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TESI DI LAUREA

Comparative and safety of Ustekinumab in Crohn's disease and Ulcerative colitis: retrospective study of real-life

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To the young women in Kabul
who can’t go to University anymore
To their dreams, silenced
May they find a voice, one day

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Abstract

Background & aims: Ustekinumab is an effective biological treatment for patients with Crohn's disease (CD) and Ulcerative Colitis (UC). Real-world effectiveness and safety studies are warranted especially in the bio-experienced inflammatory bowel disease (IBD) population and in patients with UC vs CD, where the evidence is still limited. The aim of our study was to assess clinical, biochemical and endoscopic remission as well as the safety profile of Ustekinumab after 3 months, 6 months and 1 year.

Methods: all consecutive moderate-to-severe IBD patients who started Ustekinumab from 01/01/2019 to 12/04/2023 were included. We collected demographic and clinical data, including disease location and behavior, previous treatments, previous surgery, concomitant medications, smoke habit. In addition, data on partial Mayo (p-Mayo) Score, endoscopic Mayo (e-Mayo) Score, Harvey-Bradshaw Index (HBI), SES-CD Score, Rutgeerts Score, fecal calprotectin were collected. Serious infections and adverse events were defined as those requiring hospitalization or treatment discontinuation. Continuous and categorical variables were expressed as mean with standard deviation (SD) and frequency with percentages respectively. Comparisons among variables were conducted using one-way ANOVA and Chi-square. All data analyses were performed with SPSS Version 26.0 statistic software package.

Results: overall, 131 IBD (84 with CD and 47 with UC) adult patients were enrolled. Among the patients included, all CD patients and 89.36% (42/47) UC patients were bio-experienced; most of them had previous experience with Infliximab (80,95% CD patients; 72,34% UC patients). Ten CD (11,90%) and 19 UC (40,42%) patients were taking immunosuppressants at baseline; 53 CD patients (63,10%) and 3 UC patients (3,75%) had undergone bowel resective surgery.
After 1 year, 27% of CD patients and 38% of UC patients obtained a clinical response (p=0,423), while 64% of CD and 22% of UC patients obtained clinical remission; after 12 months 100% of CD patients and 50% of UC were in steroid-free clinical remission (p=0,02). In addition, 51,6% of CD patients and 20% of UC patients reached biochemical remission (p=0,028), while 27,87% of CD patients

and 20% of UC patients obtained an endoscopic remission (p=0,583). 44% of CD patients and 7% of UC patients found endoscopic response.
A total of 15 adverse events occurred during follow-up in both groups. The majority of them were related to infection (9/15) and 4 were classified as serious adverse events. Serious adverse reactions occurred only in CD patients.

Conclusions: Ustekinumab was an effective therapy in both patients with CD and UC. However, CD patients had better clinical and endoscopic outcomes than UC subjects, but biochemical remission did not differ between the two groups. Ustekinumab was safe and well-tolerated in the short and in the long term, with only 4 severe adverse reactions out of 131 patients in both groups. Infections were the most frequent adverse events encountered.

Riassunto

Introduzione e scopo dello studio: Ustekinumab è una terapia biologica efficace per i pazienti affetti da morbo di Crohn (MC) e colite ulcerosa (CU). Sono necessari studi sull'efficacia e sulla sicurezza nel mondo reale, soprattutto in un campione di pazienti affetti da malattia infiammatoria intestinale (IBD) già precedentemente esposto a terapie biologiche. Risulta inoltre importante eseguire un confronto tra pazienti con CU vs MC, dove le evidenze sono ancora scarse. Lo scopo del nostro studio è stato quello di valutare la remissione clinica, biochimica ed endoscopica e il profilo di sicurezza di Ustekinumab a distanza di 3 mesi, 6 mesi e 1 anno.

Metodi: sono stati inclusi tutti i pazienti affetti da IBD di grado moderato-severo che hanno iniziato la terapia con Ustekinumab dal 01/01/2019 al 12/04/2023. Abbiamo raccolto dati demografici e clinici, tra cui localizzazione e comportamento della malattia, trattamenti precedenti, interventi chirurgici precedenti, farmaci concomitanti, tabagismo. Inoltre, sono stati raccolti i dati relativi al Mayo Score parziale (p-Mayo), al Mayo Score endoscopico (e-Mayo), all'indice di Harvey-Bradshaw (HBI), allo score di SES-CD, allo score di Rutgeerts, alla calprotectina fecale. Le infezioni gravi e gli eventi avversi sono stati identificati in quei casi che hanno richiesto l'ospedalizzazione o l'interruzione del trattamento. Le variabili continue e categoriche sono state espresse rispettivamente come media con deviazione standard (SD) e frequenza con percentuali. I confronti tra le variabili sono stati effettuati utilizzando le tecniche statistiche ANOVA e il Chi-quadrato. Tutte le analisi dei dati sono state eseguite con il pacchetto software statistico SPSS versione 26.0.

Risultati: complessivamente sono stati arruolati 131 pazienti adulti affetti da IBD (84 con MC e 47 con CU). Tra i pazienti inclusi, il 100% dei pazienti MC e l'89,36% (42/47) con CU aveva già fatto precedente uso di farmaci biologici; la maggior parte di loro aveva già assunto Infliximab (80,95% pazienti con MC; 72,34% pazienti con CU). Dieci pazienti MC (11,90%) e 19 CU (40,42%) assumevano immunosoppressori allo stadio basale; 53 pazienti MC (63,10%) e 3 pazienti CU

(3,75%) erano stati precedentemente sottoposti a un intervento chirurgico di resezione intestinale.

Dopo 1 anno, il 27% dei pazienti con MC e il 38% dei pazienti con CU ha ottenuto una risposta clinica (p=0,423), mentre il 64% dei pazienti con MC e il 22% dei pazienti con CU ha ottenuto una remissione clinica; dopo 12 mesi il 100% dei pazienti con MC e il 50% dei pazienti con CU era in remissione clinica senza l'utilizzo di steroidi (p=0,02). Inoltre, il 51,6% dei pazienti con MC e il 20% dei pazienti con CU ha raggiunto una remissione biochimica (p=0,028), mentre il 27,87% dei pazienti con MC e il 20% dei pazienti con CU ha ottenuto una remissione endoscopica (p=0,583). Il 44% dei pazienti con MC e il 7% dei pazienti con CU ha ottenuto una risposta endoscopica.

Durante il follow-up si sono verificati in entrambi i gruppi un totale di 15 eventi avversi (11,45%), di cui la maggior parte infettivi (9/15) e 4 classificati come eventi avversi gravi (3,05%). Le reazioni avverse gravi si sono verificate solo nei pazienti con MC e hanno richiesto l'interruzione del farmaco.

Conclusioni: Ustekinumab è risultata una terapia efficace sia nei pazienti con MC che con CU. Tuttavia, i pazienti con MC hanno avuto risultati clinici ed endoscopici migliori. La remissione biochimica tra MC e CU è statisticamente non significativa. Ustekinumab si dimostra un farmaco sicuro e ben tollerato in entrambe le patologie, con sole 15 reazioni avverse, di cui 4 gravi. Le infezioni sono state gli eventi avversi più frequenti e meno severi.

Introduction

Inflammatory bowel disease

1.1 Definition

Inflammatory bowel disease (IBD) is a chronic idiopathic inflammatory disease affecting the gastrointestinal tract. The two most common forms of IBD are Crohn's disease (CD) and Ulcerative Colitis (UC); they share some common features and differ in other aspects that allow them to be distinguished.

Some common characteristics include familial tendency, chronic and intermittent course, frequent association with extraintestinal manifestations, and good response to steroids.

On the contrary, these two pathologies differ in other features, such as location and extension, macroscopic and microscopic appearance, and symptoms (Table I) [1].

CHARACTERISTICS OF CROHN'S DISEASE AND ULCERATIVE COLITIS

Characteristics	Crohn's disease	Ulcerative colitis
Location	Any area of gastrointestinal tract	Continuous lesions starting in rectum Generally occurs only in the colon
Thickness	Transmural involvement	Mucosa and submucosa only
Colonscopy findings	Skip lesions, cobble-stoning, ulcerations, strictures	Pseudopolyps, continuous areas of inflammations
Anemia	+	++
Abdominal pain	++	+
Rectal bleeding	+	++
Colon cancer risk	++	++++

Table I. Comparison of Crohn's disease and Ulcerative colitis' main features [1]

Crohn’s disease

2.2 Definition

Crohn's disease (CD) is a chronic inflammatory bowel disease in which lesions can affect any segment of the digestive tract, from the mouth to the anus.

This disease was first described by Burril Crohn and his colleagues as one characteristic finding of terminal ileitis in their initial publication in 1932, called “creeping fat” [2], but only subsequently, in 1960, Henry Lockhart-Mummery and Basil Morson first discussed the possible involvement of the colon.

Nowadays, it is well-known that this disease affects the whole gastrointestinal tract, involving more frequently the distal ileum and colon, with a segmented distribution of injured areas interspersed with lesion-free areas.

The inflammation in Crohn’s disease involves all layers of the intestinal wall, unlike in Ulcerative Colitis. The lesions can be mainly fibrosthenous, penetrating, or inflammatory. These three lesion characteristics may also coexist in the same patient or evolve into each other during the natural history of the disease.

Crohn's disease has a chronic-recurrent course, with intestinal symptoms and, in some cases, extra-intestinal manifestations. It is characterized by alternating phases of clinical activity and remission of symptoms: for this reason, patients with Crohn's disease still require lifelong clinical check-ups, medical therapy, and often also surgical therapy, which can be followed by post-surgical recurrence of lesions.

2.3 Epidemiology

Crohn's disease is more frequent in countries with high socio-economic development, such as North America and Northern Europe, and rarer in underdeveloped ones. This finding suggests the role of environmental factors in the pathogenesis of the disease. In fact, it is a well-known fact that white race and higher education levels are associated with an increased prevalence of the disease [3], together with urban regions and good standards of domestic hygiene during childhood. Moreover, it has been demonstrated that people who migrate from areas with a low incidence of IBD to areas with a high incidence have an *increased* risk of developing IBD; moreover, the younger they are when they migrate, the greater their risk of developing a form of IBD.

On the contrary, people who migrate from regions with a high incidence of IBD to areas with a low incidence have a *decreased* risk of developing a form of IBD [4].

2.3.1 Incidence and prevalence

The incidence of Crohn's disease has almost quadrupled in the last 25 years, particularly in Northern Europe (11.4/100,000 inhabitants/year, with a prevalence of 262/100,000) and North America
(23.8/100,000 inhabitants/year, with a prevalence of 318.5/100,000).
In Italy, the incidence is 6.9/100,000 inhabitants/year, and the prevalence of 86/100,000 inhabitants, with no significant differences between men and women.
Crohn's disease is predominantly a disease of young adults: approximately 25% of patients with IBD will present it before 20 years of age; however, it can occur at any age.
The major incidence of this form of IBD can be observed during adolescence, but it is also common to be found during childhood: approximately 20% of children with IBD present the disease before 10 years of age, and approximately 5% present it before 5 years of age. [4]
In general, the diagnosis is made in more than 66% before age of 35, and in 25% before age 20. The main peak of incidence is reported between 15 and 40 years of age, although a second peak of incidence is observed between 50 and 60 years of age.
A progressive reduction in the age of diagnosis has been noticed over the past decades. Moreover, populations previously considered with a 'low risk', such as Japanese or Indian inhabitants, are witnessing an increase in incidence. [5]

2.3.2 Risk factors

The etiology of Crohn's disease is unknown.
However, the following main risk factors for developing the disease have been identified.

2.3.2.1 Familiarity

The role of genetic and familial predisposition is supported by various observations. Approximately 12% of patients have a first or second-degree relative with Crohn's disease or Ulcerative colitis; the risk for a patient with Crohn's disease to have a child with the same disease is approximately 10 times higher than for an unaffected person and there is a high concordance for Crohn's disease in homozygotic twins.
Genetic loci associated with IBD were initially identified using linkage studies, which first demonstrated an association with a locus on chromosome 16, subsequently characterized as the *NOD2* locus, with three common variants that influence susceptibility to Crohn's disease [5 6].

2.3.2.2 Appendectomy

It carries a significantly increased risk of developing Crohn's disease, particularly within the first year after appendectomy surgery, but with a subsequent decrease in subsequent years. This finding underlines the possible diagnostic problems in Crohn's disease, as acute appendicitis may sometimes be the initial manifestation of Crohn's disease. In fact, one study suggested a later diagnosis of Crohn's disease in those patients who had undergone an appendectomy previously [7].

2.3.2.3 Cigarette smoking

Cigarette smoking is the only environmental factor significantly associated with the disease. Smoking is associated with an increased risk of the disease as well as a more aggressive course and earlier post-surgical recurrence [8].
The effect of smoking could be mediated by multiple reasons: alteration of smooth muscle tone and endothelial function through nitric oxide production, or integrity's modification of the gut mucous barrier through oxidative stress [9].

2.3.2.4 Other factors

Other risk factors include the use of peculiar medications such as *non-steroidal anti-inflammatory drugs* (NSAIDs), *oral contraceptives,* and *postmenopausal hormone therapy.* In addition, the exposure to *antibiotics* in childhood, with the perturbation of the microbiota that could influence the gut immune response and

alter susceptibility to IBD, and *dietary habits* with high-fat and low-fiber diet affect the probability to develop the disease [5].
However, none of these has a definite etiological association.

On the contrary, factors associated with decreased risk include exposure to pets and farm animals, bedroom sharing, having more than two siblings, high fiber intake, fruit consumption, and physical activity [5]. Furthermore, it is important to underline that vaccines have not been associated with the development of Crohn's disease. [10]

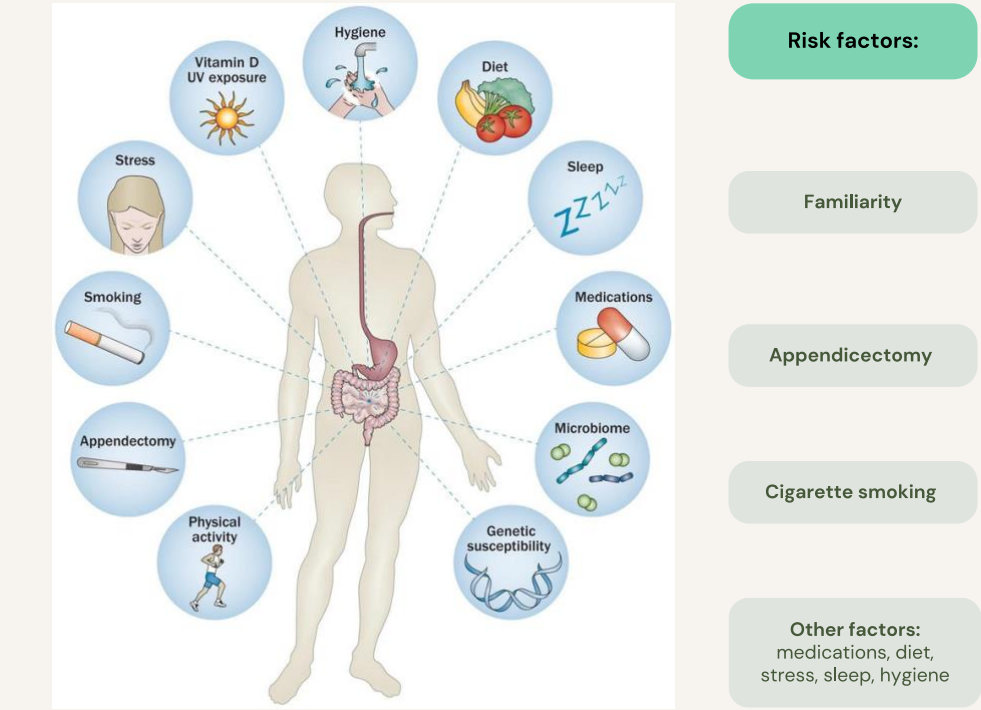


Figure 1. Main risk factors of Crohn's disease [5]

2.4 Etiology

The etiology of Crohn's disease is unknown.
Current studies suggest that, in genetically predisposed individuals, an inappropriate immune response to antigens present in the intestinal lumen (microbiome) may induce and/or perpetuate an inflammatory process. The hypotheses take into account various agents, although for none of them, a precise etiologic role has been demonstrated.

2.4.1 Microbiota

The dysbiosis condition evidenced in patients with Crohn's disease includes a reduction in Firmicutes and Bacteroides and an increase in *Actinobacteria* and *Gammaproteobacteria*. Approximately one-third of the patients also show an increase in invasive adherent *Escherichia coli*, which are able to cross the mucosal barrier, adhere and invade intestinal epithelial cells and finally replicate in macrophages, causing the secretion of high amounts of INF alpha.

It has also been documented how concentrations of *Faecalibacterium prausnitzii*, a commensal bacterium with anti-inflammatory properties, are reduced in Crohn's disease patients.

Finally, it has been reported that in the microbiota of Crohn's disease patients, there is a relative expansion of certain viral and fungal species [11] [12].

This hypothesis is supported in part by two observations: on the one hand, therapies capable of modulating intestinal bacterial composition such as probiotics and antibiotics may modify Crohn's disease activity; on the other hand, surgical diversion of the luminal content improves the clinical course and prevents post-surgical recurrence.

In addition, surgical recanalization, with the restoration of intestinal continuity, is followed by post-surgical recurrence.

So far, however, no agents responsible for the etiology of Crohn's disease have been identified [13] [14].

2.4.2 Genetic susceptibility

In approximately 15-20% of patients with IBD-like symptoms, it is possible to detect an underlying monogenic defect, that can be identified through the new techniques of Next Generation Sequencing. So far, more than 50 genes have been identified, together with more than 200 alleles associated with inflammatory bowel disease, 37 of which are specific to Crohn's disease.

The discovery of genes related to bacterial components, innate immunity, and related to Th17 immune response, such as NOD2, RK2, IRGM, ATG16LI, HLA, I23R, STAT3, JAK2, has clearly shown how altered processing of intestinal bacterial components plays a key role in the pathogenesis of the disease.

In particular, three loci (NOD2, MHC, and MST1 3p21) were associated with subphenotypes of inflammatory bowel disease, in the mainly location of ileal and colonic Crohn's disease.

However, these results show that Crohn's disease is heritable in only a low percentage of cases (about 13%), while in the rest of the cases, the involvement of epigenetics and environmental factors plays a bigger role [15] [13] [16].

2.4.3 Immune response

The equilibrium between the luminal content and the mucosal immune system in the lamina propria is maintained by the intestinal epithelium, which orchestrates this equilibrium with its mechanical function of physical barrier and its role in immune responses.

Specialized intestinal epithelial cells (IECs) have important roles in intestinal immunity: for example, *Paneth cells* at the base of crypts of Lieberkühn constitutively produce antimicrobial peptides, *microfold cells* in the gut-associated lymphoid tissue sample luminal antigens and present them to cells of the adaptive immune system.

After contact with an antigen, antigen-presenting cells (APCs), such as dendritic cells, present antigen to T cells and B cells to initiate a controlled inflammatory response.

In inflammatory conditions such as Crohn's disease, epithelial barrier dysfunction related for example to polymorphisms in *NOD2* and NFκB signaling pathway genes, results in the luminal contents entering the lamina propria, leading the dendritic cells to activate inflammatory T cell types, such as naive T helper ($T_H 0$) cells, T helper 1, ($T_H 1$) cells, $T_H 17$ cells and $T_H 2$ cells, which produce proinflammatory cytokines, such as IFNγ and tumor necrosis factor (TNF), targeted of most recent biological drugs.

Furthermore, in response to luminal contents, macrophages produce the proinflammatory cytokines IL-12 and IL-23, which activate natural killer (NK) cells, resulting in perpetuation of the intestinal inflammation with the production of proinflammatory cytokines. These proinflammatory cytokines are targeted by many of the currently available treatments for moderate to severe CD, including monoclonal antibody such as Ustekinumab.

