„CAROL DAVILA", UNIVERSITY OF MEDICINE AND PHARMACY,
BUCHAREST
DOCTORAL SCHOOL
MEDICINE

Predictive factors for inflammatory bowel diseases course

ABSTRACT OF DOCTORAL THESIS

PhD supervisor:
PROF.UNIV.DR. NEGREANU LUCIAN

PhD student:
STATE ROXANA MONICA

2024

Table of contents

Introduction

Inflammatory bowel diseases are chronic, progressive, and debilitating conditions. Questions regarding the natural course and prognosis of the disease are among the most common both among patients and physicians. A better understanding of the natural progression of the disease would allow the identification of characteristics that confer an unfavorable prognosis and the stratification of patients based on their risk for a severe disease course.

Thus, identifying predictive factors that allow the assessment of individual risk for a severe disease course, defined by a high rate of relapses, episodes of severe clinical activity, disease progression in terms of extension, the need for surgical intervention, frequent hospitalization, colorectal cancer, the presence of extraintestinal manifestations, or other complications, has become one of the important research directions in this field.

I chose to continue the research activity conducted at Colentina Clinical Hospital in the field of inflammatory bowel diseases, in the form of prospective study that included all patients with this pathology that were evaluated in the Gastroenterology Department. In this setting, patients benefited from a careful, multidisciplinary monitoring approach.

I conducted a descriptive analysis of the cohort of patients with inflammatory bowel diseases prospectively followed in the clinic, aiming to assess the severity and extent of the disease using dedicated tools and to identify changes in the disease's progression based on patient characteristics, disease features, or treatment.

Another important part of the research activity was the investigation of predictive factors for disease severity and relapses (clinical, serological, fecal, and tissue factors). Finally, I contributed to the establishment of a biological sample bank from patients with inflammatory bowel diseases, unique in Romania, which could be used for studies carried out during the doctoral program and could serve as the basis for many future studies.

The prospective study on which this work is based benefited from material resources and logistical support from the academic environment. From the "Carol Davila" University of Medicine and Pharmacy, I received support through a doctoral scholarship. Additionally, during the doctoral program, we obtained funding from the Ministry of Research and Innovation by winning a research grant developed by the research team of the Gastroenterology Department, alongside colleagues from the Pathology Department (CCCDI-UEFISCDI, Project Number PN-III-P1-1.1-TE-2021-0801/2022).

I. General Part

1. Key elements regarding the natural history of inflammatory bowel diseases

Inflammatory bowel diseases, represented by ulcerative colitis (UC), Crohn's disease (CD), and unclassified colitis, are chronic entities with a course marked by periods of remission and relapse. They are characterized by chronic inflammation in the gastrointestinal tract, and in the absence of treatment, both Crohn's disease and ulcerative colitis can lead to progressive and irreversible damage to the digestive tract [1].

1.1 Epidemiological data

The incidence and prevalence of these pathologies have been increasing globally in recent decades, both in the pediatric and adult populations [2,3]. The progression of these diseases is unpredictable.

1.2 Natural history of inflammatory bowel diseases

The clinical course of CD and UC is unpredictable and characterized by alternating periods of clinical remission and active disease, most commonly manifested by abdominal pain, diarrhea, and weight loss. Although the two diseases share many similarities, they differ in disease phenotype and progression. While CD can affect the entire digestive tube (from the oral cavity to the anus) with transmural involvement, UC is limited to the colonic mucosa [4].

1.3 The role of advanced therapies in disease progression

Considering the chronic nature of these pathologies, therapy aims at both inducing remission and maintaining it in the long term. Untreated chronic inflammation translates into unfavorable long-term patient outcome [5], which is why treatment strategies and patient monitoring have changed in recent years. Recent studies show that early aggressive treatment and careful patient monitoring can prevent complications [6,7]. The hypothesis that stratifying patients based on negative prognostic factors and individualizing treatment are crucial steps in optimizing management is based on intuitive reasoning, but there is no evidence confirming that this approach is correct. For Crohn's disease, numerous factors influence treatment choice: disease extension, activity and severity, previous treatment response, penetrating phenotype, or the presence of perianal disease. Additionally, gender, smoking, and age are other individual risk factors for disease progression and complication development, often considered in treatment decisions. Currently, advanced therapies such as anti-TNF monoclonal antibodies, vedolizumab, and ustekinumab are available, with no clear strategies for choosing a treatment

class throughout the disease's progression. Comparative studies on the effeciency and safety of these molecules do not yet exist, and the sequence in which they should be introduced and how treatment can be individualized remain unclear. Available data come from heterogeneous studies, usually with a small number of patients. The latest recommendations suggest using any of the mentioned molecules in the first line for moderate to severe forms of the disease (CD or UC), with tofacitinib being useful only in UC. The long-term impact of these medications is challenging to quantify, usually discussed in terms of hospitalization/surgery rates, periods of remission or endoscopic activity. However, these events in a patient's disease progression are reported using scores or classifications that are either unvalidated or not commonly used in clinical practice. For example, for mucosal healing, considered one of the main goals of therapy, there is no universally accepted and uniformly used definition. Some authors discuss mucosal healing in Crohn's disease as "absence of ulcers" or if "endoscopic appearance improves." In clinical trials and inflammatory bowel disease dedicated centers, recording an endoscopic Mayo score of 0 or 1 (for ulcerative colitis) and an SES-CD (Simple Endoscopic Score for Crohn's Disease) of less than 3 are currently considered sufficient to assert that the patient has mucosal healing [8].

In the absence of objective parameters widely used by the medical community to evaluate therapeutic effectiveness in real-life situations, some authors have recently proposed evaluating "treatment persistence." Treatment persistence is an indirect indicator of the effectiveness of medical intervention and could be used to assess long-term patient outcomes.

2. Predictive medicine

2.1 Definition

Predictive medicine refers to the use of technology, advanced data analysis, and genetic information to assess the risk of onset or progression of a disease, as well as the response to treatment at an individual level. The goal of predictive medicine is to tailor medical interventions to the specific characteristics of each patient, making it possible to provide healthcare services in an efficient and personalized manner.

2.2 General aspects

Personalized medicine is based on a set of principles and tools that contribute to fulfilling its role, namely making predictions, developing prevention strategies, and adapting the treatment for each individual. The use of biomarkers, genomics studies, and analysis of data

using machine learning methods forms the basis for developing personalized healthcare strategies.

2.3 The importance of predictive factors in chronic diseases management

Predictive medicine plays a crucial role in managing chronic diseases by combining existing data on disease progression with advanced analysis tools to anticipate the likelihood of developing the disease, disease progression, and complications. Key aspects of predictive medicine in chronic diseases include [9]: early diagnosis, risk stratification, creation of individualized treatment plans, monitoring disease progression, and developing prevention strategies.

2.4 Current state of knowledge on predictive factors in the evolution of inflammatory bowel diseases

The etiopathogenesis of inflammatory bowel diseases is complex and involves a combination of genetic, environmental, and immunological factors. Predicting the disease's progression and long-term outcomes for patients is one of the current major challenges in IBD management due to their heterogeneity. However, there is already a solid core of data in the literature regarding predictive factors for the most important elements of disease progression. Several elements have been proposed to define a severe disease course:

For ulcerative colitis: high relapse rate, episodes of severe acute colitis, disease progression in terms of extension, need for surgical intervention, frequent hospitalization, onset of colorectal cancer, presence of extraintestinal manifestations, or other complications [10].

For Crohn's disease: high relapse rate, stenotic or penetrating phenotype, perianal involvement, need for surgical intervention or frequent hospitalization, onset of colorectal cancer, presence of extraintestinal manifestations, or other complications [11,12].

In addition to identifying predictive factors for disease progression, identifying such parameters has proven to be of interest for assessing disease activity, anticipating response to different administered medications, or complications of treatment [13].

2.4.1 Predictive factors for severe disease course

Understanding the etiopathogenesis of inflammatory bowel diseases, as well as the natural progression of these diseases, forms the basis for developing strategies of predictive, personalized medicine. Initially, among the first predictive factors identified were clinical characteristics that either were not validated or lacked sufficient prognostic accuracy. Currently, there are several endoscopic, histological, serological, or fecal factors with demonstrated predictive role in the severe progression of the disease (Tables 2.1 and 2.2).

