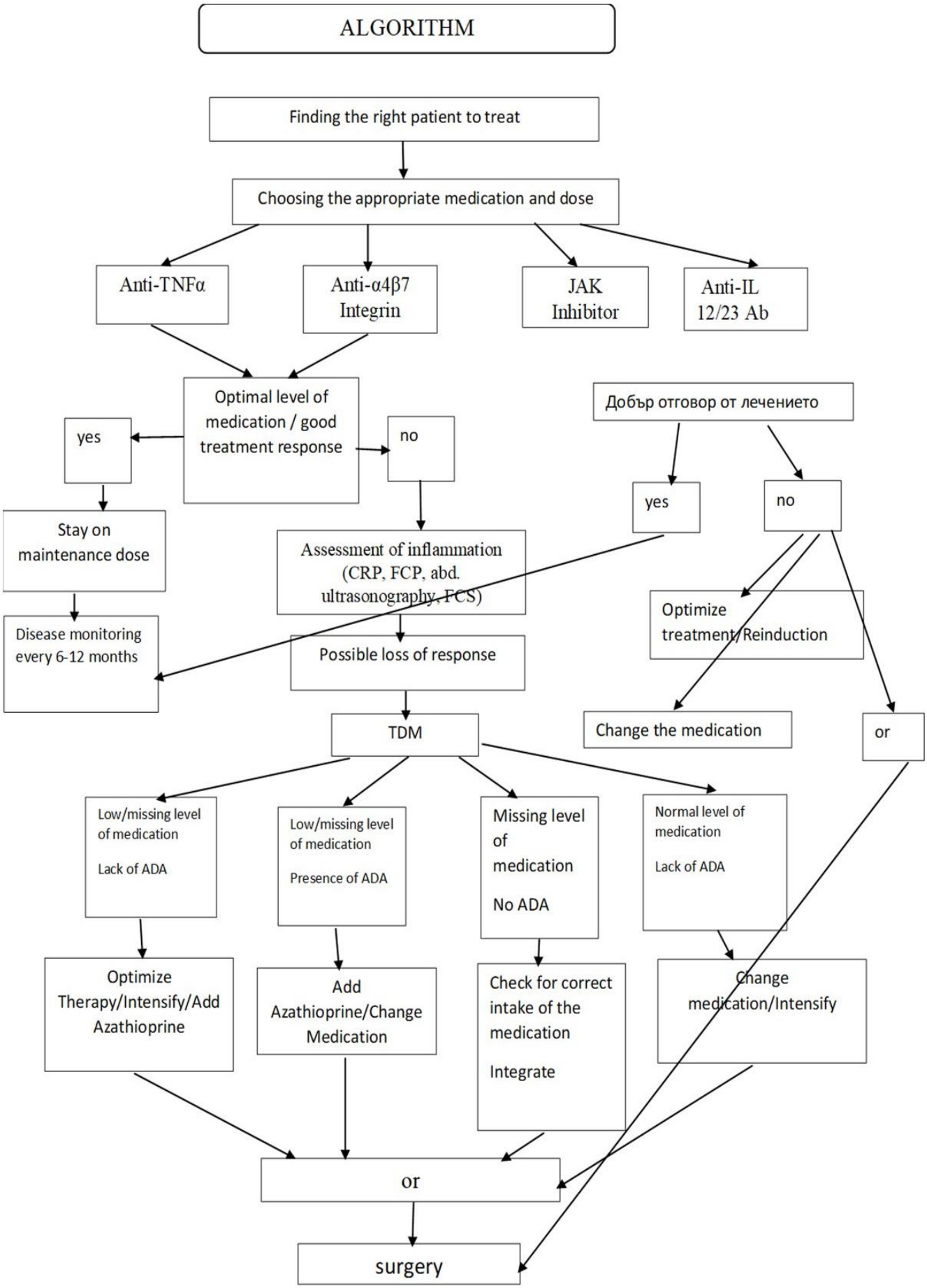
After drug selection, if there is a good response from treatment, the patient remains on a maintenance dose, subject to follow-up at the appropriate clinical center every 6 and 12 months.

In the absence of an adequate therapeutic response, it is necessary to evaluate the inflammation - examination of basic serum and fecal markers, abdominal ultrasonography, FCS and histological examination.

The next step is to conduct therapeutic drug monitoring in patients treated with Anti-TNFα and Anti-α4β7 Integrin. In case of low/missing drug level and no ADA - optimize therapy/intensify/add Azathioprine. For low/missing med level and ADA present - add Azathioprine/change med. For missing medication level, no detectable ADA - check for correct medication intake or intensify. With normal medication level and no ADA - change medication or intensify.

In patients on JAK Inhibitor and Anti-IL 12/23 Ab therapy, in the absence of adequate therapeutic response and after assessment of inflammation - change medication or reinduce.

In all cases of non-response from the described therapeutic steps, surgical treatment is proceeded.