IL-4, IL-6, IL-21 and IL-22 are also produced by T_H0 cells in response to activation of dendritic cells, with the alteration of gut homeostasis and the development of the intestinal bowel disease [17] [18] [19] [20] [21] [22].

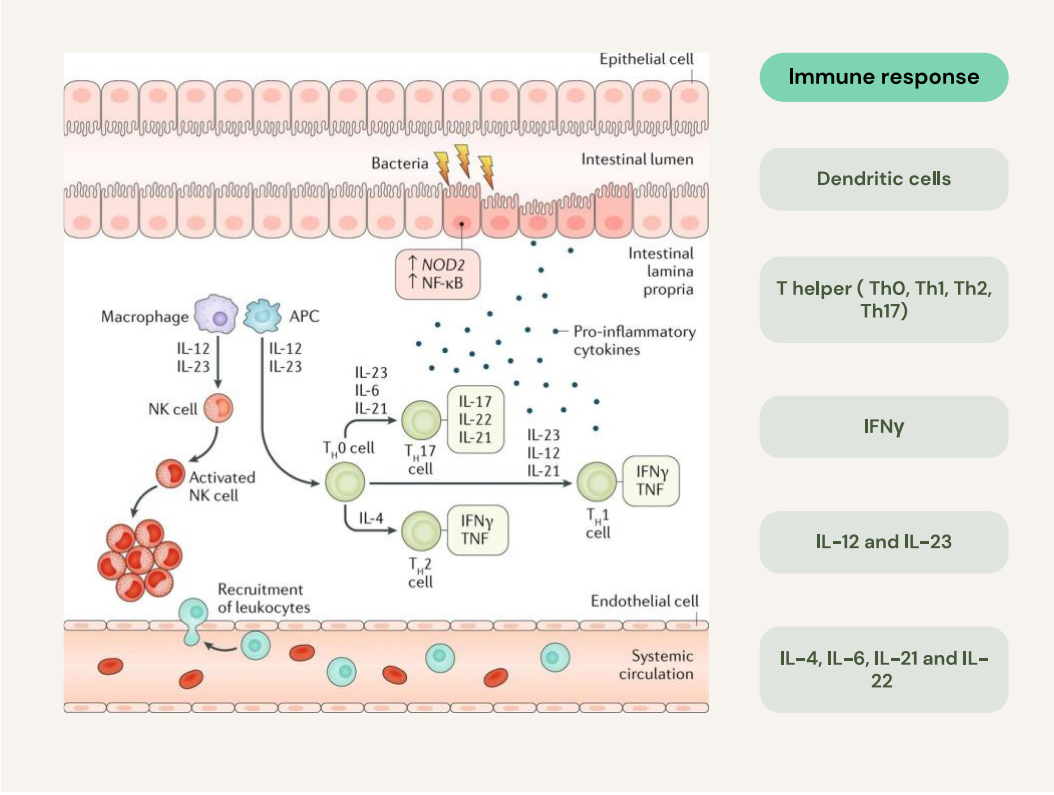


Figure 2. Schematic vision of intestinal homeostasis that is maintained by the equilibrium between the mucosal immune system in the lamina propria and the luminal content [22].

2.5 Clinical manifestations

2.5.1 Onset

The onset of the disease is acute in about 10% of cases, with a similar clinical presentation to appendicitis and population-based cohort studies have demonstrated that up to 30% of patients with CD have evidence of bowel damage at diagnosis and strong symptoms in the onset.

In some patients the onset of classic symptoms may be preceded, even by years, by the appearance of a perianal involvement, extraintestinal manifestations, or delayed growth in childhood.

The majority of patients presents non-specific symptoms, such as recurrent abdominal pain, diarrhea with or without blood, fever, chills, asthenia, weight loss.

The latency period between the onset of symptoms and diagnosis varies between 0 and 4 years, with an average of 8-9 months. During this time it is common to propose to the patient different diagnoses, such as appendicitis, irritable bowel syndrome, or adnexitis since they share many insidious symptomatic factors [23] [24] [25] [26] [22] [27].

2.5.2 Signs and symptoms

The type and severity of symptoms depend on many factors, such as the patient, the features, and the area of the lesion.

Chronic diarrhea, the main reported symptom, is often related to the presence of ileal lesions or to the malabsorption of bile salts, a condition observed in patients who underwent ileal resection.

Abdominal pain is localized mainly in the mesogastrium and right iliac fossa and is variable in intensity and frequency, occurring even at night and not receding after evacuation.

Rectorrhagia and tenesmus are instead associated with sigma-rectum lesions,

When red flag symptoms are observed, such as severe weight loss, bloody diarrhea, iron deficiency, and night-time awakenings, further evaluation is required [28].

2.5.3 Clinical course

The course of Crohn's disease is chronic recurrent, with alternating periods of relapse and remission. The probability of remission after an active phase is very high, more than 90%, and the probability of relapse at one year is also remarkable, more than 60%.

The severity of the disease can be recognized through some specific features: a juvenile onset and the need steroids already at this phase, a family history of chronic inflammatory disease, cigarette smoking habit, the presence of extensive or multiple jejunoileal strictures, and, sometimes even with free perforation of the small intestine, large intra-abdominal inflammatory masses, the fistulizing phenotype (e.g., ileosigmoid fistula) and the presence of perianal disease [21].

Once the disease is initiated, it is likely that numerous genetic and environmental factors play a role in regulating the rate of progression, but these are poorly understood.

Moreover, the progression of the disease itself may not necessarily be a linear process but rather progression may occur in a stepwise approach, with prolonged symptom-free periods over many decades. The remission after a variable length of time is followed by the reappearance of lesions, initially aphthoid ulcers, and subsequently, more extensive and severe lesions, not necessarily associated with the recurrence of symptoms. These may lead to the development of new complications and require new surgical resections over time.

2.5.4 Complications

It is demonstrated that half of all patients with CD develop intestinal complications within 10 years of diagnosis [27].

The complications that developed in Crohn's disease can be local or systemic.

Local complications include internal fistulas, which can be entero-enteric, entero-vesical, or recto-vaginal, and external fistulas (entero-cutaneous), obstruction, perforation, hemorrhage, and abscesses. Fistulas can be a manifestation of a penetrating disease; they are often associated with stenosis and subsequent development of abscess collections and have little tendency for spontaneous healing. Stenoses are more frequent in patients with ileal disease and manifest with sub-obstructive or occlusive symptoms. The development of adenocarcinoma of the tenuous or colon site of inflammation is a rare but possible complication.

Crohn's disease localized in the colon is more frequently likely to be complicated with anal lesions. The frequency of local complications increases with disease duration [29].

Systemic complications can extend beyond the intestinal lumen, causing abscesses, fissures, and/or fistulas, affecting also organs outside of the intestinal tract. These extraintestinal findings can also appear before the gastrointestinal symptoms. [30 31]

2.6 Diagnosis

2.6.1 Criteria for diagnosis

Despite a good comprehension of CD natural history, diagnosis can be challenging, because it does not rely on a single specific finding or a pathognomonic feature. Instead, diagnosis requires a complete assessment based on clinical history, physical examination, and complementary diagnostic tests, such as assays for

serological and faecal biomarkers, cross-sectional and endoscopic imaging, and histological evaluation of biopsy specimens [22].

2.6.1.1 *Natural history*

An objective examination of the abdomen may detect tenderness in the right iliac fossa or periumbilical fossa or an abdominal mass.
Disease's suggestions may come from the objective fundings of fistulas and extraintestinal manifestations.

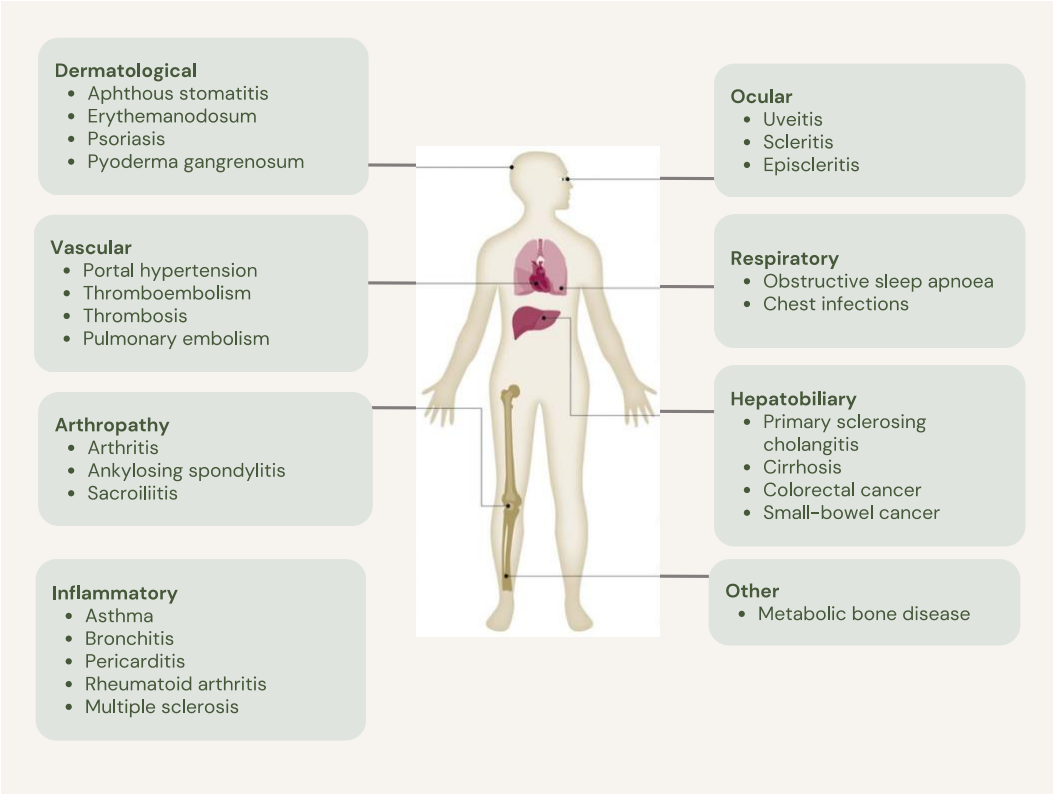


Figure 3. *Extraintestinal manifestation of Crohn's disease. Modified from GrepMed, Gerald MD*

2.6.1.2 *Biochemical investigations.*

Biomarkers are non-invasive tools that have been used to provide additional information in the management of patients with CD. Autoantibodies, such as perinuclear antineutrophil cytoplasmic antibodies (pANCAs), and antimicrobial antibodies, such as *anti-Saccharomyces cerevisiae* antibodies (ASCAs), anti-*Pseudomonas fluorescence-associated* sequence I2 antibodies, anti-outer membrane porin C antibodies, and anti-CBir1 antibodies, are useful biomarkers for CD diagnosis. However, even if these antibodies are highly used to detect the

difference between CD and UC, they have a limited practical clinical utility in the general diagnosis of CD, for which they are not recommended [32].

Other laboratory investigations may be much more helpful, detecting, hypochromic sideropenic anemia from loss, increased consumption, and reduced intake of iron, or macrocytic anemia, from reduced absorption of vitamin B12 and/or folic acid.

Neutrophilic leukocytosis, increased ESR, C-reactive protein, and mucoproteins are frequently observed during the active phases of the disease.

Hypoalbuminemia, hypocholesterolaemia and hypotriglyceridaemia, vitamin D3, folic acid and vitamin B12 deficiency due to malabsorption and hydro-electrolyte alterations, secondary to chronic diarrhoea, can be found.

Moreover, faecal biomarkers are potential non-invasive tools to aid in differential diagnosis, such as other infectious diseases, or as indicators of CD disease activity [12 34 35].

Calprotectin is a calcium-containing antimicrobial protein complex that makes up 60% of the cytosolic protein in neutrophils and is released during acute and chronic inflammation of the gastrointestinal tract wall. Faecal calprotectin has high sensitivity and specificity in the diagnosis of CD and can also be used to monitor the effect of ongoing therapy [36 37].

2.6.1.4 *Endoscopic investigations*

Colonoscopy with retrograde ileocolonoscopy is the reference diagnostic examination, as it allows visualization of the mucosal surface and also, if necessary, the performance of the biopsies. The ileocolonoscopy detects lesions alternating with macroscopically undamaged areas.

The most frequently observed lesions are aphthoid ulcers or confluent ulcers of varying morphology, interspersed with areas of edematous mucosa with a typical 'cobblestone' appearance and stenosis, sometimes not valved with the endoscope.

Esophagogastroduodenoscopy is recommended in the presence of symptoms referable to the upper gastrointestinal tract, such as nausea, vomiting, and epigastralgia, to assess the possible presence of esophageal, gastric and/or duodenal lesions, associated with typical ileal and/or colic localization, in 1-3% of patients.

Moreover, wireless video capsule endoscopy first approved in 2001, has developed into a safe and effective technology to image the small intestine with a noninvasive

approach, which has led to a significant advantage over enteroscopy with a greater sensitivity[13 38] .

2.6.1.4 *Radiological investigations*

Radiological investigations are crucial to support or confirm the diagnostic suspicion of Crohn's disease, to define its site, extension, abdominal complications, and disease activity, identifying extraluminal pathology and examining the gastrointestinal tract not accessible to endoscopic procedures.

For this purpose, *entero-TAC*, entero-computerized axial tomography and *entero-RM*, entero-nuclear magnetic resonance imaging and *intestinal ultrasound* are today the investigations of reference, preferred with contrast load.

Through these diagnostic tools it is possible to detect the phlogistic thickening of the intestinal wall. Furthermore, through the previous introduction of oral electrolyte solutions such as polyethylene glycol, it is possible to observe the distension of the loops and to accurately diagnose complications such as stenosis, fistulas and abscesses.

The three techniques presented share an equivalent diagnostic precision, however because of their differences, they are used for various purposes: entero-RM and ultrasound, due to their non-invasiveness and lack of ionizing radiation, are recommended as preliminary investigations for the diagnosis of the disease and for the follow-up of the lesions. MRI is particularly good at demonstrating the perianal complication of Crohn's disease [39].

On the contrary, the entero-TC is primarily used in the diagnosis of complications and in patients with acute symptoms, such as a toxic presentation. However, in case of suspicion of occlusion or bowel perforation, direct radiographic assessment of the abdomen turns out to be still useful [40 12].

2.6.2 Differential diagnosis

Instrumental investigations do not provide data specific to Crohn's disease but are often useful in differentiating it from other diseases with a similar clinical picture. The differential diagnosis of Crohn's disease includes numerous pathologies:

Ulcerative colitis, infectious enterocolitis of bacterial origin, pseudomembranous colitis due to Cl. difficile, colitis of viral and parasitic origin, irritable bowel syndrome, ischemic and radiation enterocolitis, colon carcinoma, colitis associated with diverticula, appendicitis, intestinal and pelvic endometriosis, coeliac disease and chronic Ulcerative non-granuloma digiuno-ileitis [13].

In the developing world, other differential diagnoses have to be firstly considered: Behçet's disease, intestinal lymphoma, and intestinal tuberculosis.
Behçet's disease might present intestinal inflammation characterized by solitary ulcers and extra intestinal manifestations, such as uveitis and skin involvement.
The diagnosis of lymphoma is instead possible only with histological confirmation.
Intestinal tuberculosis is characterized by fever and night sweats, ulcers of the transverse colon, patulous ileocecal valve, and unique histological features, such as caseating and/or confluent and/or large granulomas. These features, together with a positive smear test result for acid-fast bacillus and detection of necrotic lymph nodes by cross-sectional imaging, can make an effective differential diagnosis.

Crohn's disease and Ulcerative colitis show some common clinical and aetiopathogenetic aspects, but often different anatomical features, always more visible also thank to the introduction of new diagnostic procedures, such as hydrocolonic sonography [41] [42]. In fact, Crohn's disease, presents a segmental disease distribution, transmural inflammation and non-caseating epithelioid granulomas [43]. The discrimination with Ulcerative colitis is particularly important for IBD patients who require surgery, since the difference of the surgical procedure, while medical therapy remains similar.

Unclassified colitis and undetermined colitis, the initial diagnosis in 10% of patients with Crohn's disease, describe the cases in which the diagnosis is not possible through both clinical and endoscopic pictures, but also after histological examination on endoscopic biopsies alone. In these patients, an endoscopic video capsule study may be indicated, which allows visualization of aphthous ulcers compatible with Crohn's disease and undetectable by conventional radiology, as well a as histological review of the surgical specimen. This diagnosis is made when all the other previous hypotheses have been excluded [44] [45] [46] [47] [48].

2.6.3 Activity indices in Crohn's disease

Crohn's disease is characterized clinically by phases of activity and remission.
The clinical picture, the objective findings and the biochemical and instrumental investigations contribute not only to diagnose the disease but also to determine its activity.
Clinical activity can be defined by measuring specific indices, which are useful for standardizing and quantifying the doctor's subjective judgment.
The most widely used clinical index is the *Crohn's Disease Activity Index (CDAI),* developed in 1976 and used to quantify the symptoms in patients with CD by assigning a weighted score for eight clinical or laboratory variables, including general well-being, loose stool, abdominal pain, presence of abdominal mass, weight change, low hematocrit, and opiate use for diarrhea.
The score identifies patients in remission (<150) or in the active phase (>150).
Furthermore, other indices have been proposed to assess disease activity and monitor response to treatment: clinical, such as the *Harvey-Bradshaw index, HBI*, endoscopic, such as the *Simple endoscopic score for Crohn's disease, SES-CD*, and radiological, such as the *Magnetic Resonance Imaging index of Activity, MARiA*.
These indices are applied in clinical trials to define response to treatment or disease remission because the signs and the symptoms can differ consistently. 50% of patients in clinical remission have endoscopic and/or C-reactive protein (CRP) evidence of residual, active CD, whereas other patients have normal endoscopic findings and CRP levels despite having symptoms [49].
Montreal classification is used to classify the severity of Crohn's disease, in comparison to the Montreal classification used to classify the severity of Ulcerative colitis (Table II).

MONTREAL SCORE CD

AGE OF ONSET	≤16 years	A1			
	17–40 years	A2			
	> 40 years	A3			
LOCATION	Terminal ileum	L1	BEHAVIOUR	Non-stricturing, non- penetrating	B1
	Colon	L2		Stricturing	B2
	Ileocolon	L3		Penetrating	B3
	Upper gastrointestinal tract	L4			

Table II. Montreal classification in Crohn's disease

2.6.4 Endoscopic manifestations

2.6.4.1 *Macroscopic appearance*

The lesions may involve any tract of the alimentary canal; however, the most frequently affected sites are the distal ileum (30-40%), the distal ileum and colon (40-55%), or the colon alone (5-10%) [2] (Table 3).

LOCATION OF CROHN'S DISEASE AND ASSOCIATED SYMPTOMS

Location	Symptoms	Comments	Frequency (%)
Ileum and colon	Diarrhea, cramping, abdominal pain, weight loss	Most common form	35
Colon only	Diarrhea, rectal bleeding, perirectal abscess, fistula, perirectal ulcer	Skip lesions and arthralgias more common	32
Small bowel only	Diarrhea, cramping, abdominal pain, weight loss	Complications may include fistula or abscess formation	28
Gastro-duodenal region	Anorexia, weight loss, nausea, vomiting	Rare form; May cause bowel obstruction	5

Table 3. Involvement of the different areas of the gastrointestinal tract and the relative symptoms associated.

Lesions show a typical segmental distribution, with injured tracts alternating with uninjured tracts.

The macroscopic picture of the injured areas is mainly represented by a thickening of the wall secondary to edema, inflammatory filtrate, and fibrosis. This parietal thickening and fibrotic outcomes of inflammation may lead to the development of stenosis, varying in extent, severity, and number [50].