Table 2.1 – Characteristics associated with aggressive Crohn's disease progression

Clinical factors	Age at diagnosis < 40 ani
	Corticosteroid administration at diagnosis
	Perianal involvement
	Ileo-colonic extension
	Upper gastrointestinal tract involvement
	Stenosing/penetrating phenotype
	Smoking
Endoscopic Factors	Deep ulcers
	Involvement of a large mucosal surface
	Persistence of endoscopic lesions after induction treatment/first year of disease evolution
Histological Factors	Basal plasmacytosis
	Inflammatory lymphocytic infiltrate in the lamina propria
	Paneth cells metaplasia
Serological Factors	ASCA
	anti-GM-CSF
	anti-CBir1
	anti-OmpC
Fecal Factors	Calprotectin >150ug/g
	Positive lactoferin

Table 2.2 – Characteristics associated with aggressive ulcerative colitis progression

Clinical factors	Age at diagnosis < 40 ani
	Female gender
	Extensive colitis
	Non-smoker status
Endoscopic Factors	Severity of inflammatory activity
Histological Factors	Basal plasmacytosis
	Acute (neutrophilic) infiltrate in the lamina propria
	Architectural distortion of crypts
Serological Factors	pANCA

	anti-GM-CSF
	Hypoalbuminemia
Fecal Factors	Calprotectin>150ug/g
	Positive Lactoferin
	M2-pyruvate kinase

2.4.2 Predictive factors for disease activity

Disease activity is primarily evaluated and quantified by clinical activity scores, with the most commonly used being the CDAI (Crohn's disease activity index) and HBI (Harvey-Bradshaw index) for CD and the partial Mayo score for UC. Endoscopy plays a pivotal role in diagnosing and monitoring patients with inflammatory bowel diseases (IBD). According to current recommendations, patients with IBD are repeatedly evaluated by colonoscopy throughout the disease's progression, sometimes at relatively close intervals. The main objective for many research endeavors has been to identify non-invasive surrogate markers for endoscopic disease activity. Given the importance of the subject and its relevance to the doctoral theme, I decided to systematically investigate the literature published to date regarding the utility of non-invasive markers for confirming mucosal healing [14]. In the conducted research, I identified several serological markers investigated in the last 10 years, with variable results for detecting mucosal healing. Encouraging results have been obtained, for example, for a series of interleukins (IL-6, IL-7, IL-10, IL-17), matrix metalloproteinases, lipocalin, visfatin. However, none of these independently investigated markers have shown a sufficiently high performance to replace endoscopy**.**

2.4.3 Predictive factors for treatment response

Identifying predictive factors for treatment response is one of the main objectives of current research in the field of IBD. The justification lies in the fact that anticipating the response to a certain therapeutic class is useful in choosing the appropriate treatment, preventing disease-related complications, especially frequent hospitalizations and surgeries. Data are available on factors influencing the response to most treatment classes used in IBD: 5-aminosalicylic acid derivatives, corticosteroids, biological therapies, or small molecules (such as tofacitinib).

II. Personal contributions

3. Working hypothesis and general objectives

I chose inflammatory bowel diseases for my doctoral research because, in the medical approach to this multifactorial, chronic, and fluctuating pathology, there are still numerous unknowns that do not allow adequate long-term disease control for a significant number of patients. Thus, the identification of predictive factors that allow the assessment of individual risk for a severe disease course, defined by a high relapse rate, episodes of severe activity, disease progression in terms of extension, the need for surgical intervention, frequent hospitalization, onset of colorectal cancer, presence of extraintestinal manifestations, or other complications, has become one of the important directions of research in this field. Additionally, understanding information about patients with chronic diseases in general and IBD in particular is justified in designing truly useful and functional health programs and medical facilities tailored to patients' needs through judicious resource utilization. I chose to continue the activity conducted at Colentina Clinical Hospital in the field of inflammatory bowel diseases in the form of prospective research that included all patients with this pathology who addressed the Gastroenterology Department, benefiting from careful and multidisciplinary monitoring.

The specific objectives of the conducted study were:

- To conduct a descriptive analysis of a cohort of patients with inflammatory bowel diseases;
- To identify changes in the disease's progression based on patient characteristics, disease characteristics, or its treatment;
- To investigate predictive factors for disease severity course and relapses (clinical, serological, fecal, tissue);
- To investigate the association of disease- or treatment-related parameters with the presence of a more severe disease course;
- To establish a biological sample bank from patients with IBD for analysis in subsequent studies.

4. Feneral research methodology

4.1 Study design

To fulfill the established objectives and appropriately investigate predictive factors for disease progression, I conducted a prospective observational cohort study (MAID –

Multimodal Approach in Inflammatory Bowel Disease). This study included patients with IBD regardless of clinical activity or type of disease at the time of their first presentation at Colentina Clinical Hospital. The study protocol involved the assessment of patients at predetermined time intervals (12 months), with clinical, endoscopic, and biological data being collected at each visit. The study design was approved by the Local Ethics Committee (November 10, 2011), and it took place from 2012 to 2019 (Figure 4.1). Considering that data collection was discontinued in 2019 when many newly developed therapies were not yet available, and I aimed to investigate the impact of advanced therapies on the outcomes for patients, I decided to compile a second database, analyzed separately (Chapter 8).

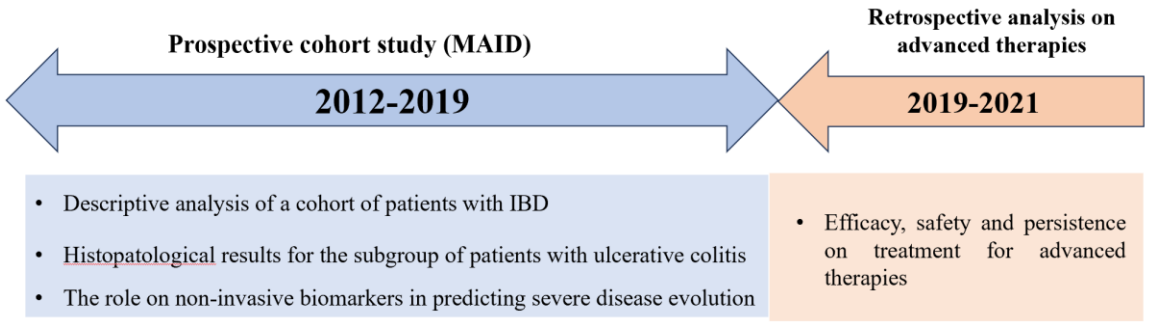

Figure 4.1 Research plan

4.2 Patients

In this study, we enrolled patients with ulcerative colitis or Crohn's disease, consecutively, at the time of their first presentation to the Gastroenterology Department of Colentina Clinical Hospital.

4.3 Patient follow-up

Study participants were evaluated every 12 months, except in cases of disease relapse, where additional assessments took place.

4.4 Clinical evaluation

Each patient was assessed at both the initial visit and follow-up visits through a comprehensive clinical examination and we recorded any significant changes. To assess the clinical activity of the disease, the Mayo partial score for ulcerative colitis and the Crohn's Disease Activity Index (CDAI) score for Crohn's disease were calculated.

4.5 Endoscopic evaluation

Endoscopic activity was assessed using the Mayo score for ulcerative colitis and the Simple Endoscopic Score for Crohn's Disease (SESCD) score.

4.6 Biological samples

For each study visit, blood, fecal, and tissue samples were collected. Blood samples were processed immediately and stored as whole blood and serum at low temperatures for long-term assays.

4.7 Quality of life assessment

At each visit, patients also completed a patient quality of life questionnaire (SIBDQ – The Short Inflammatory Bowel Disease Questionnaire – translated into Romanian).

4.8 Statistical analysis

The collected data were recorded and processed using the SPSS 20.0 program.

4.9 Ethical considerations in doctoral research

The study was conducted in accordance with medical ethics standards, with enrolled patients providing written informed consent. The study received approval from the Colentina Hospital Ethics Committee.

5. Results

5.1 Patient cohort

We enrolled 219 patients with inflammatory bowel diseases in the study, completing a total of 449 visits. Patients were followed for a median period of 24 months. The biological sample bank currently holds approximately 6000 specimens, which can be used to investigate a panel of serological, fecal, or histopathological markers. The majority of included patients were diagnosed with ulcerative colitis (131 patients, 60%), and 88 (40%) with Crohn's disease. The average disease duration at enrollment was 4.8 years (SD 6.4). Regarding disease extent, in the subgroup of Crohn's disease patients, most had colonic involvement (L2 according to the Montreal classification), followed by ileocolonic and ileal involvement in frequency (Figure 5.4). Among them, 7 patients (8%) presented perianal involvement, and 9 (10%) had a stenosing phenotype.

For patients with ulcerative colitis, half had extensive pancolonic disease at enrollment, while the others had limited involvement in the left colon (E2, according to the Montreal classification) or proctitis. In contrast to patients with ulcerative colitis, 30 (34%) of Crohn's disease patients were active smokers at enrollment.

5.2 Clinical, biological, and endoscopic profile of the cohort at enrollment

5.2.1 Subgroup of ulcerative colitis patients

At enrollment, 53 (40%) patients were in clinical remission, while 77 (60%) had clinical disease activity defined using partial Mayo scores ≥2. Among those with symptoms, 39 (30%)

had mild activity, 22 (17%) had moderate activity, and the rest (6, 5%) had a severe disease flare. When evaluating clinical activity based on the anatomical segments' involvement, the partial Mayo score was higher in patients with left-sided colitis or pancolitis compared to those with limited disease (proctitis) (Figure 5.8).