ALGORITHM
Finding the right patient to treat
Choosing the appropriate medication and dose
Anti-TNFα
Anti-α4β7 Integrin
JAK Inhibitor
Anti-IL 12/23 Ab
Optimal level of medication / good treatment response
yes
no
Stay on maintenance dose
Disease monitoring every 6-12 months
Assessment of inflammation (CRP, FCP, abd. ultrasonography, FCS)
Possible loss of response
TDM
Добър отговор от лечението
yes
no
Optimize treatment/Reinduction
Change the medication
or
Low/missing level of medication
Lack of ADA
Low/missing level of medication
Presence of ADA
Missing level of medication
No ADA
Normal level of medication
Lack of ADA
Optimize Therapy/Intensify/Add Azathioprine
Add Azathioprine/Change Medication
Check for correct intake of the medication
Integrate
Change medication/Intensify
or
surgery

6. CONCLUSION

Ulcerative colitis is an idiopathic disease and although much research has been devoted to the study of its pathophysiology, the exact etiology is still unknown. The clinical course of UC is unpredictable, with alternating periods of exacerbation and remission.

The introduction of biologics has revolutionized the treatment of patients with active UC. They represent an essential component of modern personalized therapy for patients with UC. In those with marked inflammatory activity, biologic therapy has the potential to achieve remission. In the absence of an appropriate therapeutic regimen, patients with UC have a poor quality of life, with a high morbidity that often leads to complications requiring hospitalization and surgical treatment.

In the short term, clinical remission is the most important goal of conventional and biological treatment. Our experience in the use of biologic agents has shown that proper patient selection and dosing regimen, as well as strict clinical and therapeutic monitoring of patients with active UC, contribute to the occurrence of the desired long-term remission. The present observation established the expected high efficiency of biologics as well as their safety in the treatment of active ulcerative colitis, a disease of social importance and a challenge in modern gastroenterology.

7. CONCLUSIONS

Based on the results of the clinical observation we can draw the following conclusions:

1. Early use of biologic agents or small molecules allows patients with active UC to achieve clinical, endoscopic and histological remission.

2. Biological agents show high efficacy and safety for induction and maintenance of remission in patients with moderate-to-severe UC.

3. The non-invasive serum biomarker, CRP, and fecal inflammatory biomarker, FCP, are convenient and effective for monitoring patients with active form of UC undergoing biologic treatment.

4. Therapeutic drug monitoring is finding increasing application as a potential strategy to optimize therapy.

5. Colonic ultrasonography can serve as a non-invasive marker for the assessment of histological activity in patients with active UC.

6. In our study, there was no statistically significant difference in patients achieving remission in monotherapy or concurrent with Azathioprine.

7. Clinical symptoms in patients with an active form of UC were directly associated with biochemical and endoscopic parameters, and their positive dynamic during biologic treatment was a predictor of reduced risk of relapse and colectomy.

8. Introduction of biologic therapy reduces the need for surgery and associated complications in patients with active UC.

9. Proper choice of biologic medication promotes better quality of life in patients with UC.

8. CONTRIBUTIONS

Contributions of scientific and confirmatory nature:

1. For the first time in Bulgaria, a complex assessment of patients with active ulcerative colitis on biological therapy was performed.

2. A comprehensive clinical evaluation of patients with UC in the course of biological treatment was performed.

3. The significance of non-invasive inflammatory markers CRP and FCP for determining disease activity and for patient follow-up is confirmed.

4. The role of abdominal ultrasound as a rapid and reliable method for diagnosing active disease and monitoring the outcome of treatment was assessed.

5. The importance of endoscopic examination of the colon, with the complex evaluation of activity using a scoring system - Mayo scoring, as well as the need for histomorphological follow-up in the course of biological treatment to establish mucosal healing was confirmed.

6. A comparative analysis of clinical symptoms, laboratory parameters, endoscopic and morphological findings before and during biological treatment was performed.

7. Questionnaires used to assess quality of life and fatigue in patients undergoing biologic treatment contributed to the overall evaluation of the therapy being delivered.

8. An algorithm for biological treatment of patients with active ulcerative colitis not responding to standard therapy is proposed.

9. A registry of patients with active ulcerative colitis undergoing biologic therapy was established.

9. LIST OF SCIENTIFIC PUBLICATIONS AND COMMUNICATIONS RELATED TO THE THESIS

Publications

1. Petrova A, Stamboliyska M, Tsaneva M, Boykova P. Three years of experience in the treatment of patients with severe ulcerative colitis with biosimilar Infliximab(Remsima). Proceedings of the Union of Scientists - Varna, Series Medicine and Ecology. 2019; 2:24-28.

2. Petrova A, Stamboliyska M. JAK inhibitors and their place in the treatment of ulcerative colitis. Medinfo J. 2021; 34-37.

Scientific presentations at international and national symposia and conferences

1. FALK Symposium, Prague, Czech Republic-Poster presentation on "One year experience in treating patients with severe ulcerative colitis with biosimilar (Infliximab)- Remsima, 2016

2. Gastroforum-Novosti, Varna, participation with presentation of a solo paper - "Tuberculosis and IBD", 2019

3. IV Takeda GI Academy, Sofia-participation with presentation of a solo paper - "Clinical case of a patient with early stage UC", 2022

Thank you to everyone who has contributed to making this work happen:

My supervisor Prof. Diana Todorova Gancheva-Tomova, Ph.D. for her guidance and help.

My teacher Assoc. Prof. Dr. Miglena Stamboliyska, PhD for her courage and support.

The colleagues from the Clinic of Gastroenterology at the University Hospital "St. Marina". Varna.

Colleagues from the Clinical Laboratory at the University Hospital "St. Marina", Sofia. Marina. Varna.

Colleagues from Clinical Pathology at University Hospital "St. Marina", Sofia. Marina. Varna

Assoc. Prof. Dr. Silvia Nikolova, PhD for the expert statistical processing of the data.

My family for the endless love.