The inner surface of the bowel shows serpiginous ulcers, often extended in depth. Inflammatory involvement of the serosa and extension to this level of the fissures may produce adhesions and predispose to fistula formation. All the three layers of the wall are involved.

Three main disease subgroups can be identified, which may coexist in the same patient or evolve into each other over the years [32 51].

2.6.4.1.1 Fibrosthenous disease

This subgroup shows narrowing of the lumen and the presence of stenosis, a consequence of the parietal thickening due to wall inflammation and the fibrotic outcome of ulcerations.

Stenoses may be single or multiple, of variable length, often located in the distal ileum and associated with dilatation of the proximal loops.

2.6.4.1.2 Fistulising or perforating disease

This subgroup shows fistulas, described as passageways between intestinal loops or with the surrounding viscera. Fistulas originate from fissures and adhesions caused by inflammation of the serosa and may connect the intestinal loops with each other (entero-enteric), with the skin (entero-cutaneous), with the bladder (entero-vesical), with the ureter (entero-ureteral) or with the vagina (recto-vaginal). Fistulas may also extend into the retroperitoneum or, more often, into the mesentery, giving rise to abscess collections.

2.6.4.1.3 Inflammatory disease

This subgroup shows inflammatory thickening of the walls in the absence of fistulae or stenosis, small, superficial Ulcerative lesions that represent recent onset disease,

and deep Ulcerative lesions and fissures, surrounded by areas of mucosa raised by edema, sometimes with a typical 'cobblestone' appearance.

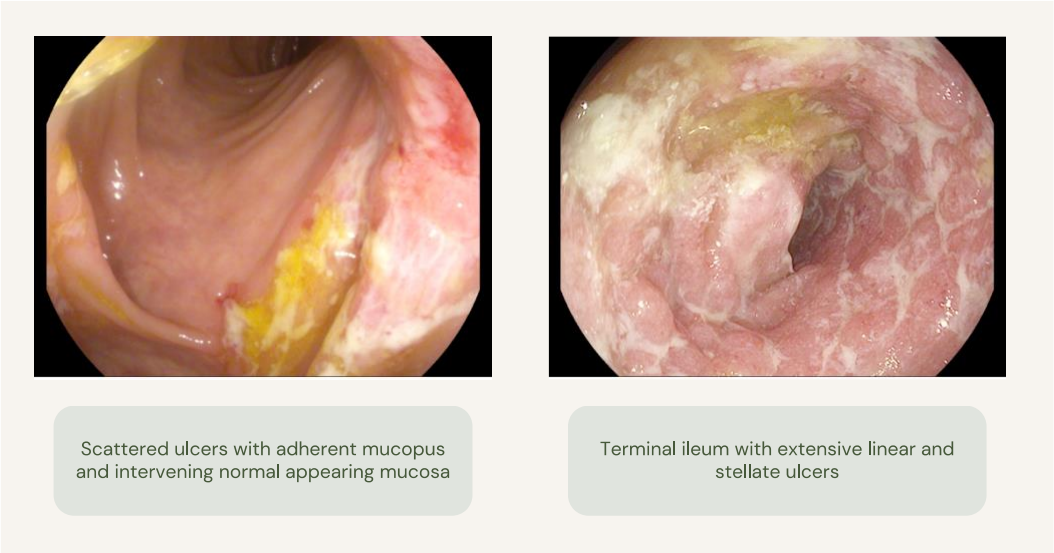


Figure 4. *CD signs at endoscopic examination. Modified from Maxine A. Papadakis, Stephen J. McPhee, Michael W. Rabow, Kenneth R. McQuaid: Current Medical Diagnosis & Treatment 2023*.

2.6.4.2 *Microscopic appearance*

At a microscopic level, Crohn's disease is histologically characterized by chronic granulomatous inflammation. The main lesions consist of non-caseating granulomas and discontinuous and transmural inflammation with focal lymphocytic aggregates and fissures. Granulomas, found in about 70% of CD patients, consist of epithelioid cells, multinucleated Langhans-type giant cells, and lymphocytes, without the presence of necrosis. Epithelioid cells and giant cells simultaneously expressed class II molecules and costimulatory molecules for the antigen-specific activation to T cells.

For this reason, granuloma in Crohn's disease may act as an inducer of the antigen-specific immune response of the disease.

Although the presence of granulomas is a specific finding in Crohn's disease, their absence is not a diagnostic criterion for excluding the disease, as they are often present in small numbers and only in some patients [52] [53].

Wall inflammation presents aggregates of inflammatory cells, such as T lymphocytes and macrophages, with focal and simultaneous distribution in multiple parietal layers, predominantly in the submucosa. In some patients, it is also possible

to observe the development of fibrosis, secondary to scar repair of the lesions and subsequent stenosis formation.

It is important to underline that the extent and severity of the macroscopic and histological lesions are independent of the clinical disease activity.

2.7 Medical treatment

Since the etiology of the disease is unknown, there are no curative therapies available to target a specific pathogenetic process.

However, the growing knowledge of the pathogenesis of Crohn's disease has led to the development of many drugs that are effective in treating the disease, with two therapeutic goals: *clinical remission*, such as the resolution of abdominal pain/ diarrhea, and *endoscopic remission*, such as the disappearance of ulcers and the resolution of inflammation on cross-sectional imaging, with a clear sign of mucosal healing. Patients who achieve mucosal healing have improved outcomes, including decreased risk of surgery, lower relapse rates, and improved quality of life [54].

As CD is a lifelong disease, the goals of the medical therapy consist of induction of remission in the short term and maintenance of remission in the long term, without using corticosteroids.

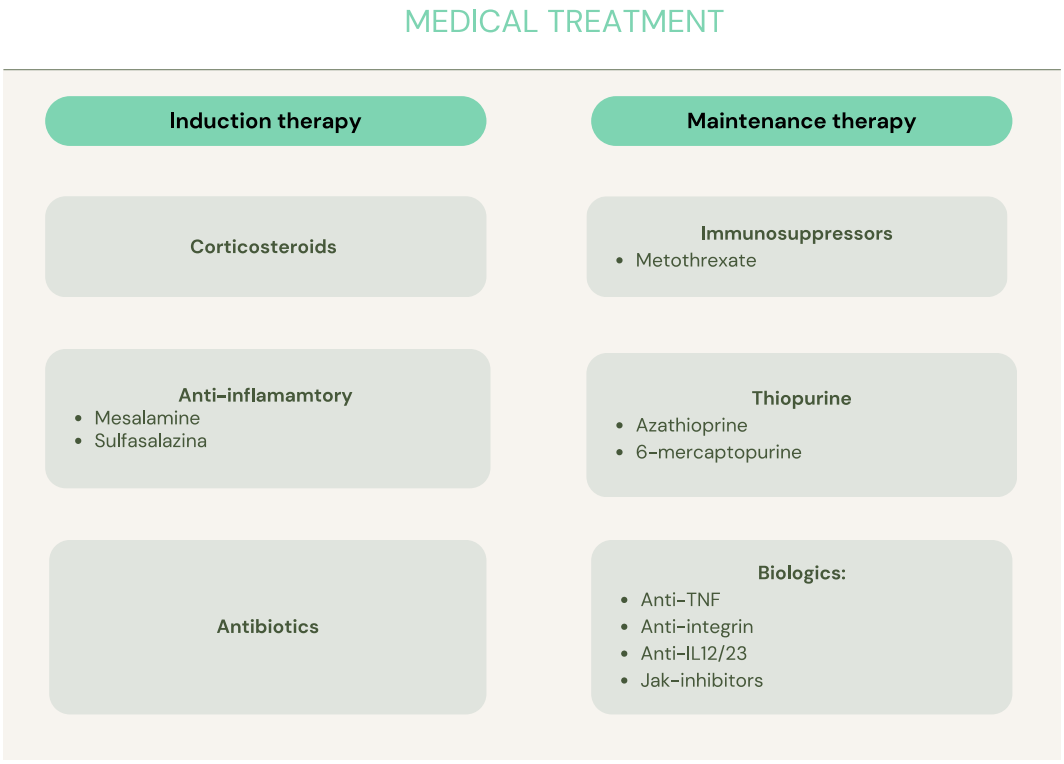


Table III. Schematic visualization of the medical treatment, presented subsequently.

2.7.1 Induction therapy

2.7.1.1 Corticosteroids

Corticosteroids have been the cornerstone of CD management for many decades, and due to their rapid onset of action are nowadays indicated for induction therapy. Corticosteroids down-regulate the transcription of genes involved in proinflammatory cytokine production, such as interleukin IL-1, IL-6, NF-κB, and TNF, and inhibit the expression of adhesion molecules in inflamed tissues and the trafficking of activated immune cells. [55]

Prednisone, with an oral tapering administration of 40 to 60 mg as an initial dose, based on disease severity, and budesonide, 9 mg, are recommended for the treatment of mild-to-moderate ileal and moderate to-severe ileocolonic CD, and induce the remission in 65-85% of the patients [56].

In case of disease's diffusion in the left colon, prednisone is preferred, whereas, in case of disease's diffusion in the ileum and/or proximal colon, formulations of controlled ileal-release such as budesonide (Entocort) are preferred, because of their unique delivery specifically to that area. Since corticosteroids induce but do not maintain remission and may cause some adverse effects, such as perforating complications, they are most often used to treat symptom flare-ups while patients transition to more effective therapies [57].

Several clinical studies have defined the response to corticosteroids as a clinical improvement after treatment with high-dose corticosteroids (40–60 mg prednisone/day) within 30 days for an oral administration and 7–10 days for an intravenous one. Conversely, patients who do not respond to corticosteroids within this time frame have been defined as corticosteroid refractory.

It has been demonstrated that unfortunately, more than 50 % of patients treated acutely with corticosteroids will become corticosteroid dependent or corticosteroid resistant, particularly smokers, or those with the colonic disease [58] [59] [60] [30].

2.7.1.2 Anti-inflammatory

Aminosalicylates, derivates from 5-aminosalicylic acid, can also be useful for induction of remission in Crohn's disease.

Mesalamine, 5-ASA, has been approved by the FDA in 1987 for the treatment of CD, since its positive anti-inflammatory action on the bowel mucosa.

It is still largely prescribed as the first-line therapy for IBD due to its safety and its low rate of side effects compared to other therapies.

In conclusion, there is a lack of evidence for the efficacy of mesalamine in either induction or maintenance therapy in CD [61] [62].

Sulfasalazine, a molecule obtained by the chemical fusion of an antibiotic belonging to the category of sulfonamides such as sulphapyridine, and an anti-inflammatory such as salicylic acid, has also been approved for induction therapy.

On the one hand sulphapyridine inhibits the synthesis of bacterial folic acid and therefore performs first locally and then systemically a bacteriostatic action, directed at the intestinal level both against the resident flora, pathogenic agents, and Clostridia; on the other hand the 5-aminosalicylic acid, able to inhibit the enzymes cyclooxygenase responsible for the synthesis of inflammatory mediators such as prostaglandins, prostacyclins and thromboxanes, thus reducing the persistent inflammatory stimulus during CD.

However, some studies show only a modestly effect of sulfasalazine, and demonstrate its inferiority to corticosteroids for the treatment of mildly to moderately active Crohn's disease [63] [64].

2.7.1.3 Antibiotics

Antibiotic intake, such as clarithromycin, metronidazole, and ciprofloxacin, can improve the efficacy of the therapy. The combination of rifabutin and clofazimine has shown efficacy in a consistent randomized controlled trial of patients with CD. The duration of the antibiotic therapy should range between 6 months and 1 year in these trials of CD [65]. However, physicians who have used antibiotics in an extensive way in CD have observed that most patients will relapse during the therapy and that for this reason antibiotics are indicated in more specific cases, such as perianal complications [66].

In fact, in general, there is a lack of vast evidence for the efficacy of antibiotics in really reducing inflammation in CD, and their administration should be limited only to specific cases [65] [67] .

2.7.2 Maintenance therapy

Immunomodulators, such as immunosuppressors and thiopurine and biologics, have shown efficacy in maintenance therapy in CD as adjunctive therapy, because of their slow onset of action.

2.7.2.1 Immunosuppressors

Immunosuppressors, such as methotrexate, have been used for many years as effective therapy of oncological or rheumatologic diseases. Over the past 30 years, several studies have also evaluated its efficacy in the treatment of inflammatory bowel disease. In fact, they are used as a maintenance of remission as a steroid-sparing agent in Crohn's disease.

Randomized clinical trials support the use of the methotrexate only in steroid-dependent CD, with similar outcomes to thiopurines. Furthermore, immunosuppressors could be used in patients who do already take biologics, not for induction, but only for maintenance, as an adjunct therapy to dicrease immunogenicity risk to biologics in Crohn's disease, optimizing the immunogenic profile of the biological drug. However, the impact on long-term clinical outcomes is described only in small series of patients.

With what concerns infants, based on the results of retrospective studies, methotrexate is useful in the treatment of pediatric CD in those who fail thiopurine therapy [68] [69].

Despite all of these studies, the use of methotrexate in patients with steroid-refractoriness, failure of thiopurines, or in combination with biologics is not supported by high levels of evidence[70] [71].

2.7.2.2 Thipurine

The efficacy of thiopurine, and purine antimetabolites, is limited to the maintenance of CD remission. Azathioprine and 6-mercaptopurine are more effective than placebo only in the maintenance of remission.

In moderate- to high-risk patients, azathioprine is used in combination with biologics, such as anti–tumor necrosis factor (TNF) agents, demonstrating improved effectiveness over either agent used alone. Through this combination it is possible to reduce corticosteroid exposure and therefore adverse effects, decreasing the rate of immunogenicity against anti-TNF agents.

2.7.2.3 Biologics

Biologics are drugs of recent development; the first clinical trial was conducted in 1995. They differ from other drugs because they contain one or more active principles extracted from a biological system, such as hormones, enzymes, vaccines, immunoglobulins, and monoclonal antibodies.

Biologics have been a revolution in the way we treat inflammatory bowel disease patients.

These drugs are now considered the therapy of choice, especially for patients who risk highly disease progression.

Targeted therapies exploit specific molecular properties of a tumor's disease.

To this group belong monoclonal antibodies (end in "mab") and the "small molecules" (end in -ib"), which can modulate different targets. The "small molecules" are taken into the cells and block the enzymes' activities, while monoclonal antibodies bind to a specific antigen, such as a tumoral antigen or an immunity checkpoint, inhibiting it. In the case of Crohn's disease, the target of a monoclonal antibody consists of cytokines and cytokines receptors involved in the inflammatory process, to block it [72] [73].

Although these targeted biological therapies are a notable advance in the treatment of CD, they show also some disadvantages. They are expensive, require parenteral administration, and are associated with potential immunogenicity and an increased risk of possibly skin cancers and infections, including reactivation of tuberculosis. However, despite these risks, a recent meta-analysis indicates that patients are more likely to continue treatment with biologics than immunomodulators because of improved effectiveness and tolerability.

The production of these monoclonal antibodies consists in immunizing animals with the substance subject of the study and subsequently obtaining cell lines or clones, genetically modifying the structure of the monoclonal antibody in a laboratory, with the substitution of a human isotype of the heavy and light channel, leaving the idiotype, such as the combinatory site with the antigen [74] [21].

In the absence of head-to-head comparative trials of these agents, relative differences in efficacy and safety are suggested by network meta-analyses. The choice of biologic agent depends on the disease severity, patient age and comorbidities, patient preference, and drug cost/pharmacy tiering.

Whichever biologic agent induces remission should be continued for maintenance.

2.7.2.4 *Anti TNF*

Anti-tumor necrosis factor antibodies work better than conventional therapies at achieving consistent remission without steroids. Anti-TNF agents, such as certolizumab pegol, adalimumab, and infliximab, induce and maintain remission in moderate-to high-risk patients, or patients with inadequate response to corticosteroids and immunomodulators.

The clinical benefits can be observed within two weeks of therapy initiation, but to witness the overall effectiveness of anti-TNF agents two years are recommended.

The importance of TNF as a proinflammatory cytokine in IBD has long been appreciated, and for this reason, turns out to be a perfect target for CD therapy [75] [76].

Infliximab, the first biologic to be approved for inflammatory bowel diseases, is a chimeric immunoglobulin G monoclonal antibody directed against TNF, which has revolutionized the medical therapy of moderate to severe Crohn's disease. It is administered intravenously, with a starting dose of 5 mg/kg at weeks 0, 2, and 6, it is effective for achieving and maintaining remission in Crohn disease, including disease refractory to the standard medications discussed previously. it is steroid-sparing and effective in healing fistulae.

The administration of infliximab every 8 weeks promotes Crohn's disease remission, but only 25% of patients are still in full clinical remission after one year, despite an initial response rate of 80%.

In case of loss of response, the dose should be increased to 10 mg/Kg without changing the time between infusions or shortening the time between the infusions to 6 weeks. Infliximab therapy is constantly evaluated right before the infusion through levels of drug and antibodies.
Infliximab's recognition as a foreign substance by the immune system is thought to be the trigger for antibody production[77].
In addition, the combination of infliximab with azathioprine has shown statistically significant improvement over monotherapy with infliximab or azathioprine, but no clinical benefit for patients who failed azathioprine and then started infliximab.
For maintenance therapies, the combination of infliximab and azathioprine has been ranked highest for clinical remission and lowest for adverse events.

More recently, other anti-TNF biologic agents have been introduced, such as adalimumab and certolizumab, with the same functional mechanism of action and comparably effective.
Adalimumab is administered with an induction dose of 160 mg subcutaneously, followed by 80 mg subcutaneous dose at week 2, then 40 mg subcutaneously every 2 weeks.
Certolizumab is administered with an initial dose of 400 mg subcutaneously at weeks 0, 2, and 4, and subsequently 400 mg subcutaneously every 4 weeks; they both provide a similar response and remission rates as infliximab; however it is clear the subcutaneous administration of the induction dose, compared to the intravenous administration, is simpler [78].

2.7.2.5 *Anti-integrin*

Other biologic therapies for CD include gut-selective monoclonal anti-integrin antibodies, such as vedolizumab and natalizumab. They are anti-integrin agents that target leukocyte trafficking and migration.
In fact, integrins mediate strong adhesion between leucocytes and endothelial and mucosal epithelial cells by binding to extracellular matrix components and specific receptor molecules. Adhesion molecules play a crucial role in the cell-cell interactions that are necessary for the recruitment of circulating immune cells from

the vasculature to local tissue sites and anti-integrin monoclonal antibodies prevent this process to happen, drastically reducing the inflammation[79]. Natalizumab, anti $\alpha_4\beta_1$ integrin, is associated with progressive multifocal leukoencephalopathy and should only be used in patients who are seronegative for anti–John Cunningham virus antibody.

Vedolizumab, anti $\alpha_4\beta_7$ integrin, is preferred because of its specificity to leukocyte trafficking in the gut and has demonstrated effectiveness in achieving clinical response, remission, and corticosteroid-free remission.

Vedolizumab with subcutaneous administration has maintained the same clinical efficacy of intravenous induction in patients, without increasing the safety risk. Moreover, the efficacy of the subcutaneous administration has proved to be more pronounced in patients with UC [80].

In conclusion, many observational studies demonstrated extensively the effectiveness of vedolizumab, with a reassuring safety profile.[81].

2.7.2.7 *Anti IL 12/23*

Ustekinumab, an anti-subunit p40 of the interleukin 12/23 antibody therapy an emerging treatment option for patients when standard therapies have been ineffective. It has been approved by the FDA in September 2016 for the treatment of patients with moderate to severe Crohn disease who have not responded to or are intolerant of conventional therapies [82].

It may also be appropriate as first-line induction therapy for patients with severe Crohn's disease who are deemed to be at increased risk for complications of anti-TNF therapy or in which anti-TNF therapy failed, with clinical response in 34% of patients 6 weeks after a single dose of intravenous Ustekinumab compared to 21.5% with placebo.