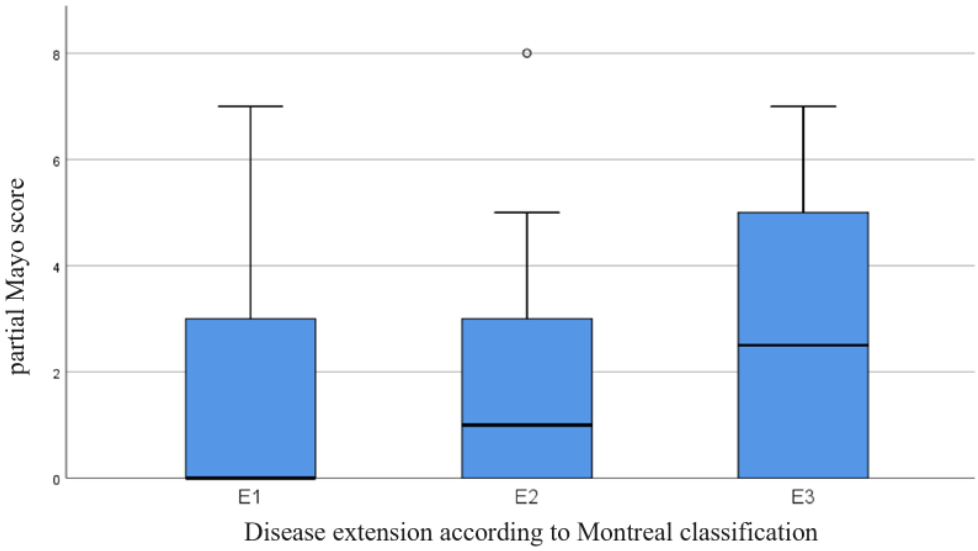

Figure 5.8 – Clinical activity expressed by the partial Mayo score according to disease extension

Regarding endoscopic activity, 23 (17%) patients had mucosal healing, 43 (32%) had a Mayo endoscopic score of 1, 29 (22%) had a Mayo endoscopic score of 2, and 27 (20%) had a Mayo endoscopic score of 3, indicating severe endoscopic lesions (Figure 5.9).

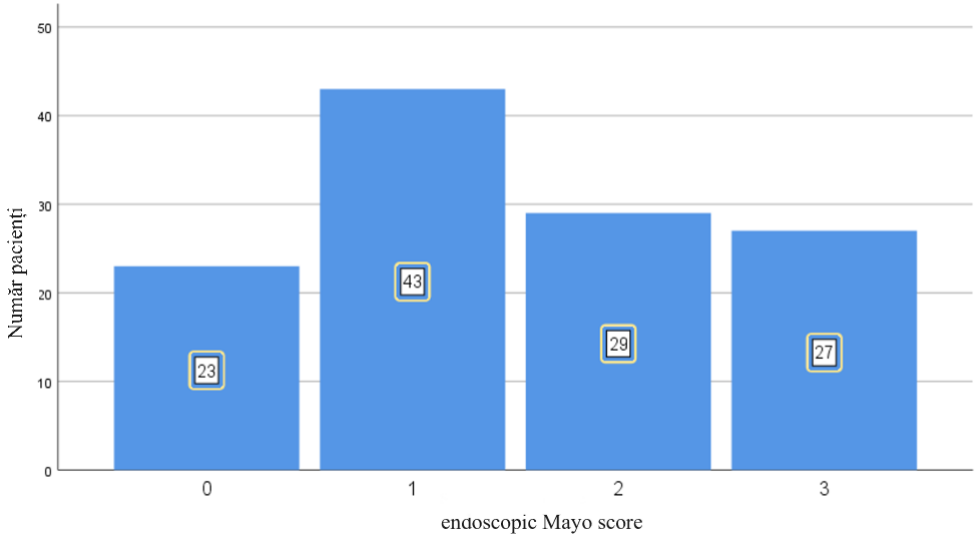

Figure 5.9 – Endoscopic activity at enrollment

Fecal calprotectin: For calprotectin determination, quantitative tests were performed. The average value at enrollment for ulcerative colitis patients was 252 µg/g (±154). I chose to briefly report the results of fecal calprotectin only for the ulcerative colitis patient subgroup for two reasons. First, results from the MAID cohort have already been published and detailed in my mentor, Dr. Theodor Alexandru Voiosu's doctoral thesis. The second reason relates to its utility, especially in ulcerative colitis patients, for whom I wanted to report it as revealed by the collected data for the followed patient cohort, highlighting the limitations of this method and the need to identify other non-invasive markers for disease activity assessment.

The average values of this fecal marker were significantly lower in those in clinical remission compared to those with clinically active disease (163 µg/g versus 230 µg/g, p=0.021) (Figure 5.13).

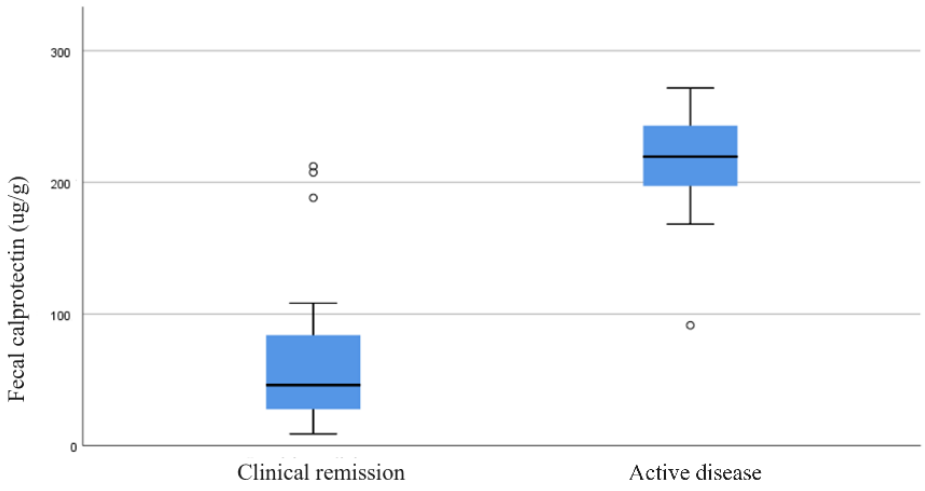

Figure 5.13 – Fecal calprotectin values based on clinical disease activity

This difference was even more pronounced when analyzing the average calprotectin values based on the endoscopic activity of the disease (209 µg/g in those with endoscopic lesions versus 30 µg/g in those with mucosal healing, p<0.01).

Calculating the diagnostic accuracy of fecal calprotectin in detecting clinical activity in IBD patients, we find an area under the curve of 0.8 (95% CI 0.62-0.99). At a threshold value of 250 µg/g, fecal calprotectin presents a sensitivity of 58% and a specificity of 98% in detecting endoscopic activity in this patient group. Comparing the obtained results with those reported in the literature, we observe concordance.

5.2.2 Subgroup of Crohn's disease patients

At enrollment, 44/88 (50%) presented clinical activity, while the rest were in clinical remission with a CDAI score <150. Among those with clinically active disease, 19/44 (43%)

had mild involvement (CDAI between 151-219), 18/44 (40%) had moderate involvement (CDAI between 220-450), and 7/44 (15%) had severe involvement (CDAI >450). Regarding endoscopic activity, at enrollment, 69/88 (78%) presented inflammatory activity on colonoscopy, defined by an SESCD score greater than 3.

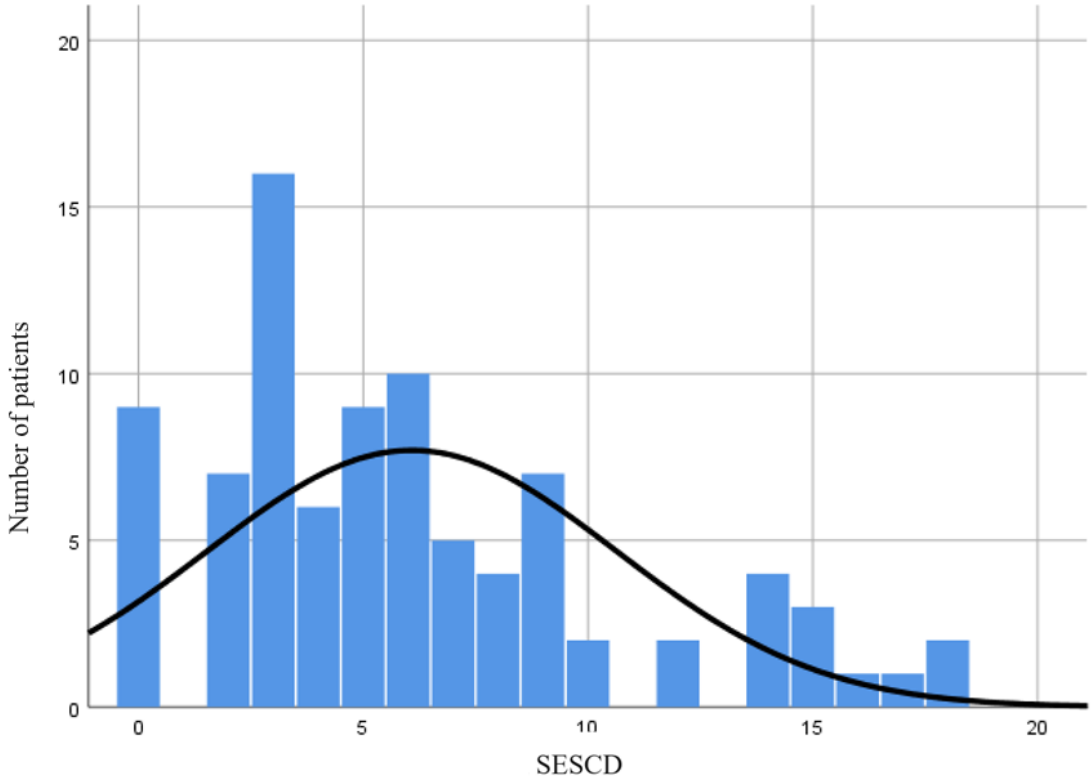

Figure 5.16 – Endoscopic activity at enrollment

Median PCR values were high in patients with clinical activity (median values of 9.3 versus 2.13 in those with clinical remission, p=0.02, Chi-square test) and present endoscopic lesions (8.14 versus 1.18 mg/dL, p=0.02, Chi-square test). Unlike ulcerative colitis, for Crohn's disease patients, PCR values more accurately reflect the presence of clinical and endoscopic disease activity.

5.3 Evolution of the Patient Cohort over Follow-up

For the entire cohort, the average follow-up duration was 24 months (minimum 12 months, maximum 60 months). Out of the total of 219 patients, 115 (52%) completed the 12-month follow-up visit. At study enrollment, 45% (97/219) of patients were in clinical remission, and at the 12-month follow-up visit, 64% (74/115) had achieved this therapeutic target. Among patients in remission at the initial visit, 8 experienced a disease relapse during the first 12 months of treatment.

Analyzing the results by disease, it is observed that for ulcerative colitis patients, both clinical remission rates (41% vs. 61%) and mucosal healing rates (21% versus 29%) increased after 12 months of monitoring.

Regarding the group of Crohn's disease patients, 44/88 (50%) were in clinical remission at enrollment, and this proportion increased at the 12-month assessment visit to 72%. In terms of endoscopic activity, we also observed an increase in mucosal healing rates, from 21% to 39%.

It is worth mentioning that therapeutic success after 12 months of follow-up is reflected in an approximately 20% increase in the percentage of patients in clinical remission, regardless of the type of disease, and an approximately 10% increase in the mucosal healing rate for ulcerative colitis patients and a 20% increase for Crohn's disease patients.

5.4 Quality of life during follow-up

At the time of enrollment, the quality of life of patients was suboptimal, with a median SIBDQ score of 4.9 (SIBDQ values <5.5 were used to define poor quality of life).

The presence of clinical activity of the disease and systemic inflammation (values above the normal limit of PCR) are associated with a lower quality of life for patients included in our analysis. The median SIBDQ score was significantly lower in those with current clinical activity compared to those in clinical remission (4.3 versus 5.7, $p<0.001$, Mann-Whitney u). The SIBDQ score was also significantly higher in patients with mucosal healing compared to those with active endoscopic disease (4.7 versus 5.7, $p<0.001$, Mann-Whitney u). Regarding the evolution of the quality of life of patients over time, a primary observation would be that the quality of life score increase over the follow-up period, without exceeding a median value of 56 points (Figure 5.26)

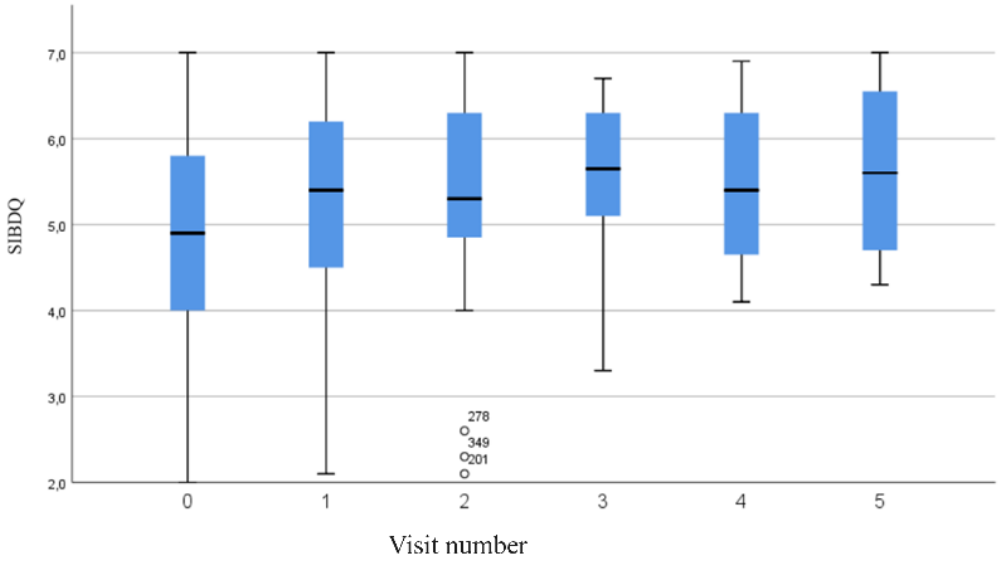

Figure 5.26 – Variation of the SIBDQ score over the follow-up period

From the enrollment visit, we observed a significant increase in the SIBDQ score, from 49 to 55 points, at the next monitoring visit ($p<0.005$, Mann-Whitney u). This increase is important because it implies a shift of median values towards scores indicating a good quality of life. The plateauing of values after this visit could be interpreted either by the reduced number of patients in the subsequent study visits or by the existence of an impairment of quality of life even after achieving usual therapeutic targets (clinical remission, mucosal healing). In the logistic regression analysis, after adjusting for corticosteroid therapy and biological therapy, age, PCR, and disease type, only female sex (OR 1.97 CI 95% 1.1-3.5), initial visit (OR 2.01 95% CI 1.13-3.58), and clinical activity (OR 5.81, 95% CI 3.1-10.88) were independent predictors for a quality of life score < 5.5.

6. Histopathological results for the subgroup of patients with ulcerative colitis

6.1 Introduction

Histological normalization is not one of the mandatory therapeutic targets set by the current guidelines for any type of inflammatory bowel disease. However, published data argue for its use to confirm profound disease control. The absence of endoscopic inflammatory activity does not exclude the persistence of microscopic inflammation, correlated in turn with disease relapse. Considering these aspects, we decided to analyze the subgroup of patients with ulcerative colitis included in the cohort. The main objective was to report the rate of histological

healing in the monitored cohort, as well as to identify clinical and endoscopic parameters with predictive potential for this therapeutic outcome.

6.2 Materials and methods

From the monitored cohort, patients with UC were selected, for whom at least one visit with endoscopic inflammatory activity followed by a visit with documented endoscopic healing was recorded. Histological healing was defined as a Geboes score ≤ 2.0 (absence of neutrophils in the epithelium). Histological response was defined as a Geboes GS score < 3.0[36]. The expression and distribution of CD4+ and CD8+ lymphocytes were analyzed by immunohistochemical tests for samples taken both at the visit with present endoscopic activity and at one of the visits with documented mucosal healing.

6.3 Results

Among the patients included in the study, we selected the 131 with a diagnosis of ulcerative colitis. They were followed for a median period of 2 years (0-5), with a total of 266 visits performed. At the time of enrollment, most patients presented endoscopic inflammatory activity (99.75%), and only half also had clinically active disease (77.58%). At the initial visit, the severity of endoscopic inflammatory activity assessed by the Mayo score was mild (Mayo score 1). Univariate analysis showed that long-term clinical remission (>12 months), SIBDQ ≥5.5, PCR≤5mg/dl, and absence of corticosteroid therapy were associated with mucosal healing.

Out of the total 70 visits where no endoscopic lesions were reported, histopathological results could be evaluated for 48 of them. At 18/48 (37%) visits where no endoscopic lesions were reported, histological healing was confirmed. Univariate analysis showed that SIBDQ score ≥5.5 and normal PCR values were associated with histological healing. Furthermore, we identified a subgroup of 20 UC patients for whom mucosal healing was recorded during the follow-up. Among the evaluated patients, 9 had a Geboes score between 0.3-1.3, indicating histological healing, and 8 had a score below 3.1.

6.4 Discussions

The results obtained for the monitored cohort demonstrate that rates of endoscopic and histological healing in current practice in Romania are low. Only 47/131 (35%) of patients had mucosal healing at some point during monitoring, and an even smaller proportion also reported histological normalization (18/131, 13%). Available data in the literature indicate a wide variability in histological healing rates, with microscopic lesions present in 16-100% of patients without endoscopic inflammatory activity.

6.5 Conclusions

The most important conclusion from this subanalysis is that the healing of endoscopic lesions and histological normalization is achieved only for a minority of UC patients, results that are suboptimal and discouraging. Simple tools, such as quality of life assessment questionnaires and PCR measurement, could prove useful in the non-invasive assessment of treatment targets, especially in association with other factors, by building predictive models.