However, studies haven't demonstrated a significant difference in comparison to anti TNF alpha, such as adalimumab, with the placebo; also a study from the Swedish routine care hasn't shown clinically relevant differences in effectiveness or safety of second-line Ustekinumab vs anti-TNF treatment in patients with CD with prior exposure to anti-TNF [83].

In a second phase 3 trial composed of patients in whom conventional therapy with immunomodulators or corticosteroids (but not anti-TNF) had failed, clinical improvement occurred in 55% compared to 28.7% with placebo.

In addition, a chronic maintenance trial that involved the administration of Ustekinumab versus placebo subcutaneously every 8 weeks, has shown that 53% of those given Ustekinumab were in clinical remission at week 44, versus 36% given the placebo.

In general, it has been noticed that subcutaneous Ustekinumab maintained remission in patients who had a clinical response to induction therapy [84].

In the magnetic resonance enterography it has been possible to observe early mucosal healing in about 1/5 of the patients with Crohn's disease after Ustekinumab treatment; the assessment lasted 26 weeks and included many indexes, such as bowel ball thickness and apparent diffusion coefficient, clear positive signs of transmural healing [85]. Moreover, higher Ustekinumab serum levels have been confirmed, in other studies, to be associated with a greater likelihood of achieving mucosal healing and mucosal response in patients with Crohn's disease regardless of prior biologic exposure [86].

There has been demonstrated also a remarkable health-related quality of life's improvement in patients with moderate-to-severe Crohn's disease, both in treat-to-target and standard of care treatment strategies, over a period of 2 years. [87]

Ustekinumab has also the highest persistence between biological treatments; followed by vedolizumab, infliximab, and adalimumab, in a follow-up study of 12 months, with a considerable optimized patients' management cost for the sanitary system[88] [89].

In conclusion, the last Ustekinumab guidelines suggest the timely escalation of Ustekinumab in Crohn's disease, based on early endoscopic response, clinical symptoms, and biomarkers, not better endoscopic outcomes at week 48 than symptom-driven decisions alone. [90]

In addition, adalimumab and Ustekinumab monotherapies are highly effective in CD biologic-naïve, with no difference in the clinical remission between the drugs [91]

Ustekinumab has been demonstrated to be effective in real-world use in the short- and long-term treatment in a refractory cohort of CD patients. Safety has been consistent with the known profile of Ustekinumab [92].

2.7.2.8 *Jak inhibitors*

JAKs are intracellular tyrosine kinases that play a crucial role in the signaling pathways of many cytokines involved in immunity and haematopoiesis. On receptor-cytokine binding and receptor dimerization, receptor-associated JAKs cross-phosphorylate one another; subsequently, phosphorylation of receptor-associated tyrosine residues provides docking sites for STAT proteins, which are also phosphorylated by JAKs. Phosphorylated STAT molecules then dimerize and translocate to the nucleus, where they act as potent regulators of gene expression.

The development and recent application of Janus kinase (JAK) inhibitors in the treatment of IBD, has led to the creation of first-generation pan-JAK inhibitors, such as tofacitinib, baricitinib, ruxolitinib, peficitinib, and second-generation of selective JAK inhibitors, such as decernotinib, filgotinib and upadacitinib, with limited adverse events [79] [93].

These novel drugs differ because of their lower molecular weights, usually <1 kDa, and rapid absorption and distribution to the systemic circulation, that make a more efficient and cost-effective therapeutic alternative for IBD treatment, compared to biologics with greater molecular weight, such as 150 kDa.

The administration of small-molecule JAK inhibitors can be oral or topical, thereby avoiding the requirement of specialized staff. In addition, their stability in terms of structure reduces storage costs, and their rapid onset of action, without eliciting antidrug antibodies, and their shorter elimination half-lives, in comparison with other biologics., make them a simpler alternative therapy.

In fact, they can be especially useful in the case of concurrent infections or before surgery, because of the requirement of a rapid drug elimination.

These newer small molecules also have more predictable pharmacogenetics and are less expensive to manufacture than biologics [93] [94] [95].

2.7.2.9 *Efficacy*

With what concerns the efficacy of biologics in luminal CD, infliximab and adalimumab are the most used biologic drugs in CD, with a similar effectiveness confirmed in several real-world studies.

Subsequently have been introduced, in naïve to biological CD patients, other monoclonal antibodies with a different target, such as vedolizumab or Ustekinumab; however, data seem to indicate no differences with anti TNFs in terms of effectiveness [96].

2.7.2.10 *Safety*

Safety of a biologic depends mainly on two factors: on the one hand, its intrinsic immunosuppressive effect with consequent serious infections and malignancies and, on the other hand, its ability to reduce inflammation, decreasing the use of corticosteroids and the risk of IBD complications.

With what concerns infections, the largest amount of evidence on biologic drug safety has been accumulated for anti-TNFs and registry studies have suggested that anti-TNFs may double the risk of serious infections, compared with other immunomodulators [96] .

2.8 Surgical Treatment

In approximately 70-80% of cases within 20 years of diagnosis, due to acute and chronic complications, such as bowel occlusion, perforation, abscessing or uncontrolled bleeding, and development of neoplasia, or due to failure of medical therapy, surgery is required.

After a first approach of nonsurgical resolution, resective operation proves to be the final solution. Risk factors include previous strictures, perianal disease, and emergency indications [97].

The most widely used surgical solution is the bowel resection, with conservative margins of 2 cm [12 98]. There is an increased risk for short bowel syndrom, if there is less than 200 cm of small bowel that remains in situ.

In case of short length stenosis, previous resections > 100 cm or circumscribed duodenal stenosis, the most widely surgical solution is stricuroplasty, as alternative to resection.

Stricturoplasty is a technique to resolve an internal stenosis without removal of the affected tract, reducing the risk of short bowel syndrome, a well-known complication due to insufficient food absorption [99 100] . There are three types of techniques in which this procedure is performed:

Heineke-Mikulicz type, the most straightforward for short segments, Finney type for intermediate segments and Michelassi technique, used in cases of long stenotic tracts or a series of many short stenoses [101 102].

Laparoscopic techniques are associated with a decreased length of stay and incisional benefits.

Ulcerative colitis

3.1 Definition

Ulcerative colitis (UC) is a chronic inflammatory disease of the large intestine in which lesions invariably can affect the rectum and extend proximally in a continuous and uniform manner to the entire colon.

This disease was first described in 1875 by two English physicians, Wilks and Moxon, who found in the ulceration and inflammation of the entire colon in a young woman who had succumbed to severe bloody diarrhea an early instance of Ulcerative colitis, to be distinguished from diarrheal diseases caused by infectious agents. [103]

Nowadays it is recognized as an inflammatory disease in which the extent of involvement differs for an intraindividual and interindividual variability: in proctitis, the disease is extended to the rectum proctitis, in proctosigmoiditis to the rectus-sigma, in left colitis to the left fissure, subtotal colitis to the transverse colon and in the pancolitis or the entire colon. [13]

The inflammation in UC is limited only to the most superficial layer of the intestinal wall, namely the mucosa, unlike Crohn's disease; the extent and intensity of the inflammatory lesions determine the severity of the clinical picture.[104]

Ulcerative colitis has a chronic relapsing and remitting course, with specific intestinal symptoms such as rectal bleeding and tenesmus, and more general symptoms, such as abdominal pain, diarrhea, fever, and weight loss, involving also extraintestinal manifestations. In the long term, Ulcerative colitis is also associated with the risk of developing adenocarcinoma of the colon [4, 5]

3.2 Epidemiology

This geographical distribution likely reflects the co-participation of genetic and environmental factors associated with increased risk of UC. They include diet, in particular the western one, medications, intestinal bacterial flora, and lifestyle factors such as cigarette smoking that may influence the host's microbiome or immune response to antigens [107].

Surprisingly, in the twenty-first century, studies have found a stabilization in the incidence of UC in developed nations and, in some cases, even a decrease.

However, despite this stabilization, the prevalence of UC has risen dramatically, especially in the Western industrialized countries, potentially due to inclusion of phenotypes with lower mortality, younger ages of onset, and no current definable cure for the disease in an aging cohort of patients [108].

As a consequence, factoring morbidity and mortality related to UC, healthcare and societal costs are substantial.

Although the incidence of UC has reached a stable trend in developed nations, it has risen in many newly industrialized countries, within South America, Asia, Africa, and the Middle East [1 110 111].

Previous studies have shown that IBD tends to occur more commonly in urban regions, correlated with the population to important environmental factors, pollutants, and lifestyle changes, such as in the dietary patterns, which subsequently changes in the host microbiome and adaptive immunity, as it happened in China where the diet began to resemble a western diet and the incidence of UC rose precipitously [112].

Nowadays in Italy, the incidence amounts to 5-10/100,000/ year, with a calculated prevalence of 80-100 patients/100,000 inhabitants, with no significant differences between North and South. Based on the disease exemptions issued in Italy, it can be assumed that there are between 80 and 100,000 patients suffering from Colitis ulcerative colitis [12].

In Europe the situation doesn't differ consistently: the incidence ranges from 0,6 to 24,3 per 100.000 person-years [107 113].

The disease can occur at all ages, but it most frequently begins between the ages of 20 and 40, with no gender predisposition; a positive family history of chronic inflammatory bowel disease is the major risk factor to become ill with OCR, and other recognized factors are abstention from cigarette smoking and a history of failed appendectomy. Approximately 3-5% of patients have a blood relative with Crohn's disease or Ulcerative colitis.

Whether these observational associations reflect actual causal associations, confounding, or reverse causation is unclear [114]. In fact, according to an important metanalysis, the risk of Ulcerative colitis and Crohn's disease in smokers lacks causal evidence [115].

Despite the few epidemiologic data from developing countries, it is a well-known fact that the incidence and prevalence of IBD are increasing over time and in different regions around the world, underlining its emergence as a global disease [114].

3.2.1 Risk factors

3.2.1.1 *Cigarette smoke*

Some studies have shown that cigarette smoking has a protective effect on the development and severity of Ulcerative colitis, which in fact is more common and dolent in non-smokers and especially in ex-smokers, who a 70% higher risk of developing the disease, which is more likely to have a refractory and extensive course, interestingly also compared to patients who have never smoked. Moreover, ex-smokers who resume smoking appear to experience a milder course of disease. However, whether these observational associations reflect actual causal associations, confounding, or reverse causation is unclear [115].

3.2.1.2 *Appendicectomy*

Another association seems to exist epidemiologically between appendectomy and the development of Ulcerative colitis. Cohort studies and a meta-analysis indicate that appendectomy in childhood has a protective effect, with a 69% risk reduction on the incidence and severity of later Ulcerative colitis, based on the hypothesis that the appendix is a gut microbiome reservoir that shapes IgA production and modulates T helper cell function and Interleukin-4 expression [116]. However, not all follow-up studies have confirmed the protective effect of appendectomy and lead it back to the effect of smoking, without a significant beneficial effect. The data on appendectomy are epidemiologically and pathophysiologically interesting, but they have no therapeutic consequence in clinical practice and are therefore not considered in the recommendations [117].

3.3 Etiology

The etiology of Ulcerative colitis is unknown. At present it has been hypothesized that the cause at its origin consists of an abnormal immune response against intestinal lumen antigens in genetically predisposed patients [118].

The mucosa of the gastrointestinal tract is usually exposed to millions of antigens from the food, environment, and microbiome. Normally the outermost layer of the mucosa is covered by a thick layer of mucin, that provides the first line of the gut immune system through both physical separation and antimicrobial properties. However, in UC, the synthesis and secretion of mucin are impaired, resulting in an increased uptaking of these antigens and correlated stimulation of the gut immune system [119], activation of the innate and adaptive immune response, with particularly stimulation of the dendritic cells. These dendritic cells express Toll-Like Receptors, which use pattern recognition of pathogens to signal activation of multiple transcription factors, such as NFkB. NFkB triggers inflammatory cascades, that result in the production of proinflammatory cytokines, such as TNFα, IL 12, and 23. These inflammatory cytokines own the fundamental function of transducing messages through intracellular proteins, such as Janus kinases (JAK), which further potentiate lymphocyte activation and proliferation. It is important to underline that these proinflammatory cytokines are used by many of our currently available treatments for moderate to severe CU, including monoclonal antibodies [120] [121] [107] . However, the expression levels of many cytokines highly expressed in UC, such as IL-1β, IL-6, TNFα, T helper (Th) 1, Th2, and Th17-associated cytokines, depends on disease stage and patient characteristics, inducing many differences in responses to therapy [122] [123].

3.3.1 Genetic

First-degree relatives of people diagnosed with UC have 10-14 times the risk of developing UC themselves: these statistics corroborate the hypothesis that Ulcerative colitis is a polygenic disease, even though only around 10% of patients with UC have a family history of IBD. Moreover, familial cases of Ulcerative colitis appear to be more likely to affect the female sex, and further, the age of onset appears to be lower compared to sporadic cases [124] [123] [125].

3.3.2 Diet

Many studies have taken into consideration the role that diet may play in the development of IBD. Most fibers are fermented within the colon by enzymes, producing short-chain fatty amino acids that can be used as energy by the colonic mucosa. On the one hand some of these fibers may reduce bacterial adherence and translocation, while one the other hand a low intake of them is associated with the consumption of protective colon mucosa, with an increased risk of inflammatory changes.

Moreover, increased consumption of artificial sweeteners has been correlated with a rising incidence of UC [126] [127].

3.3.3 Microbiome

In UC patients it is common to observe disturbances in the composition of the gut microbiome, genetically characterized from the large collection of microbes of the human gut microbiota.

This dysbiosis, with a reduction in bacteria diversity, with high proportions of *Enterobacteriaceae* and a lower proportion of *Firmicutes*/ *Bacteroides*. [128] [129]

The role of intestinal bacterial flora is suggested by several experimental data, and variations in bacterial populations have been identified in humans' gut; it has been proven that probiotics improve intestinal mucosa barrier and immune system function, promoting the secretion of anti-inflammatory factors, thereby inhibiting the growth of harmful bacteria in the intestine. Fecal microbiota transplantation in UC patients has also shown some benefits. However, [130] [131] [132] [133]

3.3.4 Medications

It is a well-known fact that the use of antibiotics alters the gut microbiome, which may have a role in the pathogenesis of UC, especially in childhood, a period in which disturbances to the microbiota in the earliest year of life may affect gut immunity and therefore susceptibility to IBD.

In addition to the use of antibiotics, also the administration of NSAIDs, nonsteroidal anti-inflammatory drugs, increases the risk of UC [134] [123]

3.4 Clinical manifestations

3.4.1 Onset

At the onset, in most cases, symptoms are relatively mild; they can progressively worse if left untreated. In some cases, however, the disease can also begin very acutely, and this is especially the case of extensive colitis already at diagnosis [135]. After the eventual resolution of the onset episode, the natural evolution of UC is characterized by alternating phases of exacerbation, with the reappearance of symptoms and periods of quiescence, with even complete regression of them.

3.4.2 Sign and symptoms

UC symptoms include rectal blood, diarrhea, and tenesmus. In severe episodes, it is also possible to observe tachycardia, weight loss, abdominal guarding, attenuated bowel sounds, and perianal disease [123].

If the extent of inflammation is important, bleeding may be accompanied by diarrhea, even nocturnal, and containing large amounts of visible blood, abdominal pain, and rectal tenesmus. The abdominal pain is usually not very intense, is often spread to all but predominantly left quadrants, and is only partially relieved by evacuation.

3.4.3 Clinical course

The natural course of Ulcerative colitis is characterized by episodes of disease flares alternating with periods of remission, while in only about 5% of patients, the course of the disease may be continuous.

During the acute phase, in which the characteristic symptoms and signs of the clinical picture are evident, rectal bleeding is the most characteristic sign of the acute manifestations [136].

In about 1,1% of cases, the onset is asymptomatic, leading to a better prognosis [137]; in 10 - 20% of cases the disease has a chronically active course without quiescent; in a small percentage of cases, after the initial acute phase, no further relapses are observed and the disease shows no further sign of itself, meaning that probably the real diagnosis may be another form of colitis, such as the acute self-limiting colitis;

in about 15 % of cases the evolution is toward the grave form, requiring intensive or semi-intensive medical management, despite the prevention of relapses through medical therapy[138] [139].

In patients with the typical course, the acute phases following onset are also characterized by signs and symptoms whose intensity is generally proportional to the extent and severity of organ involvement.

In some cases, the bleeding may be of such intensity that it represents a true emergency in the management of the patient, with the necessity of numerous hemotransfusions and a possible indication for emergency surgery [140].

3.4.4 Complications

Evidence has shown complications of UC in esophageal, stomachi, and duodenal mucosal areas of damage [141]. Also, perimyocarditis can be observed, even if it is not very clear if it is a complication of the disease or a hypersensitivity idiosyncratic reaction from the use of mesalazine [142].

However, the two main complications of Ulcerative colitis are hemorrhage and toxic megacolon.

The main characteristics of toxic megacolon are signs of systemic toxicity and severe colonic distension, even up to 7 cm, because of the extension of the disease to all the layers, including the muscle layer, which may undergo ischemic necrosis. Diagnosis is made by clinical evaluation for systemic toxicity and imaging studies depicting colonic dilatation, such as computed tomography scanning and transabdominal intestinal ultrasound [143].

3.5 Diagnosis

3.5.1 Criteria for diagnosis

3.5.1.2 *Diagnosis by natural history*

The diagnosis is based on a combination of typical findings in the history, endoscopy, sonographic/radiological techniques and histopathology resulted from the biopsy collected during endoscopy or from the workup of surgical specimens. Normal findings on histopathology of mucosal biopsies exclude active Ulcerative colitis.

Once the diagnosis is established, approximately 10% of patients change to Crohn's disease within the first 5 years of diagnosis, or the diagnosis of inflammatory bowel disease is discarded. For this reason, it is always important to execute an endoscopic and histopathologic confirmation in case of a doubtful diagnosis.
In a little number of patients, it is also very hard to distinguish Ulcerative colitis and Crohn's disease: this is the case of the "indeterminate colitis".[144]
The presence of rectal bleeding without diarrhea and the limitation of the inflammation to the rectum can postpone the real diagnosis, initially posed as hemorrhoids: this situation should be avoided because of the proven tendency of proctitis and more distal forms to extension to more extensive forms.
Physical examination is fundamental to the diagnosis: growth failure or pubertal delay as well as pallor suggestive of anemia should be considered for this disease. Significant objective findings are observed only in particularly severe exacerbations, with abdominal distension and decreased bowel peristalsis.

3.5.1.3 *Biochemical investigations*

In the context of haematochemical examinations, it is possible to observe, during active phases, hypochromic microcytic sideropenic anemia in more than half of the cases, secondary to chronic blood loss, hypoalbuminemia, due to intestinal protido-dispersion, an increase in indices of inflammation, particularly ESR and CRP, index of disease activity and a predictor of the course. In addition, it is common to observe, during the most intense phases of activity, leukocytosis, hypokalemia, and metabolic alkalosis, together with an increase in platelets, fibrinogen, and mucoproteins [123].
In order to exclude that the patient's clinical picture is caused by an infection or parasitic infestation of the intestine, it is necessary to perform, especially at the time of initial diagnostic framing, a stool culture and a search for parasites and their eggs, the latter on at least three samples to increase sensitivity. Moreover, it is essential to look for fecal positivity for Clostridium difficile toxin and/or mucosal positivity for Cytomegalovirus infection, because it may lead to important changes in therapy.
Another laboratory exams include the measurement of fecal excretion of proteins that are markers of local inflammation, such as calprotectin and lactoferrin. These tests seem to have both good predictivity in differentiating an organic disease from

a functional disorder and good predictive value of recurrence in patients with Ulcerative colitis in remission [123].