7. The role of non-invasive biomarkers in evaluating the severe evolution of the disease

7.1 I Introduction

The integration of biomarkers into clinical practice improves the quality of patient care by enhancing long-term outcomes and facilitating the implementation of personalized treatment strategies. These indicators of pathophysiological processes or pharmacological response can be used in several key stages of IBD patient care: diagnosis, disease monitoring, prediction of treatment response, or early detection of relapses [15]. Among these, the non-invasive assessment of inflammatory activity in the digestive tract has been the subject of numerous studies, driven by the need to replace invasive procedures, such as colonoscopy [16,17,18,19].

A small number of biomarkers have proven their utility in assessing disease progression and have addressed the need to reduce the number of colonoscopies performed by patients during monitoring. Among these, C-reactive protein and fecal calprotectin are the only markers validated in large cohorts of patients, with a clear role in assessing disease activity. The most significant limitation in their use remains the lack of correlation with the degree of inflammatory activity for some patients, according to colonoscopic evaluation [20,21].

Thus, starting from the data and samples collected in the monitored cohort, we aimed to evaluate the usefulness of a panel of serum markers in assessing inflammatory activity in patients with ulcerative colitis and to determine their potential role in predicting disease progression and long-term outcomes. The investigated markers were: visfatin, serum amiloid A, lipocalin, matrix-metaloproteinases 1 și 2, TFF3 (trefoil factor 3), LRG (alfa 2 leucine rich glicoprotein), interleukines IL-4, IL-6, IL-7, IL-17. These serum markers were selected based on the results of a systematic review conducted by our team.

7.2 Materials and methods

Two groups of patients, each with different outcomes during follow-up, were selected from the patient cohort. Thus, we conducted an exploratory, retrospective case-control study

(Figure 7.1). The study was funded by the Ministry of Research and Innovation, following the winning of a research grant, developed by the Gastroenterology Department team, along with colleagues from the Pathology Department. The study is funded by CCCDI-UEFISCDI under Project Number PN-III-P1-1.1-TE-2021-0801/2022.

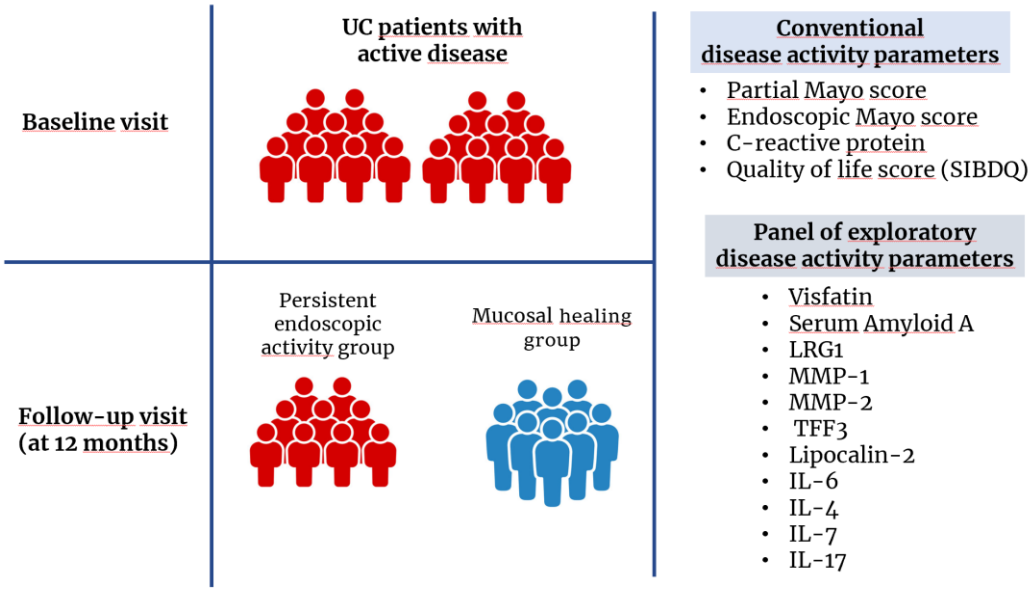

Figure 7.1 Study design

The serum concentration of selected serological parameters was determined at two separate visits for each group.

The primary objective of the study was to assess the predictive value of selected markers for persistent inflammatory activity (for a minimum of 12 months), defined by an endoscopic Mayo score greater than 0. Secondary objectives included determining the accuracy of these markers and investigating the utility of combinations of factors for detecting endoscopic inflammatory activity.

For the analysis of the selected patient subgroup, 84 serum samples from 42 patients with UC (21 with mucosal healing and another 21 with persistent endoscopic activity at the monitoring visit) were analyzed. Characteristics of the included patient cohort indicate that male gender, younger age, lower BMI, and higher endoscopic severity scores are more frequently encountered in the group of patients with persistent endoscopic activity compared to those with mucosal healing dynamics.

7.3.1 Assessment of the predictive value of selected markers

For lipocalin and interleukin 7, a significant increase was identified at the monitoring visit compared to initial values, in the case of patients with persistent endoscopic activity (Table 7.3).

Table 7.3 –Serum levels for evaluated markers in the group of patients with persistent endoscopic activity at baseline and follow-up

Parameter (measure unit)	Baseline visit (mean, SD)	Monitoring visit (mean, SD)	p
Visfatina (ng/mL),	105(34.2)	92.7(33.7)	0.5
Serum amyloid A(ng/mL)	357.1(59.5)	340.5(47.2)	0.67
LRG1(µg/mL)	81.7(17.1)	84.7(13.7)	0.67
MMP-1(ng/mL)	40.7(19.7)	44(19.2)	0.43
MMP-2(ng/mL)	48.8(150)	11.3(31.3)	0.68
TFF3 (ng/mL)	12.9(15.9)	2.35(6.68)	0.59
Lipocalin-2 (NGAL) (µg/mL)	5.9(21.6)	11.5(29.6)	**0.05**
IL-6(pg/mL)	9.9(20.6)	106(326)	0.98
IL-7(pg/mL)	6.8(15.5)	23.5(82.85)	**0.05**

The serum level of lipocalin at the initial visit showed the best performance in predicting endoscopic activity at the monitoring visit (12 months of follow-up), with a sensitivity of 90% and specificity of 95% for a threshold value of 0.421 µg/mL (AUROC 0.677). The next marker in terms of performance was interleukin 7, with a sensitivity of 95% and specificity of 95% for a threshold value of 22.6 pg/mL (AUROC 0.615).

Taking into account the performance of these two markers and the differences observed between the evaluated patient groups, predictive models were constructed, including usual clinical and biological characteristics evaluated in patients with ulcerative colitis. The combination of serum lipocalin, age, and BMI has good accuracy in predicting persistent activity at 12 months of follow-up (AUROC 0.87, 95% CI 0.762-0.979) (Figure 7.3).

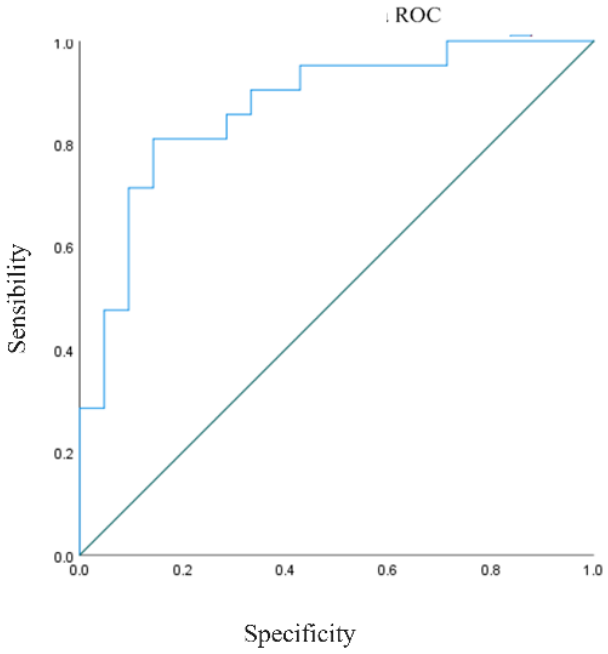

Figure 7.3 – Performance of the combination of lipocalin, age and BMI for predicting persistent endoscopic activity

7.3.2 Role of investigated markers in assessing endoscopic activity

Comparative analysis of serum levels of these parameters between the two patient groups did not show significant differences for any of the investigated markers, including CRP or quality of life scores. Similarly, in patients with mucosal healing at the monitoring visit, no significant differences were observed for the two visits.

However, in the separate analysis of the group with mucosal healing, the SIBDQ score was the only factor with a statistically significant difference at the monitoring visit (median value 5.3 versus 6.2, $p < 0.05$, Wilcoxon test).

An interesting result of our analysis is that treatment with biologics or previous exposure to this therapeutic class was not an indicator for the results obtained at the monitoring visit. At the time of data collection, the only biologics available for patients with IBD were infliximab and adalimumab, usually prescribed as part of a step-up strategy. Although the results of our analysis should be interpreted with caution, considering the limited number of patients and collected samples, lipocalin and interleukin 7 show promising results in identifying patients with future persistence of endoscopic activity. Validation of the results obtained by our team could support efforts to standardize treatment strategies in IBD and identify patients with a more aggressive disease progression.

8. Efficacy, safety and treatment persistence for advanced therapies

8.1 Introduction

To assess the impact of advanced therapies on the course of inflammatory bowel diseases, I analyzed the outcomes for patients included the MAID cohort. I retrospectively collected data for patients who received advanced therapues (biologics or small molecules) within a 24-month period (December 2019-December 2021). I chose this timeframe to include all currently used molecules, allowing for similar follow-up times. The data obtained from this analysis were collected in a multicenter, national study involving 23 other inflammatory bowel disease centers, and the results were subsequently published [22].

8.2 Materials and methods

From the previously created database, I selected relevant information that could impact the chosen treatment and disease progression, including:

- General patient data: sex, age, smoking status;
- Disease details: extension, Crohn's disease phenotype, duration, surgical history;
- Previous treatment details: failure on other treatment lines (AZA, ADA, IFX, TOFA, USTE, VEDO), duration of previous treatments, and reasons for discontinuation (primary non-response, loss of response, adverse reactions, deep remission, or other reasons).

I selected and constructed the database for this analysis, focusing on three categories of events:

1. Initiation of an advanced treatment (IFX/ADA/VEDO/USTE/TOFA) during the designated period, its duration, and the need for concurrent corticosteroid administration;
2. Recording adverse reactions (opportunistic infections, severe infections, IBD-related hospitalizations, IBD worsening, or appearance/worsening of extraintestinal manifestations, cancer, surgical interventions, allergic reactions) and the subsequent course of action (discontinuation/continuation of treatment);
3. Treatment outcomes: clinical remission, mucosal healing, and treatment persistence.

8.3 Results

In this analysis, 93 patients from the MAID cohort were included, initiating an advanced treatment with one of the five available options (ADA, IFX, TOFA, USTE, VEDO) between December 2019 and December 2021.

8.3.1. Therapy history

For the studied patients, we investigated the history of previous medication exposure, including exposure to azathioprine, biologics, or tofacitinib. Previous therapy for this analysis was considered any advanced medication received and discontinued before the database creation and current analysis (before April 2022). The results can be reviewed in Figure 8.1. Out of the total patients, 21/93 (22%) received azathioprine before the investigated period, either as monotherapy or in combination with infliximab.

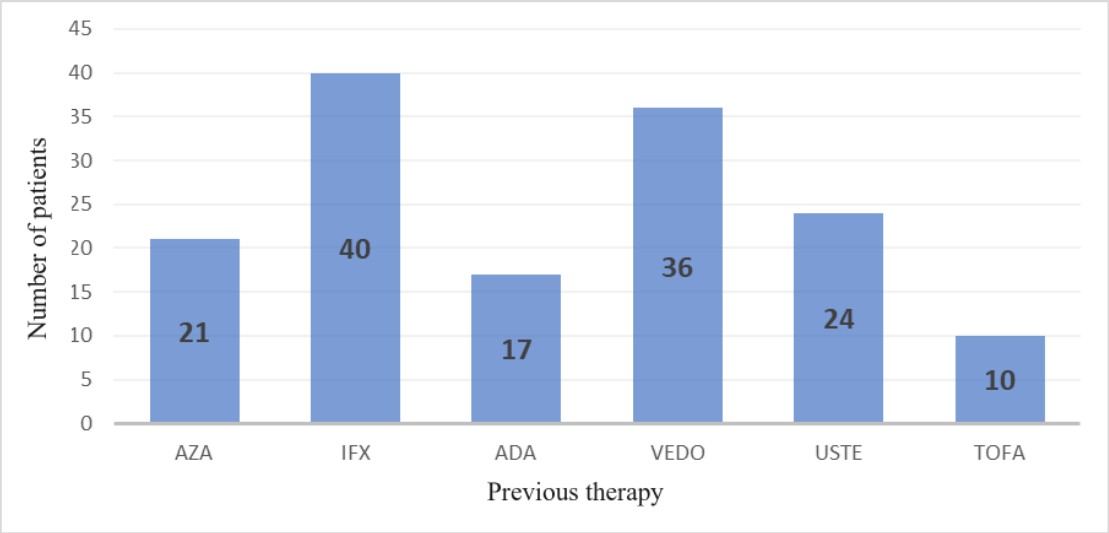

Figure 8.1 Treatment history

Regarding previous exposure to advanced therapies, only 26/93 (27%) were treatment-naive, with the rest having been treated with one or more medications before (Figure 8.2). Most failed at one (28/93, 30%) or more treatment lines, with 4 patients previously treated with 4 other medications (from those investigated: IFX/ADA/USTE/TOFA/VEDO).

The main reason for treatment discontinuation was the loss of response, followed by initial non-response to medication or adverse events. During the observed period, VEDO had the highest prescription frequency (27/93, 29%), followed by USTE, IFX, ADA, and TOFA (Figure 8.3).

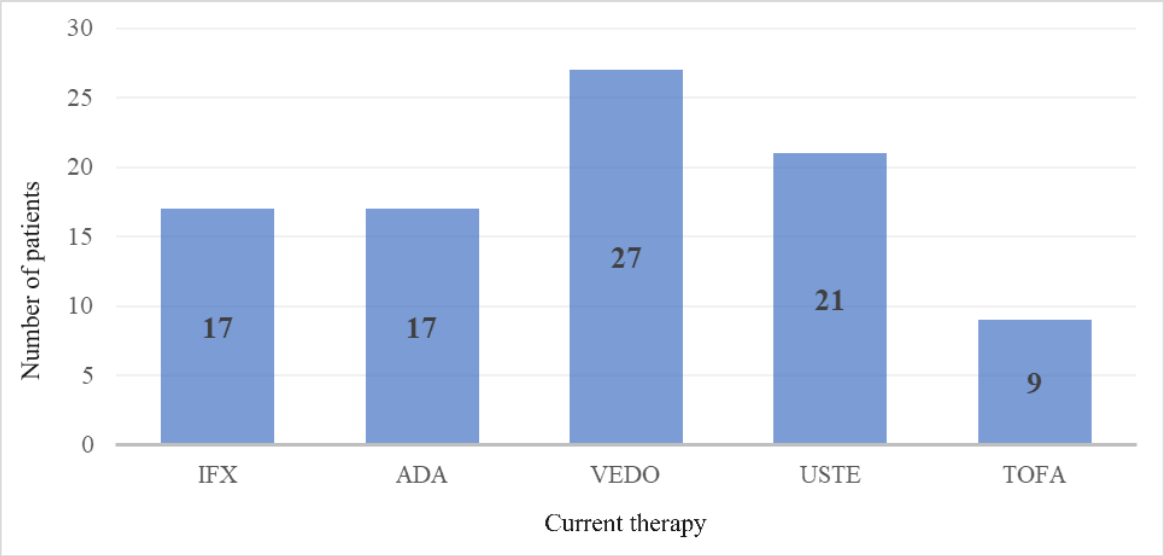

Figure 8.3 – Frequency of advanced treatment initiations

For biologic-naive patients (26/93), in all cases, initiation of an anti-TNF agent was chosen, with similar proportions for UC and CD.

For current treatments, the median follow-up period was 9 months (longest for adalimumab, 14 months, followed by infliximab, tofacitinib, and ustekinumab. Vedolizumab had the shortest follow-up period (6 months). In multivariable analysis, need for corticosteroids and Crohn's disease diagnosis were predictors for treatment persistence at 6 and 12 months of follow-up.

To evaluate the results obtained with current treatments, the achievement of the most important therapeutic targets recommended by current guidelines (clinical remission and mucosal healing) was monitored (Figures 8.6 and 8.7).