3.5.1.4 *Endoscopic investigations*

The key examination in the diagnosis of UC is ileocolonoscopy, with at least 2 biopsies from each inflamed area. Its purpose is to distinguish it from other clinical forms with a similar presentation, to evaluate the extent and endoscopic activity and to obtain biopsy specimens for histologic evaluation, which is important for diagnostic confirmation.

In more severe colitis, moreover, prudent endoscopic examination, perhaps limited to distal tracts, also has prognostic utility because certain findings, such as the presence of deep ulcers and denudation of the colic mucosa, are associated with an increased risk of surgical intervention. [145]

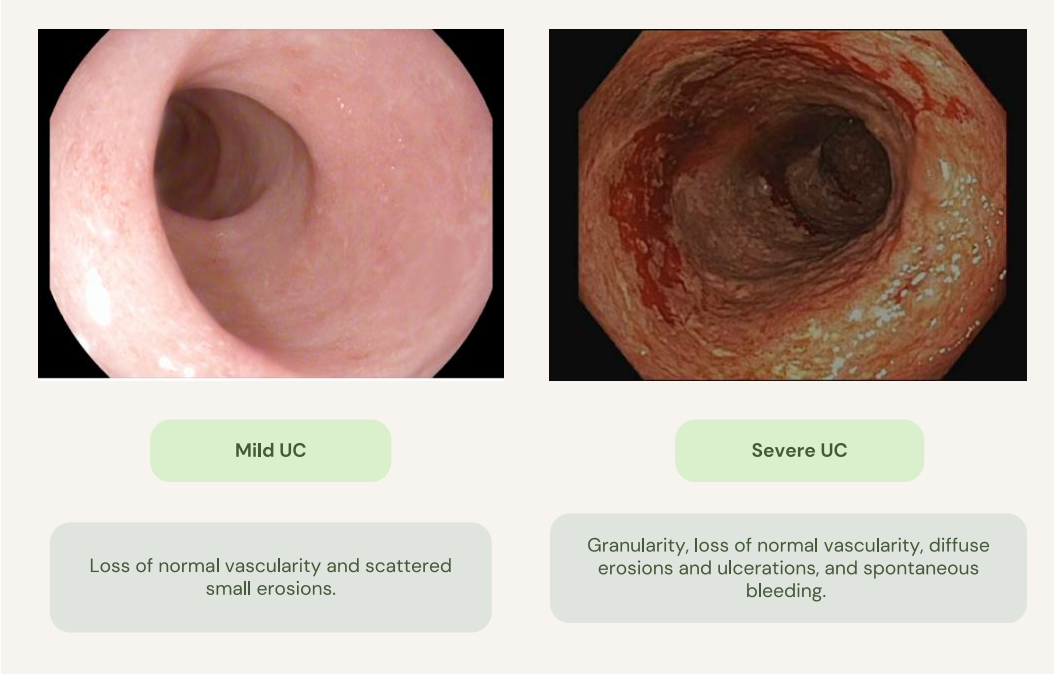


Figure 5. *Mild UC, left, and severe UC, right, at endoscopic examination.*
Modified from Maxine A. Papadakis, Stephen J. McPhee, Michael W. Rabow, Kenneth R. McQuaid: Current Medical Diagnosis & Treatment 2023.

In conclusion, UC diagnosis and therapy are based on a combination of histological and endoscopic scorings which are difficult to evaluate in an objective manner. Artificial intelligence might overcome the current issues of inter-observer variability, repetitive need for biopsies, and estimation of disease activity medicine currently encourages [146].

3.5.1.5 *Radiological investigations*

Transabdominal ultrasound examination has emerged as an imaging modality in IBD given the benefits of being noninvasive, less expensive, and well-tolerated, with an accuracy of 95% in combination with color Doppler imaging: in UC it is possible to observe an increased bowel wall thickness greater than 4 mm.
Moreover, in patients with relevant gastrointestinal symptoms not explained by endoscopic fundings, cross-sectional imaging such as CT scans or MRI or video capsule endoscopy may be warranted. A CT scan allows the detection of bowel abnormalities, such as thickness and abnormal enhancement, but with the main disadvantage of ionizing radiations.
On the contrary, MRI, lacks radiation and has a superior soft tissue contrast, but it is more expensive and requires a longer scan duration. [147]
In general, radiologic examination of the abdomen without contrast medium is useful in patients in the acute severe or fulminant phase, to evaluate for signs of perforation, gaseous distention of the colon, or other premonitory signs of probable adverse evolution of the picture.

3.5.2 Differential diagnosis

The initial presentation of Ulcerative colitis is indistinguishable from other causes of colitis, clinically as well as endoscopically. Thus, the diagnosis of idiopathic Ulcerative colitis is reached after excluding other known causes of colitis. Infectious colitis should be excluded by sending stool specimens for routine testing to exclude *Salmonella, Shigella*, *Campylobacter*, *E coli*, *C difficile*, and amebiasis[148].
Colitis from *Cytomegalovirus* occurs in immunocompromised patients, including patients receiving prolonged corticosteroid therapy, and is diagnosed on mucosal biopsy.
Gonorrhea, *Chlamydial* infection, *Herpes*, and *Syphilis* have to be considered in sexually active patients with proctitis. The clinical picture may mimic that of an acute infectious colitis also because of the possible presence of systemic signs, such as fever, dehydration, electrolyte imbalances, and tachycardia. Moreover, on some occasions, an infection with intestine may promote the onset or flare-up of UC symptoms, making differential diagnosis even more difficult. However, in most of

cases, the course reveals the chronic character of the Ulcerative colitis, it this is the case.

It is possible to confuse it with Crohn's disease involving the colon but not the small intestine; moreover, in about 10% of patients, a distinction between Crohn's disease and Ulcerative colitis may not be possible. The absence of rectal blood discharge, symptoms in active smokers, granuloma, patchy disease, cecal patch, upper GT tract involvement should be more suggestive of Crohn's disease. However sometimes it's very difficult to differentiating Crohn's disease from Ulcerative colitis [147].

Infectious or drug-induced colitis should be differentiated anamnestically, if possible.

3.5.3 Extraintestinal manifestations

About one-third of patients with Ulcerative colitis also have extraintestinal manifestations.

Some examples are hepatic diseases, such as steatosis and sclerosant cholangitis, cutaneous ones, such as nodoses erythema, and gangrenous pyoderma, articular diseases, such as peripheric arthritis and anchyloses spondylitis and ocular diseases, such as episcleritis and uveitis.

Many of them, particularly cutaneous and ocular ones, are often related to disease activity and tend to improve in the quiescent stages, with a resolution after colectomy [149] [12].

3.5.4 Activity and severity indices in Ulcerative Colitis

Disease activity refers to a cross-sectional, moment-in-time assessment of inflammation.

On the contrary, disease severity may include more longitudinal and historical factors, such as prior biologic failure, history of maximum disease extent, and health care use metrics such as hospitalization and disability scoring tools. [150]

The three main areas to be assessed in UC are clinical activity, endoscopic disease distribution, and gross endoscopic severity.

Truelove and Witts's classification (Table IV) of clinical disease defines mild disease as less than 4 bloody stools per day, with minimal systemic signs and symptoms.

Severe disease is characterized by having more than 6 bloody stools per day, with evidence of systemic signs and symptoms.

Fulminant disease entails more than 10 bloody stools per day with systemic signs and symptoms; complications such as abdominal distension and anemia may be developed [147].

TRUELOVE AND WITT

	Mild	Moderate	Severe
Frequency of stool (n^/day)	< 4	4-6	>6
Blood in stool	None or small	Streaks	Visible
Fever	< 37,1 °C	37,1 - 37,8°C	> 37,8°C
Tachycardia	<70/ min	70 - 90/ min	< 90/ min
Anemia	> 11 g/dl	10,5 - 11 g/dl	< 10,5 g/dl
ESR	< 30	< 30	> 30

Table IV. Truelove and Witt's classification.

Since the Truelove and Witts study in 1955, several severity scores have been developed using several variables, including clinical symptoms, laboratory studies, and endoscopic assessment, to influence the correct choice of therapy and monitor the response.

One of the most popular and commonly used is the *Mayo score* [151]. It combines symptoms with endoscopic findings and physician global assessment (Table V).

It is organized into four subscores, each scored 0, 1, 2, and 3: rectal bleeding, stool frequency, physician's global assessment, and endoscopy subscore.

The 4 subscores are added to give the Mayo score, which can range from 0 to 12, where 12 indicates the highest disease severity.

It is also possible to calculate a *partial Mayo score*, excluding the endoscopic subscore, and a *modified Mayo score*, excluding the physician' global assessment.

The use of the modified MCS, without the subjective assessment of the physician, is recommended; however, the availability he repeated endoscopies to calculate the endoscopic subscores is limited, due to the invasiveness of the procedure, resulting in incomplete longitudinal data [150].

MAYO SCORE

Stool frequency	Normal number of stool for this patient	0
	1-2 stools more than normal	1
	3-4 stools more than normal	2
	5 or more stools than normal	3
Rectal bleeding	No blood seen	0
	Streaks of blood with stool less than half of the time	1
	Obvious blood with stool most of the time	2
	Blood alone passed	3

Table V. Mayo Score classification: stool frequency, rectal bleeding, sigmoidoscopy and physician's global assessment.

Findings of flexible sigmoidoscopy	Normal or inactive disease	0
	Mild disease (erythema, decreased vascular pattern, mild friability)	1
	Moderate disease (marked erythema, absent vascular pattern, friability, erosions)	2
	Severe disease (spontaneous bleeding, ulceration)	3
Physician's global assessment	Normal	0
	Mild disease	1
	Moderate disease	2
	Severe disease	3

Table V. Mayo Score classification: stool frequency, rectal bleeding, sigmoidoscopy and physician's global assessment.

3.5.6 Endoscopic manifestations

3.5.6.1 *Macroscopic appearance*

At the time of diagnosis, lesions affect only the rectum in 10-15%, rectum-sigma in 30-40%, the colon up to the splenic flexure in 20%, up to the transverse in 15%, and the entire colon in 15% of cases.

Depending on the onset situation, the *Montreal classification* divides the disease in proctitis, left-sided colitis, and extensive colitis with extension beyond the left flexure. [152] The rectum is typically affected in all patients, and lesions may extend proximally in a uniform and continuous manner.

In general, the macroscopic appearance of mild UC includes continuous colonic inflammation starting at the rectum characterized by erythema, loss of normal vascular pattern, and granularity.

Ulcerations are common, usually extensive on the surface but shallow in depth.

As the severity of the disease increases, it is possible to note erosions, friability, bleeding, and ulcerations [147]. Over time, the development of polypoid lesions, such as pseudopolypes, of varying shape, can be a result of the exuberant epithelial regeneration on flaps of previous ulcers.

Changes in the extent of the lesions may be observed in the same patient as the disease progresses (Table VI).

Classification of Ulcerative colitis according to the extent of the disease is useful for two main reasons: on the one hand, the localization of the disease determines the use of topical and/or systemic application of medication, especially 5-ASA preparations.

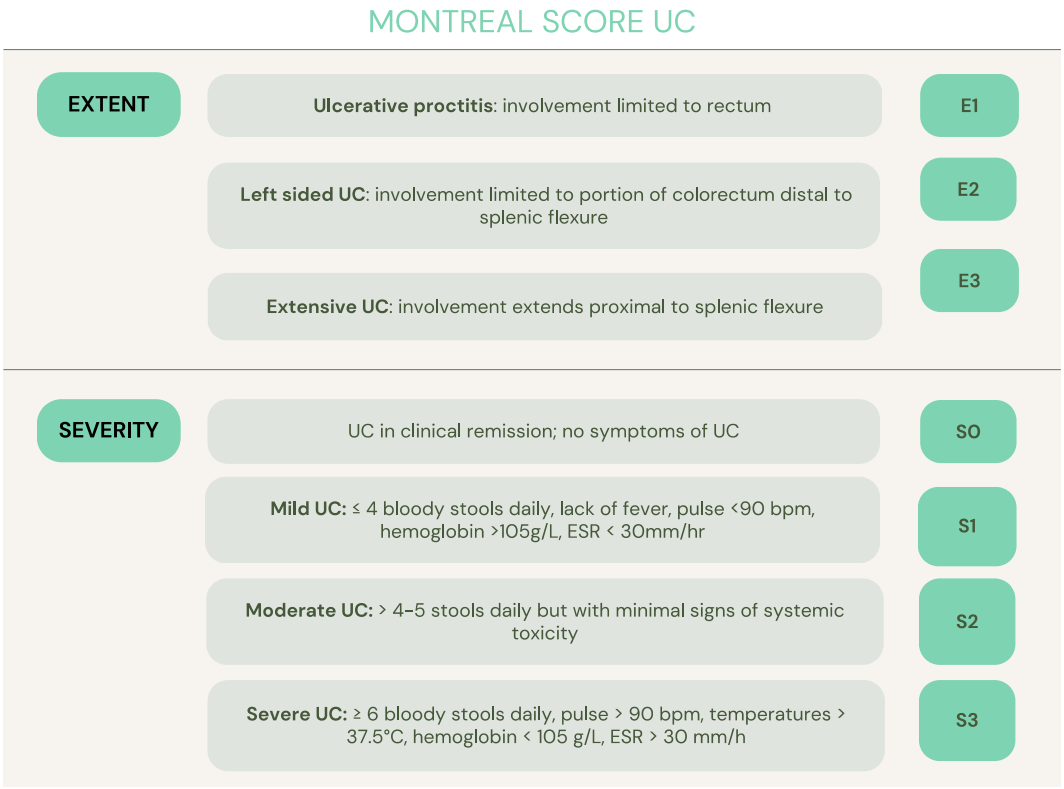


Table VI. Montreal score UC classification: extent and severity of the disease.

3.5.6.2 *Microscopic appearance*

The microscopic appearance of UC shows that inflammation is typically limited to the mucosa and the most superficial part of the submucosa, in a continuous pattern without skipping.

Histologic changes consist of edema of the lamina propria with dilated and congested vessels and extravasation of hematins and leukocytes, resulting in an inflammatory infiltrate in the lamina propria.

Neutrophil granulocytes tend to invade the crypt epithelium, resulting in the cryptic abscesses characteristic but not specific to the disease.

Damage to the epithelium is a clear hallmark of Ulcerative colitis and, in addition to the evidence of erosive lesions and ulcers, is also indicated by the reduction of the secretion of caliciform cells and the disruption of glandular architecture by the reduction and distortion of crypts.

Finding a single feature of chronic inflammation is not diagnostic of UC, yet there are no clear criteria for the features to establish the diagnosis.

Once complete remission is induced, the histologic picture no longer shows signs of active inflammation; however, changes in the glandular architecture remain, with atrophic and distorted crypts, indicative of a previous episode of activity.

In patients with long-standing disease, dysplasia may be observed, divided into low and high grades, with an increased risk of developing adenocarcinoma [147].

Histopathologic criteria that should be used when evaluating biopsies for the diagnosis of Ulcerative colitis are [123]:

- diffuse panmucosal chronic inflammation, with the presence of lymphocytes and plasmacells, in combination with disruption of crypt architecture or crypt atrophy;
- plasmacytosis in the basal mucosal stroma;
- Paneth cell metaplasia distal to the right colonic flexure;
- reduction in the number of goblet cells or mucin content of single cells, continuous distribution of inflammatory and structural mucosal changes, decreasing gradient from distal to proximal.

3.6 Medical treatment

Since the etiology of the disease is unknown, there are no curative therapies available to target a specific pathogenetic process.
Medical therapy of Ulcerative colitis aims to control and regress acute phase inflammation through induction therapy and maintain the quiescent state of disease through maintenance therapy [153].

From a practical point of view, it can be very useful to distinguish the activity of Ulcerative colitis into mild, moderate, and severe, according to the criteria of Truelove and Witts [151].
In addition to clinical disease activity, the therapeutic approach in patients with UC is also guided by the extent of the disease, in order to decide the most appropriate drug or formulation.

3.6.1 Mild - moderate forms of distal colitis

If Ulcerative colitis is confined to the rectum or rectosigmoid region, the symptoms are generally mild to moderate, but distressing.
Patients may be treated with topical mesalamine, topical corticosteroids, or oral aminosalicylates, such as mesalamine or 5-ASA, the active metabolite of sulfasalazine, according to patient preference and cost considerations.
Topical mesalamine is the drug of choice and is superior to topical corticosteroids and oral sulfasalazine; it is administered as a suppository, for 4 - 8 weeks, with 75% of patients improving.
In case of mesalamine refusal or impossibility to manage topical therapy, oral mesalamine is the drug of choice. Topical corticosteroids, such as hydrocortisone and budesonide foam, cost less than mesalamine; however, they are also less effective, and for this reason they are administered in addiction in case of failed improvement.
Most patients with proctitis or proctosigmoiditis who achieve a complete remission with oral or rectal sulfasalazine should continue indefinitely on the same therapy to reduce the likelihood of symptomatic relapse, decreasing the 12-month relapse rate from 75% to less than 40% [154] [148].

3.6.2 Mild - moderate forms of colitis

Disease extending above the sigmoid colon is best treated with both an oral and rectal 5-ASA therapy. For induction of remission, the optimal dose is sulfasalazine 2–3 g oral once daily, in combination with mesalamine 1g suppository for 4–8 weeks, with 75% of patients improving. Some patients may prefer to initiate therapy with an oral agent, adding topical therapy if initial response is inadequate; however, sulfasalazine has many side effects, and for this reason should be always administered with folic acid.

Patients with mild to moderate colitis who do not improve within 4–8 weeks of 5-ASA therapy should have an oral corticosteroid therapy added with budesonide, preferred for the few corticosteroid-associated side effects, or prednisone.

For patients who require more than one course of corticosteroid therapy every 1–2 years for symptomatic relapse, treatment should be "stepped up" to include a thiopurine, such as azathioprine, or a biologic agent [154] [148].

3.6.3 Moderate - severe forms of colitis

The treatment of outpatients with moderate to severe Ulcerative colitis is evolving quickly due to the recent advent of several biologic therapies, that have also been included as a first-line therapy to induce remission, together with corticosteroids [155].

An oral corticosteroid, such as prednisone or methylprednisolone, is commonly prescribed as the first-line agent for non-hospitalized patients with moderate to severe colitis or as second-line therapy in patients in whom initial 5-ASA therapy was ineffective.

Prednisone is administered with an initial oral dose of 40 mg daily, with a subsequent tapering of 5–10 mg/week, but should not be continued long-term because of an unacceptable risk of adverse side effect. The maintenance therapy consists of oral mesalamine, 2–4 g/day.

In case of failed response to prednisone, in about 30% of patients, the addition of a thiopurine is sometimes used to promote complete steroid withdrawal and maintain long-term remission.

Biologic agents or small molecules are recommended for patients in whom corticosteroids cannot be completely withdrawn or who require more than one course of corticosteroids every 1- 2 years.

3.6.3.1 Biologics

Due to their more favorable safety profile and efficacy in maintaining long-term remission, anti-TNF antibodies, such as infliximab, adalimumab, golimumab or anti-integrin antibody, such as vedolizumab or anti IL-12/23 antibody, such as Ustekinumab, or JAK inhibitors such as tofacitinib, are become a promising new form of therapy for moderate to severe colitis.

3.6.3.1.1 Patients naïve to other biological therapies

A 2020 guideline from AGA, the American Gastroenterological Association, recommends either vedolizumab or infliximab, because of their highest rankings of all biologic agents for induction of clinical remission [156].

On the one hand, infliximab proves to be the more effective agent, on the other hand, vedolizumab ends up being the preferred first-line therapy in older adults with medical comorbidities, since its reduced infectious complications.

Infliximab is administered with an induction dose of 5 mg/kg intravenously at 0, 2, and 6 weeks, resulting in clinical response in 65% of patients. The maintenance treatment with the same drug, every 4–8 weeks, shows clinical improvement or remission in approximately 50% of patients, with a clear superiority of intravenous infliximab over the other anti-TNF agents, such as golimumab and adalimumab, administered through a self-injection in the subcutis.