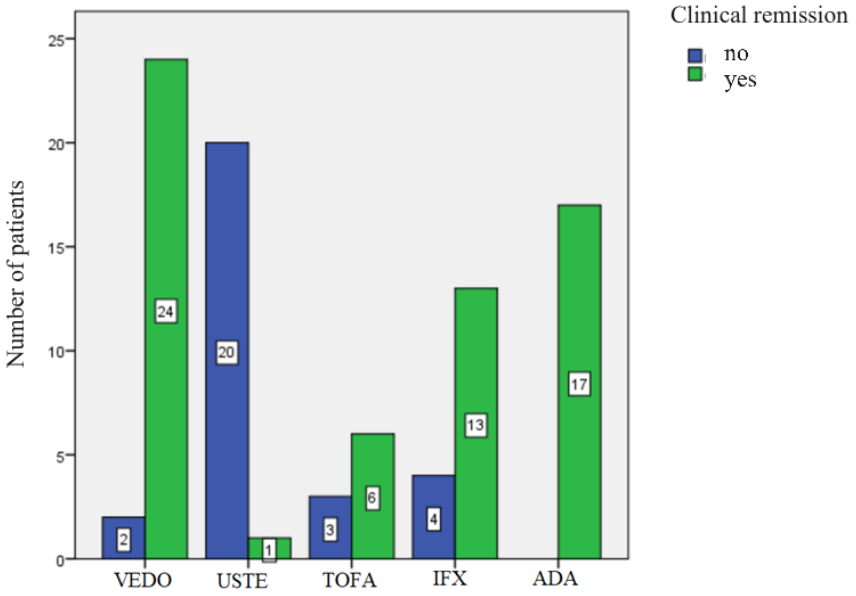

Figure 8.6 – Patients in clinical remission under current treatments

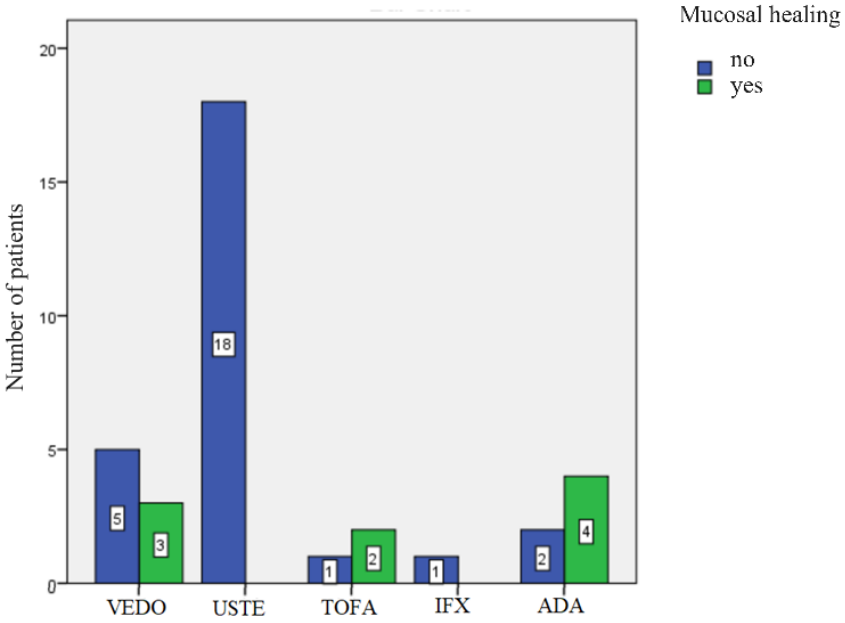

Figure 8.7– Patients with mucosal healing under current treatments

At the time of evaluating results under current treatments, 65% (61 patients) achieved clinical remission, and in 10% (9 patients), mucosal healing was observed. The study's design and the small number of patients did not allow for a comparative analysis of the effectiveness of the investigated molecules.

8.3.3 Data regarding treatment persistence

For a more accurate evaluation of treatment persistence, data collection should be prospective and over an extended duration (>36 months). The available data in this evaluation are subject to selection bias, as included patients are considered "difficult" cases, having failed multiple treatment lines and currently treated with one of the advanced therapies. Out of all included patients, most were treated with an anti-TNF agent (40/93 with IFX, 17/93 with ADA). Among the 57 patients treated with anti-TNF agents, 13 received both available molecules throughout their evolution (IFX and ADA).

The median treatment period with IFX for patients in the MAID cohort is 18 months, with 15/40 (37%) discontinuing treatment by 12 months. The median treatment period with ADA for patients in the MAID cohort is 7 months, with 14/17 (82%) discontinuing treatment by 12 months. For patients with VEDO failure, the median treatment period was 7 months, with 25/36 (69%) discontinuing treatment by 12 months. The median treatment period with USTE was 7.5 months, with 22/24 (91%) discontinuing treatment by 12 months (Figure 8.11), and for TOFA, the median treatment period is 9 months, with 9/10 (90%) discontinuing treatment by 12 months.

In a retrospective analysis of the data, five adverse events were identified (5.4%), including one case of opportunistic infection in a patient treated with IFX and four cases of worsening extraintestinal manifestations leading to medication discontinuation.

Data from the MAID cohort provide an initial insight into how these treatments are used in clinical practice but also present numerous limitations. Firstly, the patients discussed are considered "difficult to treat" with an unfavorable prognosis, having already failed multiple treatment lines. Secondly, a direct consequence of the selected patient profile in this analysis is that treatment discontinuation rates and outcomes are likely much more optimistic in heterogeneous patient populations. The data from this analysis show that among patients who started a new treatment within the 24-month follow-up, only 29-52% reached 12 months of administration. For anti-TNF agents (for which there are published literature data), the results are similar to those calculated [23].

The most relevant conclusion arising from the conducted analysis is that the population of patients with IBD exposed to biologics is increasing, making disease control increasingly challenging.

9. Conclusions and personal contributions

Throughout the doctoral study, I conducted a prospective cohort study that included 219 patients with IBD. The main conclusions of my research activitity are as follows:

1. Clinical and endoscopic remission rates are significantly higher for both Crohn's disease and ulcerative colitis patients at 12 months of treatment. However, overall disease control is suboptimal;
2. The SIBDQ has a sensitivity of 54% and specificity of 86% (AUROC 0.78, 95% CI 0.73-0.83) for assessing the presence of endoscopic activity. On the other hand, diagnostic accuracy for mucosal healing is slightly lower, with a sensitivity of 57% and specificity of 72% (AUROC 0.65, 95% CI 0.58-0.72);
3. Endoscopic and histological healing rates in current practice in Romania are low. Histological normalization was observed for a small proportion of patients during the follow-up (18/131, 13%);
4. Among the investigated serological factors (visfatin, serum amyloid A, lipocalin, matrix metalloproteinases 1 and 2, TFF3 (trefoil factor 3), LRG (leucine-rich alpha-2 glycoprotein), IL-4, IL-6, IL-7, IL-17), only lipocalin and IL-7 were identified with potential predictive roles for persistent inflammatory activity;
5. The serum level of lipocalin at the initial visit performed best in predicting endoscopic activity at the monitoring visit (12 months of follow-up), with a sensitivity of 90% and specificity of 95% for a threshold value of 0.421 μg/mL (AUROC 0.677);
6. Male gender, younger age, lower BMI, and higher endoscopic severity scores are associated with persistent endoscopic activity;
7. The combination of serum lipocalin, age, and BMI has good accuracy in predicting persistent activity at 12 months of follow-up (AUROC 0.87, 95% CI 0.762-0.979);
8. A significant proportion of monitored patients were exposed to multiple classes of advanced therapies, indicating the presence of challenging-to-control diseases;
9. Treatment discontinuation rates are high, with failure reported especially in the first 12 months of treatment.

Therefore, through the implementation of this study, I have extensively reported the results obtained for a cohort of patients with IBD in Romania. There have been few prospective observational studies conducted in our country so far. We identified multiple parameters with variable importance in the long-term prognosis assessment in ulcerative colitis and Crohn's

disease. Our results emphasize the importance of careful patient monitoring, utilizing clinical and laboratory tools, as well as evaluating histopathological biopsies taken from these patients.

Long-term prospective multicenter studies are necessary to precisely establish the role of clinical, biological, or tissue factors in the evolution of patients and to optimize current treatment strategies.

References

[1] Song EM, Yang SK. Natural history of inflammatory bowel disease: a comparison between the East and the West. Intest Res. 2022 Oct;20(4):418-430

[2] Burisch J, Jess T, Martinato M, Lakatos PL. The burden of inflammatory bowel disease in Europe. J Crohns Colitis. 2013;7(4):322–37

[3] Benchimol EI, Fortinsky KJ, Gozdyra P, Van Den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflamm Bowel Dis. 2011;17(1):423–39

[4] Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140(6):1785–94

[5] Schoepfer AM, Dehlavi MA, Fournier N, Safroneeva E, Straumann A, Pittet V, Peyrin-Biroulet L, Michetti P, Rogler G, Vavricka SR; IBD Cohort Study Group. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. Am J Gastroenterol. 2013 Nov;108(11):1744-53; quiz 1754

[6] Khanna R, Bressler B, Levesque BG, Zou G, Stitt LW, Greenberg GR, Panaccione R, Bitton A, Paré P, Vermeire S, D'Haens G, MacIntosh D, Sandborn WJ, Donner A, Vandervoort MK, Morris JC, Feagan BG; REACT Study Investigators. Early combined immunosuppression for the management of Crohn's disease (REACT): a cluster randomised controlled trial. Lancet. 2015 Nov 7;386(10006):1825-34

[7] Colombel JF, Panaccione R, Bossuyt P, Lukas M, Baert F, Vaňásek T, Danalioglu A, Novacek G, Armuzzi A, Hébuterne X, Travis S, Danese S, Reinisch W, Sandborn WJ, Rutgeerts P, Hommes D, Schreiber S, Neimark E, Huang B, Zhou Q, Mendez P, Petersson J, Wallace K, Robinson AM, Thakkar RB, D'Haens G. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. Lancet. 2017 Dec 23;390(10114):2779-2789

[8] Turner D, Ricciuto A, Lewis A, D'Amico F, Dhaliwal J, Griffiths AM, Bettenworth D, Sandborn WJ, Sands BE, Reinisch W, Schölmerich J, Bemelman W, Danese S, Mary JY, Rubin D, Colombel JF, Peyrin-Biroulet L, Dotan I, Abreu MT, Dignass A; International Organization for the Study of IBD. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. Gastroenterology. 2021 Apr;160(5):1570-1583

[9] Jen MY, Shahrokhi M, Varacallo M. Predictive Medicine. 2022 Oct 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 28722970.

[10] Wanderås MH, Moum BA, Høivik ML, Hovde Ø. Predictive factors for a severe clinical course in ulcerative colitis: Results from population-based studies. World J Gastrointest Pharmacol Ther. 2016 May 6;7(2):235-41. doi: 10.4292/wjgpt.v7.i2.235

[11] Ryan JD, Silverberg MS, Xu W, Graff LA, Targownik LE, Walker JR, Carr R, Clara I, Miller N, Rogala L, Bernstein CN. Predicting complicated Crohn's disease and surgery: phenotypes, genetics, serology and psychological characteristics of a population-based cohort. Aliment Pharmacol Ther. 2013 Aug;38(3):274-83. doi: 10.1111/apt.12368

[12] Cosnes J. Crohn's disease phenotype, prognosis, and long-term complications: what to expect? Acta Gastroenterol Belg. 2008 Jul-Sep;71(3):303-7.

[13] Annese V, Annese M. Precision Medicine in Inflammatory Bowel Disease. Diagnostics (Basel). 2023 Aug 29;13(17):2797. doi: 10.3390/diagnostics13172797.

[14] State M, Negreanu L, Voiosu T, Voiosu A, Balanescu P, Mateescu RB. Surrogate markers of mucosal healing in inflammatory bowel disease: A systematic review. *World J Gastroenterol*. 2021;27(16):1828-1840. doi:10.3748/wjg.v27.i16.1828

[15] Elhag DA, Kumar M, Saadaoui M, Akobeng AK, Al-Mudahka F, Elawad M, Al Khodor S. Inflammatory Bowel Disease Treatments and Predictive Biomarkers of Therapeutic Response. Int J Mol Sci. 2022 Jun 23;23(13):6966. doi: 10.3390/ijms23136966

[16] Sandborn WJ, Abreu MT, Dubinsky MC. A Noninvasive Method to Assess Mucosal Healing in Patients* With Crohn's Disease. Gastroenterol Hepatol (N Y). 2018 May;14(5 Suppl 2):1-12.

[17]Naganuma M, Hosoe N, Kanai T, Ogata H. Recent trends in diagnostic techniques for inflammatory bowel disease. Korean J Intern Med. 2015 May;30(3):271-8. doi: 10.3904/kjim.2015.30.3.271

[18] Kato J, Hiraoka S, Nakarai A, Ichinose M. Noninvasive evaluation of mucosal healing in inflammatory bowel diseases. Clin J Gastroenterol. 2013 Feb;6(1):1-7. doi: 10.1007/s12328-012-0346-x

[19] Bromke MA, Neubauer K, Kempiński R, Krzystek-Korpacka M. Faecal Calprotectin in Assessment of Mucosal Healing in Adults with Inflammatory Bowel Disease: A Meta-Analysis. J Clin Med. 2021 May 19;10(10):2203. doi: 10.3390/jcm10102203

[20] Chen P, Zhou G, Lin J, Li L, Zeng Z, Chen M, Zhang S. Serum Biomarkers for Inflammatory Bowel Disease. Front Med (Lausanne). 2020 Apr 22;7:123. doi: 10.3389/fmed.2020.00123.

[21] Benítez JM, García-Sánchez V. Faecal calprotectin: Management in inflammatory bowel disease. World J Gastrointest Pathophysiol. 2015 Nov 15;6(4):203-9. doi: 10.4291/wjgp.v6.i4.203

[22] Mateescu RB, Gheorghe C, Trifan AV, Saftoiu A, Seicean A, Diculescu MM, Banciu C, Gheorghe LS, Busuioc B, Goldis A, Dobru D, Fratila O, Eugen D, Bataga S, Constantinescu G, Gheonea D, Tantau A, Jinga M, Brisc C, Cijevschi Prelipcean C, Chira R, Fierbințeanu-Braticevici C, Dumitrascu D, State M, Voiosu T, Negreanu L. Safety, Efficacy and Persistence of Advanced Therapies in Inflammatory Bowel Disease: Results from ORIGINS. A Retrospective Observational Study. J Gastrointestin Liver Dis. 2023 Dec 22;32(4):444-451. doi: 10.15403/jgld-5128.

[23] Lynn Huynh, MPH, MBA, DrPH, Steve Hass, PhD, Laurent Peyrin-Biroulet, MD, Mei Sheng Duh, RPh, MPH, ScD, Heather Sipsma, PhD, Mu Cheng, MPH, Angie Lax, MPH, Arpita Nag, PhD, MBA, MS, Real-World Treatment Patterns and Physician Preferences for Biologics in Moderate-to-Severe Inflammatory Bowel Disease: Retrospective Chart Review in Europe, *Crohn's & Colitis 360*. 2022 Jan; 4(1)

Published papers

1. Mateescu RB, Gheorghe C, Trifan AV, Saftoiu A, Seicean A, Diculescu MM, Banciu C, Gheorghe LS, Busuioc B, Goldis A, Dobru D, Fratila O, Eugen D, Bataga S, Constantinescu G, Gheonea D, Tantau A, Jinga M, Brisc C, Cijevschi Prelipcean C, Chira R, Fierbințeanu-Braticevici C, Dumitrascu D, **State M**, Voiosu T, Negreanu L. Safety, Efficacy and Persistence of Advanced Therapies in Inflammatory Bowel Disease: Results from ORIGINS. A Retrospective Observational Study. J Gastrointestin Liver Dis. **2023** Dec 22;32(4):444-451. doi: 10.15403/jgld-5128 (Chapter 8, pag 84-99).

2. **State M**, Balanescu P, Voiosu T, Bengus A, Voiosu A, Coman A, Mustatea P, Negreanu L, Mateescu RB, Popp C. Real-World Endoscopic and Histologic Outcomes in Ulcerative Colitis Patients: A Retrospective Cohort Study. *Biomedicines* **2023**, *11*, 1860. doi.org/10.3390/biomedicines11071860 (Chapter 6, pag 61-69)

3. **State M**, Negreanu L, Voiosu T, Voiosu A, Balanescu P, Mateescu RB. Surrogate markers of mucosal healing in inflammatory bowel disease: A systematic review. *World J Gastroenterol*. 2021;27(16):1828-1840. doi:10.3748/wjg.v27.i16.1828 (Chapter 7, pag 70).

4. Tocia C, Dumitru A, Mateescu B, Negreanu L, **State M**, Cozaru GC, Mitroi AF, Brinzan C, Popescu R, Leopa N, Iordache MM, Manea M, Matei E, Dumitru E, Alexandrescu L. Tissue and Circulating MicroRNA-31, MicroRNA-200b, and MicroRNA-200c Reflects Disease

Activity in Crohn's Disease Patients: Results from the BIOMIR Study. J Gastrointestin Liver Dis. 2023 Mar 31;32(1):30-38. doi: 10.15403/jgld-4656. PMID: 37004230 (Chapter 2, pag 20)

5. **State M**, Negreanu L. Defining the Failure of Medical Therapy for Inflammatory Bowel Disease in the Era of Advanced Therapies: A Systematic Review. Biomedicines. 2023 Feb 13;11(2):544. doi: 10.3390/biomedicines11020544. PMID: 36831079; PMCID: PMC9953124 (C Chapter 2, pag 23)

6. Diaconu C, **State M**, Birligea M, Ifrim M, Bajdechi G, Georgescu T, Mateescu B, Voiosu T. The Role of Artificial Intelligence in Monitoring Inflammatory Bowel Disease-The Future Is Now. Diagnostics (Basel). 2023 Feb 15;13(4):735. doi: 10.3390/diagnostics13040735. PMID: 36832222; PMCID: PMC9954871 (Chapter 2, pag 24)

7. Goran L, **State M**, Negreanu A, Negreanu L. Quality of Care in Inflammatory Bowel Disease: the Role of Steroid Assessment Tool (SAT) - a Review. Medicina Moderna - Modern Medicine. 2020;27. 171-176. 10.31689/rmm.2020.27.3.171 (Chapter 5, pag 57).

8. Goran L. **State M**, Negreanu AM, Negreanu L. Pursuing therapeutic success in Crohn's disease: A matter of definition, tools and longterm outcomes. European Journal of Inflammation Volume 18: 1–10, 2020, doi.org/10.1177/2058739220962896 (Chapter 1, pag 6)

9. Negreanu L, Voiosu T, State M, Mateescu RB. Quality of colonoscopy preparation in patients with inflammatory bowel disease: retrospective analysis of 348 colonoscopies. *J Int Med Res*. 2020;48(4):300060520903654. doi:10.1177/0300060520903654 (Chapter 4, pag 34)

10. Negreanu L, Voiosu T, State M, Voiosu A, Bengus A, Mateescu BR. Endoscopy in inflammatory bowel disease: from guidelines to real life. Therap Adv Gastroenterol. 2019 Jul 24;12:1756284819865153. doi: 10.1177/1756284819865153. PMID: 31384307; PMCID: PMC6657117 (Chapter 2, pag 22)