Vedolizumab is administered with an induction dose of 300 mg intravenously at 0, 2, and 6 weeks, resulting in clinical response in 47.1% of patients. The maintenance treatment with the same drug, every 8 weeks, shows clinical remission in 31.3% and endoscopic improvement in 39.7%, significantly more consistent than in other biologics, such as adalimumab[154].

Together with an anti-TNF agent, it is sometimes common to add an immunomodulator, such as azathioprine, mercaptopurine for the first year, to

increase the likelihood of disease remission and to reduce the development of antibodies that may result in secondary loss of response to anti-TNF therapies.
If the patient prefers a monotherapy approach, proactive drug monitoring of serum trough levels and anti-drug antibody titers should be obtained during induction and maintenance therapy in order to optimize drug dosing.
Ozanimod is a once-daily oral small molecule that was approved by the FDA in 2021 for the treatment of moderate to severe Ulcerative colitis; clinical experience with this novel agent is limited, but it should be considered in non-hospitalized, biologically naive patients who prefer the convenience of oral therapy.

3.6.3.1.2 Patients not naïve to other biological therapies

In patients with moderate to severe colitis who have not responded to or lost response to infliximab, the 2020 AGA treatment guideline recommends Ustekinumab or tofacitinib rather than vedolizumab or adalimumab as second-line therapy based on network meta-analyses [157] [158].
Ustekinumab is an anti-subunit p40 of the interleukin 12/23 antibody therapy and has proved to be an emerging treatment option for patients when standard therapies have been ineffective.
In fact, in the pathogenesis of Ulcerative colitis, IL-12 drives the Th1 pathway, characterized by signature cytokines IFNγ and TNF, whereas IL-23 promotes the Th17 pathway and stimulates invariant NKT cells [159]. Consequently, blockade of IL-23 and IL-17 seemed to be an effective therapeutic strategy in animal models of intestinal bowel disease, supporting the role of these cytokines in the pathogenesis of IBD (Figure 6).
Ustekinumab is already been used in Crohn's disease treatment, and it is becoming available in the other form of IBD, Ulcerative colitis [160].
It has been statistically demonstrated that Ustekinumab is more effective than placebo for inducing and maintaining remission in patients with moderate-to-severe ulcerative colitis, with confirmation through 3 years and without safety signals [161] [162]. Moreover, its rapid efficacy and onset have demonstrated an early symptomatic improvement [163] and it is statistically confirmed that Ustekinumab decreases circulating Th17 cells in Ulcerative Colitis [164] [159].

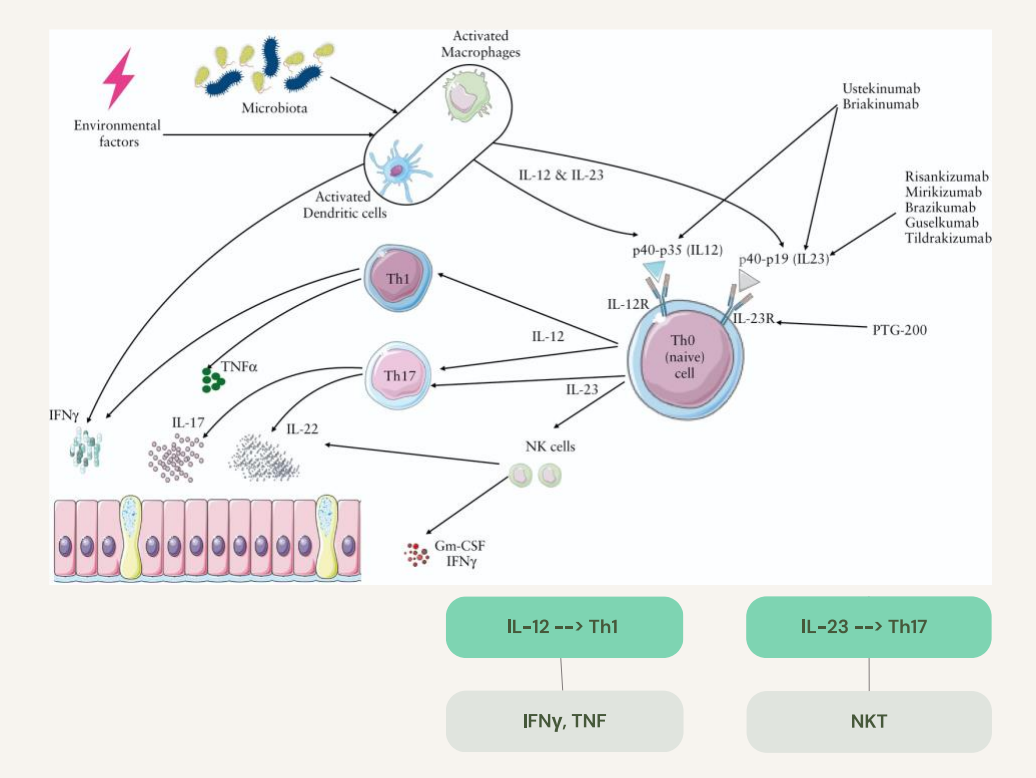


Figure 6. Schematic visualization of IL12 and IL23's pathways in the pathogenesis of inflammatory bowel disease [159]

3.6.1.4 Severe colitis

In the case of severe colitis, after the previous treatments for moderate to severe colitis, it is possible to add a continuous intravenous infusion of cyclosporine (2–4 mg/kg/day), which benefits 60–75% of patients. However, it has been reported a higher association with toxicity and adverse events, such as nephrotoxicity, seizures, infection, and hypertension.

Up to two-thirds of responders may be maintained in remission with a combination of long-term therapy with azathioprine or mercaptopurine.

3.7 Surgical treatment

In approximately 15 % of cases, due to complications or lack of medical therapy response, surgery is required. Since UC involves mainly colon and rectum, removing these two anatomical tracts proves to be a valid and curative solution [98].

The most frequent surgery is *restorative proctocolectomy with the creation of an ileoanal pouch*, as a reservoir packed with the last portion of the ileum, that is anastomosed to the anal canal.

The first part of the surgery consists in the total proctocolectomy and the creation of the pouch and the loop ileostomy, whereas the second part, after several months, involves the takedown of this continent ileostomy, with recanalization of the tract. Complications, such as sepsis, dysfunction and pouchitis may be possible; [165] contraindications to a continent ileostomy include obesity, cognitive impairment and a prior significant small bowel resection.

Another type of surgery that can be performed is the *colectomy with ileo-rectal-anastomosis*. It consists in removing the colon while preserving the rectum and intestinal transit through a colorectal anastomosis. It is indicated in cases of mildly active disease of the rectum, poor anal function, anatomical hindrances to create the pouch, plans of pregnancy, or the need for a quick return to social activities [166] [167]. The procedure is performed in a single surgical time, without a protective ostomy, and ensures good bowel function in case of a rectal disease remission.

Contraindications include active proctitis or dysplasia in the rectum [168].

Risk factor and outcomes of the restorative proctocolectomy with ileal pouch- anal anastomosis have shown that in case of preoperative extraintestinal clinical manifestations and acute proctitis, a close follow-up should be performed to identify and treat early onset of pouchitis, through a medical/microbiological prophylaxis [169].

Aim

4.1 Primary aim:

The primary objective consisted in evaluating the effectiveness of Ustekinumab (*Stelara*®) for the treatment of Crohn's Disease (CD) and Ulcerative Colitis (UC) refractory to conventional therapies.

The effectiveness was evaluated through clinical remission, clinical response, biochemical remission, endoscopic response, and endoscopic remission after 3 months, 6 months and 1 year in both conditions.

4.2 Secondary aim:

The secondary aim was to assess the safety profile of Ustekinumab, defined as the absence of adverse events during the whole follow-up, both in CD and UC. Adverse events were divided into early, which occurred during infusions, and late, which occurred at least a week after infusion, and defined as mild or severe.

Materials and methods

5.1 Study design

This study is a single-center observational retrospective study. Between January 2019 and April 2023, 131 IBD patients, referred to the regional center of Inflammatory Bowel Diseases at the Gastroenterology Unit of the University of Padua Hospital Company, have been considered because not responsive to standard medical therapy and therefore treated with Ustekinumab. Out of them, 47/131 were previously diagnosed with UC and 87/131 with MC. Of note, out of many more patients evaluated, only these 131 had all the clinical data available and that they had completed at least the induction cycle. The other patients have been excluded due to lack of data (16 in CD and 14 in UC).

5.2 Criteria for inclusion:

- Signature of informed consent;
- Age over 18 years old;
- IBD patients diagnosed with CD or UC through ECCO-ESGAR guidelines[32] in terms of endoscopic and/or radiological and/or histological criteria;
- Signature of informed consent;

5.3 Criteria for exclusion:

- Patients previously enrolled in a randomized clinical trial;
- Age under 18;
- Lack of informed consent;
- Patients with short-bowel syndrome;
- Patients with extensive bowel resection;
- Patients with neoplasia;
- Patients with psychiatric illness

5.4 Study program

All evaluated patients signed an informed written consent in order to authorize data collection and inclusion of such data in the Padua Registry of immune-mediated gastrointestinal Registry. Such consent was also given under and for the effects of D. Lgs. n. 196 of 30 June 2003 and s.m.i.

5.5 Data collection

Demographic and clinical data, before and after Ustekinumab introduction, was collected upon enrollment through an electronic collection card; subsequently this data was organized in E-Health software and a common database was created. For both CD and UC patients, the following data was collected before Ustekinumab introduction: gender, age at diagnosis, duration of illness, localization of the disease, previous surgery, previous therapies with immunosuppressants, concomitant therapies. In addition, reactive C Protein and fecal calprotectin were assessed before and after the administration of Ustekinumab.

The clinical activity has been evaluated through some clinical scores, such as the *Harvey-Bradshaw Index* (HBI, Table VII) for CD patients and the *Partial Mayo Score* (pMayo, Table X) for UC patients. The endoscopic activity has been assessed through *the Rutgeerts score* (Table VIII) for CD patients after surgical resection and the *Simple Endoscopic Score for Crohn's Disease (SES-CD,* Table IX*)* per CD patients who didn't undergo any surgical procedure, whereas through the *Endoscopic Mayo Score* in UC patients (Table XI).

Moreover, the following clinical scores, *HBI Score* in CD patients and *partial Mayo score* in UC patients, have been calculated, together with the endoscopic scores such as *Rutgeerts score* and *SES score* in CD and *endoscopic Mayo score* in UC, before and after the induction of Ustekinumab. Likewise, clinical and endoscopic data were collected after Ustekinumab administration: after 3 months, after 6 months and after 1 year.

5.6 Definitions of response and remission in CD and UC

5.6.1 Crohn's disease

- Clinical response after 3 months and 1 year is indicated by a reduction in Harvey Bradshaw Index score of at least 3 points.
- Clinical remission after 3 months and 1 year is indicated by a Harvey Bradshaw Index score <5.
- Biochemical remission after 3 months and 1 year is indicated by fecal calprotectin levels <250 µg/g.
- Endoscopic response after 1 year is indicated by a decrease in Simple Endoscopic Score or Rutgeerts Score.
- Endoscopic remission after 1 year is indicated by a Simple Endoscopic Score ≤ 2 or Rutgeerts Score ≤ 1.

Crohn's disease scores

HARVEY – BRADSHAW INDEX (HBI)

Variable	Description	Scoring
1	General- well being	0, very well; 1, slightly below average; 2, poor; 3, very poor; 4 terrible
2	Abdominal pain	0, none; 1, mild; 2, moderate; 3, severe
3	Number of liquid stools daily	1 per occurance
4	Abdominal mass	0, none; 1, dubious; 2, definite; 3, definite and tender
5	Complications	1 per item: arthralgia, uveitis, erythema nodosum, aphtous ulcer, pyoderma gangrenosum, anal fissure, new fistula, abscess

Table VII. Harvey – Bradshaw Index (HBI) Score.

RUTGEERTS SCORE

ENDOSCOPIC FINDINGS	SEVERITY
No lesions	0
<5 aphthous lesions in the neoterminal ileum	1
>5 anastomotic lesions with passable stenosis (skip lesions); or lesions in the area of the anastomosis	2
Diffuse ileitis	3
Diffuse ileitis with deep ulcerations and/or Stenosis	4

Table VIII. Endoscopic outcome assessment, Rutgeerts score classification.

SIMPLE ENDOSCOPIC SCORE (SIS-CD)

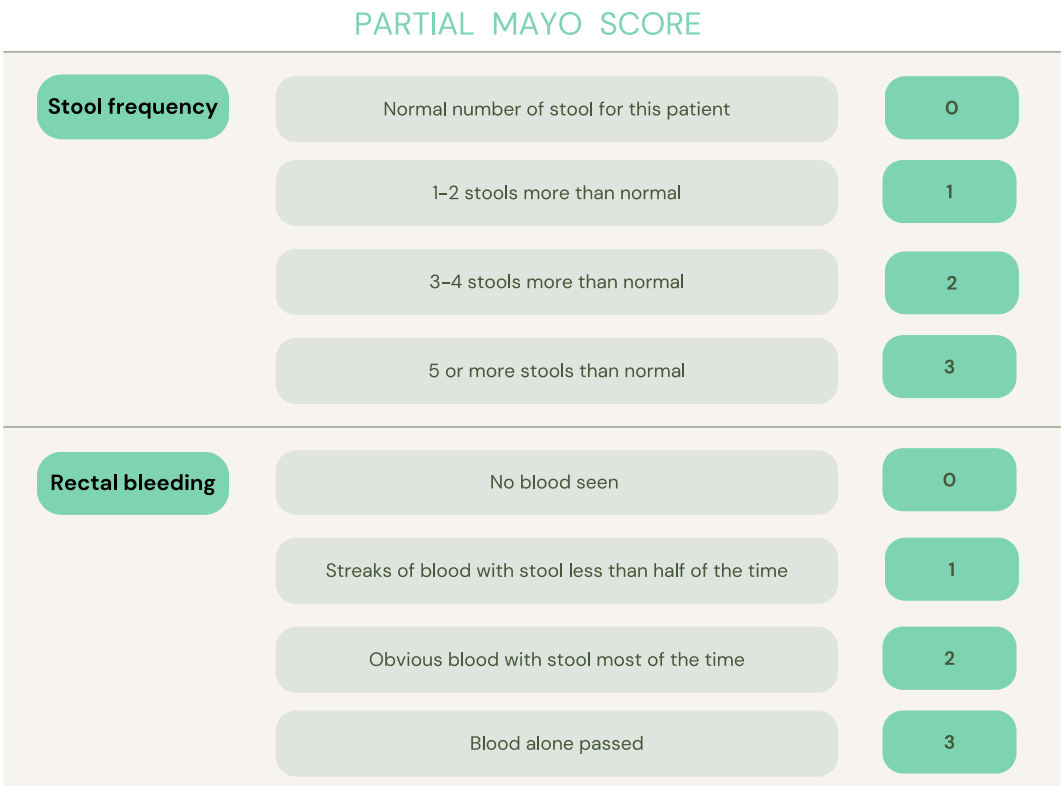
Variable	0	1	2	3
Size of ulcers	None	Aphtous ulcers	Large ulcers	Very large ulcers
Diameters of ulcers	None	0,1-0,5cm	0,5-2 cm	> 2 cm
Ulcerated surface	None	< 10%	10 - 30%	> 30%
Affected surface	Unaffected segment	< 50%	50 - 75%	> 75%
Narrowings	None	Single, can be passed	Multiple, can be passed	Cannot be passed

Table IX. Endoscopic outcome assessment, Simple Endoscopic Score.

5.6.2 Ulcerative Colitis

- Clinical response after 3 months and 1 year is indicated by a decrease in the Mayo score from baseline of 30% or more and 3 or more points.
- Clinical remission after 3 months and 1 year is indicated by a Mayo score of 2 or fewer points.
- Biochemical remission after 3 month and 1 year is indicated by fecal calprotectin levels <250 µg/g.
- Endoscopic response after 1 year is indicated by a decrease in Mayo endoscopic score.
- Endoscopic remission after 1 year is indicated by a Mayo endoscopic score of 1 or less.

Ulcerative colitis scores



Physician's global assessment	Normal	0
	Mild disease	1
	Moderate disease	2
	Severe disease	3

Table X. Clinical outcome assessment, partial Mayo Score classification.

ENDOSCOPIC MAYO SCORE

Findings of flexible sigmoidoscopy	Normal or inactive disease	0
	Mild disease (erythema, decreased vascular pattern, mild friability)	1
	Moderate disease (marked erythema, absent vascular pattern, friability, erosions)	2
	Severe disease (spontaneous bleeding, ulceration)	3

Table XI. Endoscopic outcome assessment, endoscopic Mayo Score classification.

In particular, in CD patients, it was assessed the response to the biologic through a clinical approach, thanks to the evaluation of the disease severity index (Harvey Bradshaw Index), a biochemical approach, with the measure of fecal calprotectin, and an endoscopic approach, with the assessment of the endoscopic activity (Simple Endoscopic Score). Similarly, in UC patients, the response to the biologic was evaluated through a clinical approach, with the reduction of the partial Mayo score, a biochemical approach, with the level of fecal calprotectin, and an endoscopic approach, the reduction of the endoscopic Mayo score and the mucosal healing during follow-up. Moreover, steroid-free clinical remission, defined as a Mayo score<5 and absence of steroids use for patients with UC after Ustekinumab induction was evaluated.

5.7 Data collection

Data collection has been performed through an electronic Case Report Form, which contained information on general data of the patient; habits of life; remote pathological history; characteristics of the pathology at the diagnosis, and examination of enrollment, such as localization according to the current classifications, symptoms, and signs at onset; extraintestinal manifestations; drug therapy to diagnosis and inclusion visit; possible surgical interventions.

5.8 Confidentiality of data

In compliance with the current legal provisions on the protection of individuals about the processing of personal data, under European Union Regulation 679/2016 and s.m.i. and D. Lgs. n. 196 of 30 June 2003, supplemented with the amendments introduced by Legislative Decree no. 10 August 2018, n. 101 and s.m.i., as well as the measures / general authorizations adopted by the Guarantor for the processing of personal data in this regard, the results of this study may be published scientifically without prejudice to the anonymity of the parties involved.

5.9 Statistical analysis

The statistical analysis has been performed using the IBM SPSS Statistics 26 program. A descriptive output has been created and the comparison between variables has been made by parametric and non-parametric tests with a significance level of 95%. Results were considered statistically significant at p-value ≤ 0.05. Normal variables were reported as means with standard deviations (SDs), and non normal variables as median and interquartile range or frequencies.

The normality of continuous variables was assessed by means of the Shapiro-Wilk test and Q-Q plots. Comparison between two groups for primary endpoint analysis was performed as appropriate by Student's t test, Mann-Whitney test or Kruskal-Wallis test.. Wilcoxon test and McNemar test were used to assess dependent variables.

Results

6.1 Clinical and demographic characterization of IBD patients at baseline

The main characteristics of the population of CD and UC patients at baseline are summarized in the tables below (Table XII).

Baseline characteristics	**Crohn's disease**	**Ulcerative colitis**
Males *n^ (%)*	52 (61,90%)	30 (63,83%)
Females *n^ (%)*	32 (38,10%)	17 (36,17%)
Smokers *n^ (%)*	10 (11,90%)	5 (19,64%)
Previous intestinal resection *n^ (%)*	53 (63,10%)	3 (3,75%)
Previous steroids *n^ (%)*	82 (97,62%)	47 (100%)
Median age at diagnosis *(y, min-max, IQR)*	28, 6-74, 20	37, 2-73, 25
Previous immunosuppressors *n^ (%)*	59 (69,05%)	28 (59,57%)
Previous biologics *n^ (%)*	84 (100%)	42 (89,36%)
>2 previous biologics *n^ (%)*	64 (76,19%)	32 (68,09%)
Infliximab *n^ (%)*	68 (80,95%)	34 (72,34%)
Adalimumab *n^ (%)*	71 (84,52%)	9 (19,50%)
Golimumab *n^ (%)*	0 (0%)	9 (19,50%)
Vedolizumab *n^ (%)*	42 (50%)	35 (74,47%)

Table XII. Baseline features of the two populations under study.

Among the 84 CD patients enrolled for the study, 52 of them were male (61,90%), and 32 were female (38,10%). The median age at diagnosis was 28 years old, (min-max =74-6, IQR= 20), while the median age at Ustekinumab's introduction was 47, (min-max=7-78, IQR=25). Among these patients, 10 of them were smokers (11,90%), 53 of them have undergone a previous surgical intestinal resection (63,10%). Almost all of them, 82, had previously taken steroids (97,62%) and 58 immunosuppressors (69,05%). All of them, had previously taken a biological drug, and 64 patients two or more biologics (76,19%). 68 of them (80,95%) had received Infliximab therapy, 71 patients Adalimumab (84,52%) and 42 patients

Vedolizumab (50%). No patient had taken Golimumab because it was never approved for the treatment of this disease.

Among the 47 UC patients enrolled for the study, 30 of them were male (63,83%), and 17 were female (36,17%). The median age at diagnosis was 37 years old, (min-max 2-73, IQR=25), while the median age at Ustekinumab's introduction was 59, (min-max = 18-72, IQR=25). Among these patients, 5 of them were smokers (19,64 %), only 3 of them have undergone a previous surgical intestinal resection (3,75%). All of them, 47, had previously taken steroids and 28 immunosuppressors (59,57%). Approximately all of them, 42, had previously taken a biological drug (89,36%), and 32 patients at least two of them (68,09%). This biological drug was for 34 of them (72,34%) Infliximab, for 9 patients Adalimumab (84,52%), for 9 patients Golimumab (19,15%), for 35 patients Vedolizumab (74,47 %).

Through the Montreal classification, the patients were classified depending on the framing of pathology in three aspects: Age at diagnosis (A), Behaviour (B) and Localization (L), (Table XIII).

Montreal Score	**Score**	**N^ (%)**
Montreal A	A1	1 (1,19%)
	A2	36 (42,86%)
	A3	47 (55,95%)
Montreal B	B1	22 (26,19%)
	B2	31 (36,90%)
	B3	11 (13,10%)
Montreal L	L1	17 (20,24%)
	L2	18 (21,43%)
	L3	40 (47,62%)
	L4	9 (10,71%)

Table XIII. Montreal score of Crohn's disease population at baseline.

Among CD patients, 11 of them showed a fistulizing/penetrating phenotype (13,10%), 31 a fibrosthenous (36,90%), 20 a fistolizing + fibrosthenous (23,81%) and 22 an inflammatory one (26,19%), (Table XIV).

Phenotype	**N^ (%)**
1. Fistulizing/ penetrating	11 (13,10%)
2. Fibrosthenuous	31 (36,90%)
3. Fistolizing + fibrostenuous	20 (23,81%)
4. Inflammatory	22 (26,19%)

Table XIV. Phenotype of Crohn's disease at baseline.

For what concerns UC location, in 7 UC patients (14,89%) the disease was limited to the rectum (Ulcerative proctitis) and the proximal extent of inflammation was distal to the rectosigmoid junction. In 10 UC patients (21,28%) the disease was localized in the left-side gastrointestinal tract (distal colitis); the involvement of the disease limited to the portion of the colorectum distal to the splenic flexure. In 30 patients (63,83%), the disease localization was extensive (pancolitis); in fact, the involvement extended proximal to the splenic flexure (Table XV).

Localization	**Score**	**N^ (%)**
Ulcerative proctitis	E1	7 (14,89%)
Left sided UC	E2	10 (21,28%)
Pancolitis	E3	30 (63,83%)

Table XV. Localization of Ulcerative colitis at baseline.

6.2 Characteristics of the populations at the introduction of Ustekinumab

At the introduction of Ustekinumab baseline, all UC patients were using steroids (100%), whereas the percentage of CD patients was 25%. Moreover, 40,42% of UC patients were taking immunosuppressors, whereas the percentage in CD patients was 11,90%. The median age at baseline was 47 years old in CD and 59 years old in UC. The average disease duration in years was 14,53 in CD patients and 14,81 in UC patients (Table XVI).

Characteristics at UST introduction	**Crohn's disease**	**Ulcerative colitis**
Use of steroids *n^ (%)*	19 (25%)	47 (100%)
Use of immunosuppressors *n^ (%)*	10 (11,90%)	19 (40,42%)
Median age at baseline *(y, min-max, IQR)*	47, 18-78, 25	59, 18-72, 25
Mean disease duration *(y)*	14,53	14,81

Table XVI. Baseline characteristics of the population at the introduction of Ustekinumab.

6.3 Ustekinumab efficacy in Crohn's disease

Clinical, biochemical, and endoscopic outcomes in CD patients at baseline, after 3 months, 6 months and 1 year after the introduction of Ustekinumab (UST) are summarized in the table below (XVII).

Time from Ustekinumab Introduction / Outcomes	0 M	3 M	6 M	1 Y	p
Clinical outcome					
HBI	5, 6	3	3	4	0,001
Steroids use *(%,DS)*	25%, ± 0,436	20%, ± 0,406	9%, ±0,294	5%, ±0,213	0,001
Clinical remission *(%,DS)*	53%, ± 0,502	59%, ± 0,467	70%, ± 0,460	64%, ±0,485	0,142
Clinical response *(%,DS)*		95%, ± 0,221	58%, ± 0,497	27%, ± 0,449	0,000
Biochemical outcome					
Calprotectin (*μg/mg)*	862	287	395	284	0,001
Biochemical remission *(%,DS)*	16%, ± 0,369	41%, ± 0,496	39%, ± 0,001	52%, ± 0,504	0,001
Endoscopic outcome					
SES-CD score	6,5			4	0,000
Rutgeerts-score	4			3	0,000
Endoscopic remission *(%,DS)*	22%, ± 0,416			28%, ± 0,000	0,000
Endoscopic response *(%,DS)*				44%, ± 0,502	

Table XVII. Clinical, biochemical and endoscopic outcomes over time in CD patients. M= month.

6.3.1 Clinical outcome

At baseline the median HBI was 5, (IQR= 7). After 3 months the median HBI decreased to 3, (IQR= 6, p=0,002). After 6 months the median HBI was 3, (IQR= 7, p= 0,002). After 1 year the median HBI increased to 4, (IQR= 6, p= 0,001). In general, after 1 year, it was possible to observe a statistically significant decrease, (p= 0,001) (Figure 7).

At baseline clinical remission was found in 53%, (± 0,502). After 3 months, clinical remission was found in 69% CD patients, (± 0,467, p= 0,001); while clinical response in 95%, (± 0,221, p= 0,000). After 6 months, clinical remission was found in 70% CD patients, (± 0,460, p= 0,000) and clinical response in 58% patients, (± 0,497, p= 0,000). After 1 year, clinical remission was found in 64% CD patients, (± 0,485, p= 0,142), and clinical response in 27% CD patients, (± 0,449, p= 0,000).

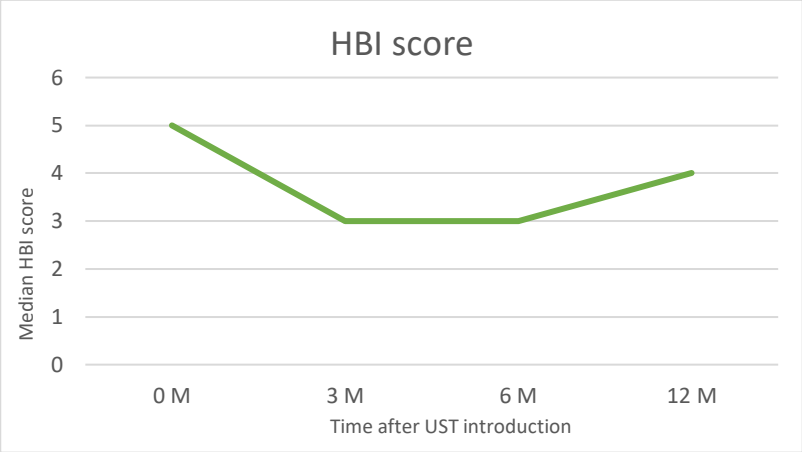


Figure 7. HBI score in CD patients over time. M=month.

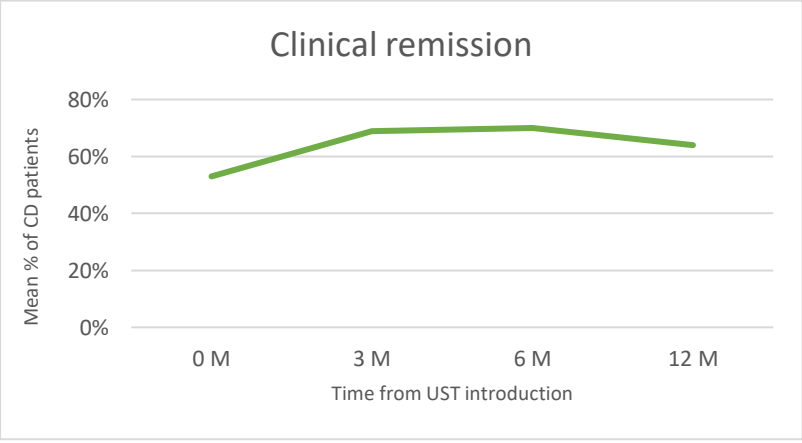


Figure 8. Clinical remission in CD patients over time. M=month.

6.3.2 Biochemical outcome

The median value of fecal calprotectin at baseline was 862 µg/mg, (min-max= 0-2500, IQR= 1354), while after 3 months was 287 µg/mg, (min-max= 8-2427, IQR= 561, p= 0,000), after 6 months is 395 µg/mg, (min-max= 5-2104, IQR= 1004, p= 0,30), and after 1 year was 284 µg/mg, (min-max= 0-6890, IQR= 845, p= 0,001) (Figure 9).

At baseline biochemical remission was found in 16% of CD patients, (± 0,369), while after 3 months in 41% CD patients, (± 0,496, p= 0,000). After 6 months biochemical remission was reported in 39% CD patients, (± 0,001, p= 0,001) and after 1 year in 52% patients, (± 0,504, p= 0,001). When biochemical remission was evaluated over time, it was possible to observe a meaningful decrease, (p= 0,001) (Figure 10).

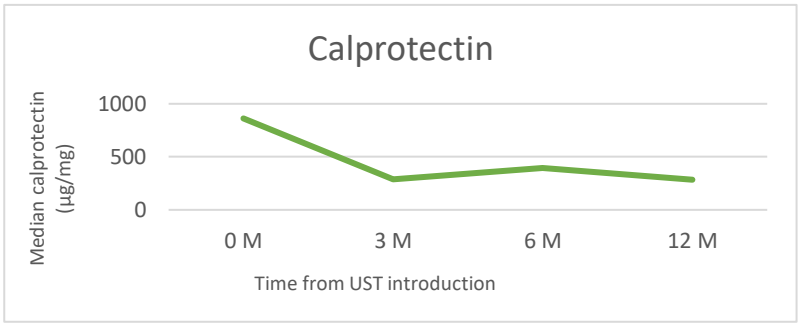


Figure 9. Calprotectin in CD patients over time. M=month.

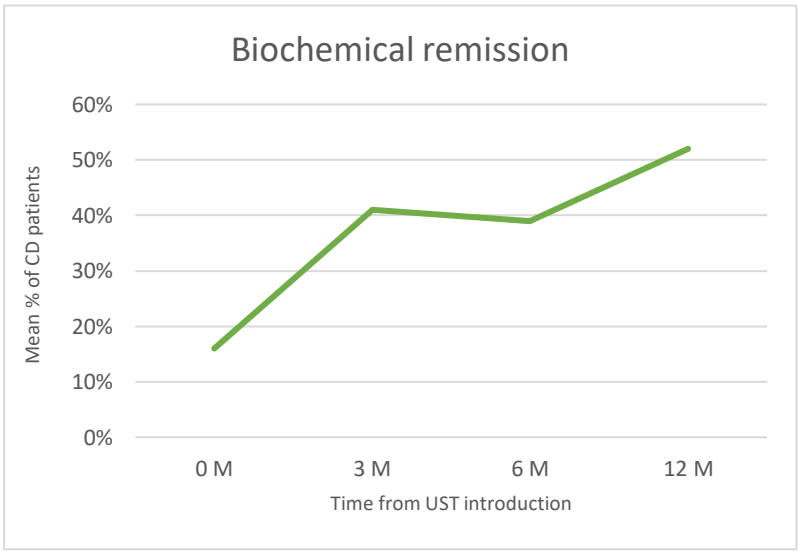


Figure 10. Biochemical remission in CD patients over time. M=month.

6.3.3 Endoscopic outcome

At baseline the median SES-CD score in CD patients was 6,5, (min-max= 0-30, IQR= 15), while after 1 year was 4, (min-max= 0-24, IQR= 14), with a statistically significant decrease, (p= 0,000). Moreover, the median Rutgeerts score, which is used after ileocolonic resection as the standard evaluation of post-surgical recurrences at ileocolic anastomosis level, at baseline was 4, (min-max= 0-7, IQR = 2), whereas after 1 year was 3, (min-max= 0-4, IQR= 1), again with a statistically significant decrease, (p=0,000) (Figure 11).

At baseline endoscopic remission was observed in 22% of CD patients, (± 0,416) , while after 1 year in 28% of CD patients, (± 0,000, p= 0,000), whereas endoscopic response in 44% of patients, (± 0,502) (Figure 12).

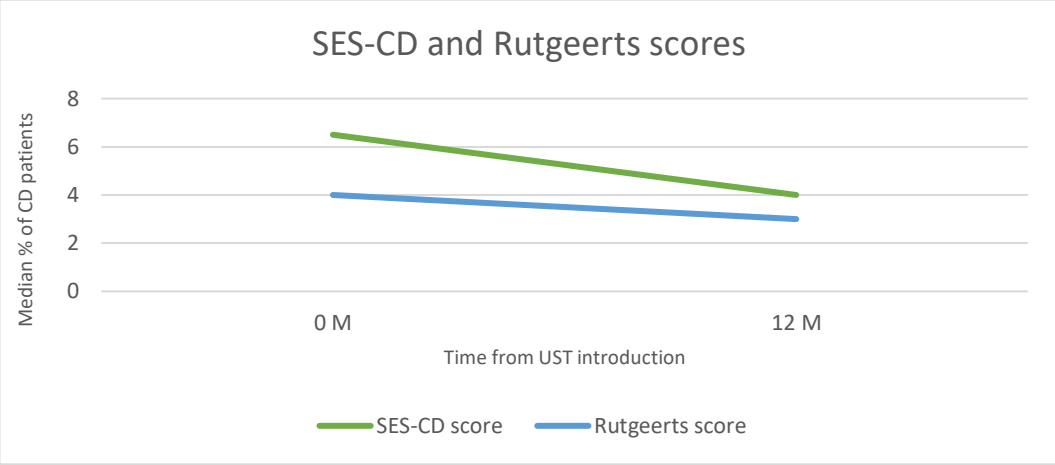


Figure 11. SES-CD and Rutgeerts scores over time in CD patients. M=month.

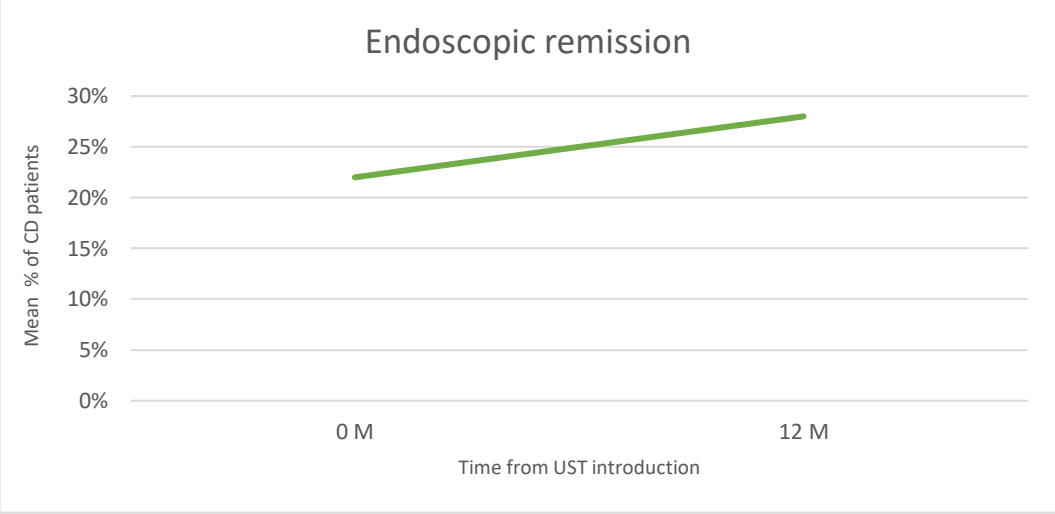


Figure 12. Endoscopic remission over time in CD patients. M=month.

6.3.4 Concomitant steroids

At baseline the number of CD patients who were taking steroids was 21 (25%), ± 0,436, whereas after 3 months it decreased to 16,8 (20%), (± 0,406, p= 0,373). After 6 months the number of CD patients who were taking steroids was 7,56 (9%), (± 0,294, p= 0,018), whereas after 1 year it statistically significantly decreased to 4,2 (5%), (± 0,213, p=0,001) (Figure 13).

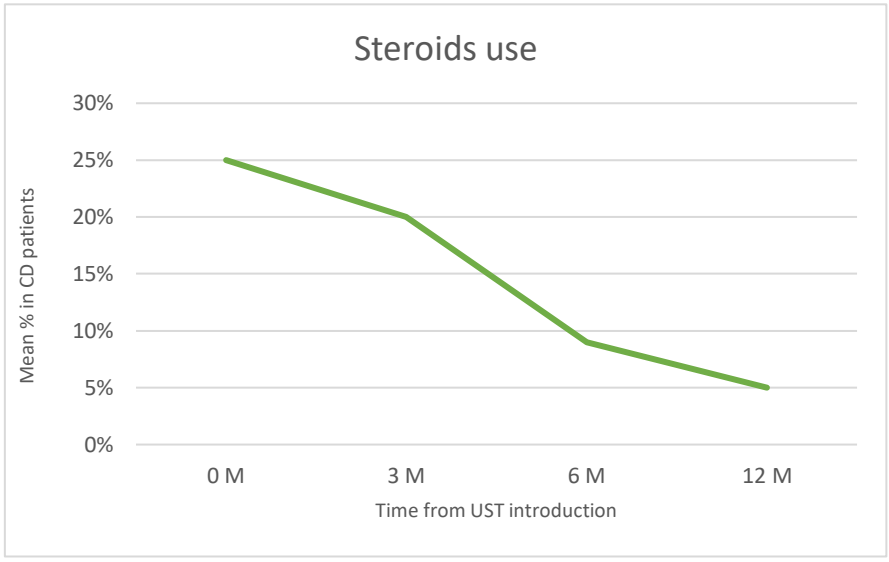


Figure 13. Use of concomitant steroids over time in CD patients. M=month.

6.4 Ustekinumab's efficacy in Ulcerative Colitis

Clinical, biochemical and endoscopic outcomes in UC patients are summarized in the table below (Table XVIII).

Ulcerative colitis	**0 M**	**3 M**	**6 M**	**1 Y**	**p**
Clinical outcome					
***p Mayo score** (n^, DS)*	5, ± 1,840	4,54, ± 1,998	3,93, ± 1,900	3,62, ± 1,758	0,195
***Steroids use** (n^, DS)*	100%, ± 0,00	62%, ± 1,392	43%, ± 0,308	47%, ± 0,851	0,005
***Clinical remission** (%,DS)*	16%, ± 0,367	24%, ± 0,431	18%, ± 0,390	64%, ±0,485	0,687
***Clinical response** (%,DS)*		50%, ± 0,506	56%, ± 0,506	38%, ± 0,500	1,000
Biochemical outcome					
***Calprotectin (**μg/mg)*	1368	790	1110	972	0,772
***Biochemical remission** (%,DS)*	11%, ± 0,318	41%, ± 0,497	42%, ± 0,504	20%, ± 0,414	0,001
Endoscopic outcome					
e Mayo score	3			2	0,000
Rutgeerts-score	4			3	0,000
***Endoscopic remission** (%,DS)*	22%, ± 0,416			20%, ± 0,414	0,054
***Endoscopic response** (%,DS)*				7%, ± 0,267	

Table XVIII. Clinical, biochemical and endoscopic outcomes over time in UC patients. M= month.

6.4.1 Clinical outcome

The average value Partial Mayo Score at baseline was 5, (± 1,840). After 3 months, it decreased to 4,54, (± 1,998, p=0,01), after 6 months it was 3,93, ± 1,900, whereas after 1 year it decreased to 3,62, (± 1,758, p= 0,195) (Figure 14). At baseline clinical remission was found in 16% UC patients, ± 0,367, while after 3 months it was reported in 24% of UC patients, (± 0,431, p=0,388) and clinical response in 50%, (± 0,506). After 6 months, clinical remission was found in 18% of UC patients, (± 0,390, p= 0,219); whereas clinical response in 56%, (± 0,506, p=1,000). After 1 year, clinical remission was found in of 22% of UC patients, (± 0,428, p= 0,687), whereas clinical response in 38%, (± 0,500, p= 1,000) (Figure 15).

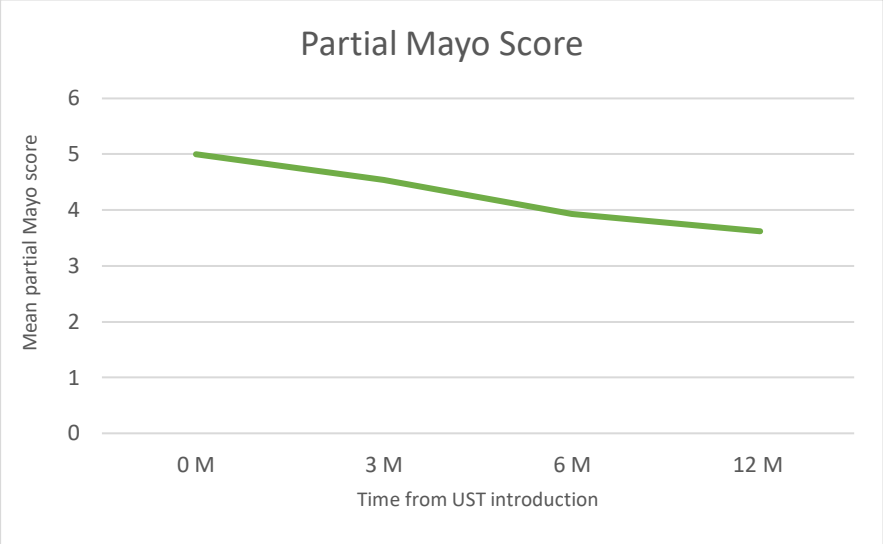


Figure 14. Partial Mayo score over time in UC patients. M=month.

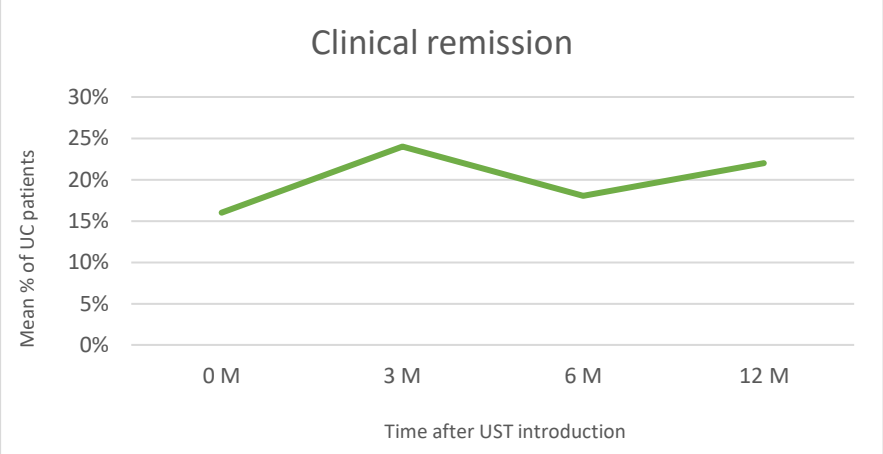


Figure 15. Clinical remission over time in UC patients. M=month.

6.4.2 Biochemical outcome

Mean fecal calprotectin at baseline was 1368 µg/mg, (min-max= 100 – 4152, IQR= 2526), whereas after 3 months the average value of fecal calprotectin was 790 µg/mg, (min-max= 248- 4448, IQR= 1652, p= 0,002). After 6 months, the average value of fecal calprotectin was 1110 µg/mg, (min-max= 194-3634, IQR= 1224, p= 0,007), whereas after 1 year was 972 µg/mg, (min-max= 106-4448, IQR= 4342, p= 0,772) (Figure 16).

At baseline, biochemical remission was found in 11% of UC patients, (± 0,318), whereas after 3 months in 41% of UC patients, (± 0,497, p= 0,004). After 6 months, biochemical remission was found in 42% of UC patients, (± 0,504, p= 0.012) and after 1 year in 20% of UC patients, (± 0,414, p= 0,625) (Figure 17).

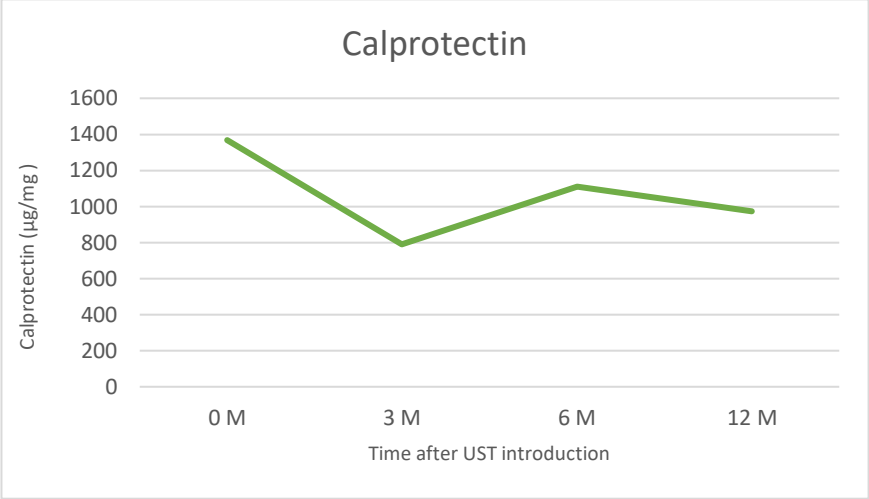


Figure 16. Calprotectin over time in UC patients. M=month.

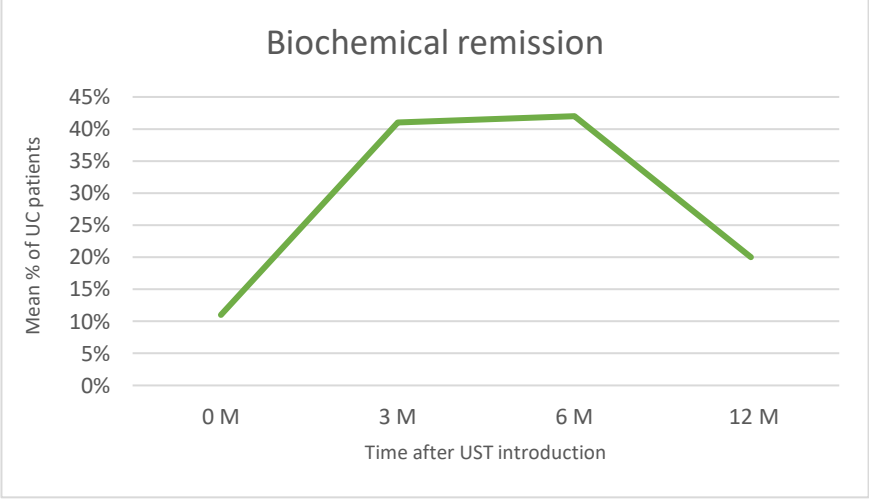


Figure 17. Biochemical remission over time in UC patients. M= month.

6.4.3 Endoscopic outcome

At baseline the median endoscopic Mayo Score was 3, (min-max= 1-3, IQR= 1). After 1 year the median endoscopic Mayo Score was 2, (min-max= 1-3, IQR=1, p= 0,054) (Figure 18). At 1 year, endoscopic remission was observed in 20% UC patients, (± 0,414, p= 0,054), whereas endoscopic response in 7%, (± 0,267) (Figure 19).

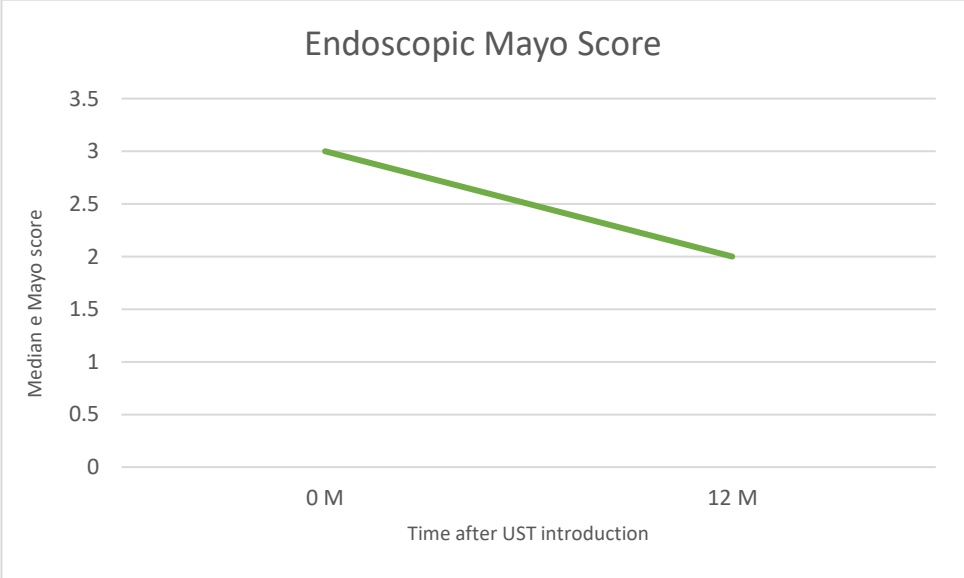


Figure 18. Endoscopic Mayo score over time in UC patients. M= month.

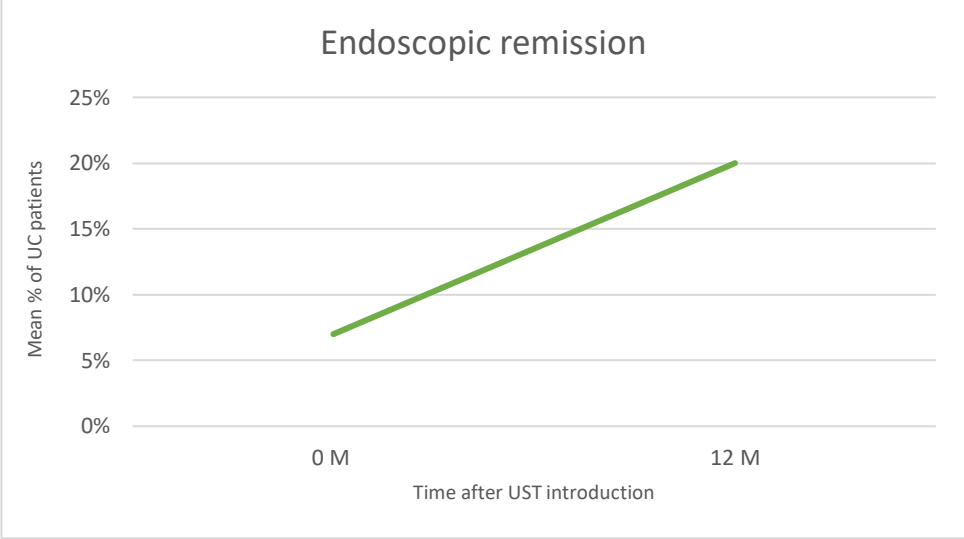


Figure 19. Endoscopic remission over time in UC patients. M= month.

6.4.4 Concomitant steroids in UC

At baseline the average number of UC patients who were taking steroids was 100%, (±0,000). After 3 months the average number of UC patients on steroids was 62%, (± 1,392, p= 0,014), whereas after 6 months the average number was 43%, (± 0,308, p=0,001). After 1 year the average number of UC patients on steroids was 47%, (± 0,851, p= 0,014). It was possible to observe a continuous decrease in the use of steroids between the first, (p= 0,014) and the second 6 months, (p= 0,327). After 1 year there was a statistically significant decrease in the use of steroids in UC, (p= 0,005) (Figure 20).

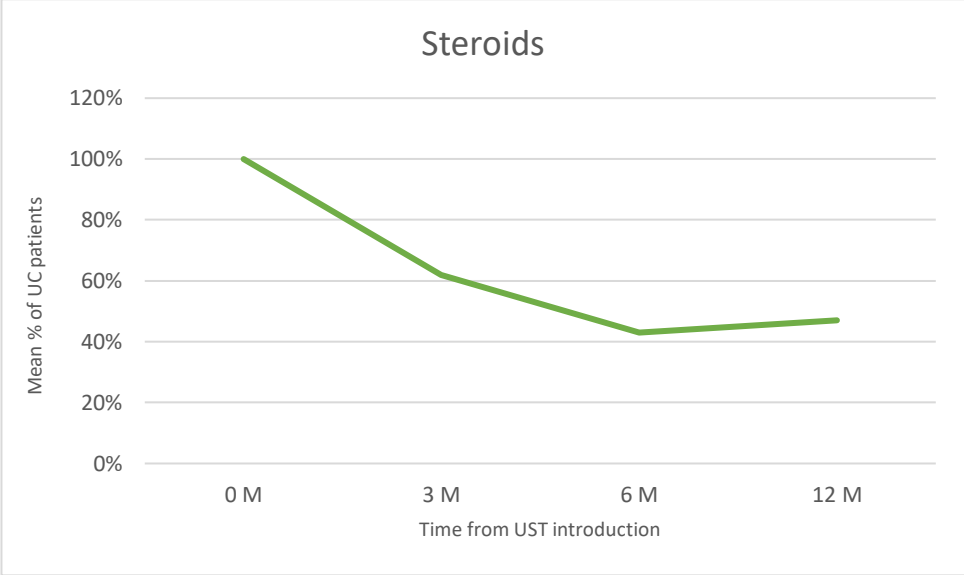


Figure 20. Concomitant steroids use in UC patients over time. M= month.

6.5 Efficacy comparison between CD and UC

6.5.1 Comparison of the two patients' populations at baseline

A comparison was made between the population of CD patients and UC patients by some preliminary statistical tests regarding specific variables, such as gender, smoking, age at diagnosis and age at Ustekinumab introduction, to determine the homogeneity of our distribution. In particular, in CD, 10 (11,90 %) of patients were smokers, whereas in UC only 5 (19,64 %) of patients were smokers. In CD the median age at diagnosis was 28 years old, (min-max= 6–74, IQR= 20), whereas in UC the median age at diagnosis was 37 years old, (min-max= 2-73, IQR= 25). Infliximab has proved to be the most administrated drug in both Inflammatory Bowel Diseases.

6.5.2 Comparison of the two patients' populations after Ustekinumab's introduction

To compare the efficacy and safety of Ustekinumab in CD and UC patients, it has been chosen to evaluate and compare the clinical remission, clinical response, biochemical remission, endoscopic response, and endoscopic remission in both conditions. The following comparisons, at 3 months, 6 months and 1 year after Ustekinumab's first administration, were done.

At baseline, clinical remission was obtained by 53% of CD and 16% of UC patients. After 3 months, clinical remission was found respectively in 69% of CD patients and in 24% of UC patients, (p= 0,000), whereas clinical response in 95% CD and in 50% UC patients, (p=0,000). After 6 months, clinical remission was found respectively in 70% of CD patients and in 18% UC patients (p= 0,000), whereas clinical response in 58% and 56% patients, (p=0,819). After 1 year, clinical remission was found in 64% CD patients and in 22% UC patients, (p= 0,002), whereas clinical response respectively in 27% and 38%, (p=0,423) (Table XIX, Figure 21).

		0 M	**3 M**	**6 M**	**1 Y**
***Clinical remission** (%)*	CD	53%	69%	70%	64%
	UC	16%	24%	18%	22%
p			0,000	0,000	0,002
***Clinical response** (%)*	CD		95%	58%	27%
	UC		50%	56%	38%
p				0,819	0,423

Table XIX. Clinical remission and response in CD and UC patients over time from Ustekinumab introduction to end of FU.

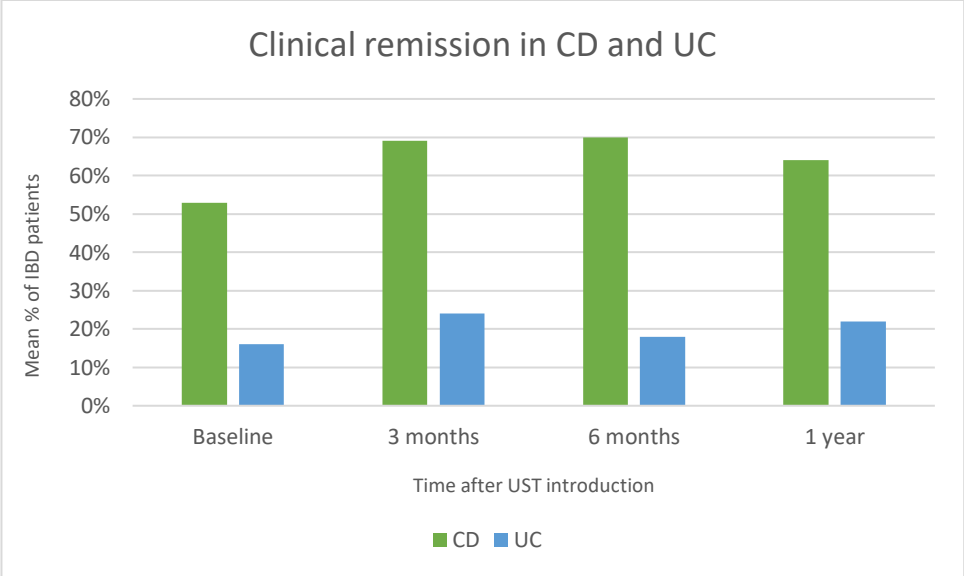


Figure 21. Comparison of clinical remission in CD and UC patients over time from Ustekinumab introduction to end of FU.

At baseline, steroid-free clinical remission was observed in 91% of CD patients, ± 0,282 and in 0% of UC patients ± 0,000, (p=0,000), while after 3 months respectively in 96% of CD patients, ± 0,204 and in 73% of UC patients, (p= 0,199). After 6 months steroid-free clinical remission was found in 96% of CD patients, ± 0,204 and in 0% of UC patients, (p= 0,000), while after 1 year respectively in 100% of CD patients, ± 0,000 and in 50% of UC patients, (p= 0,002) (Figure 22).

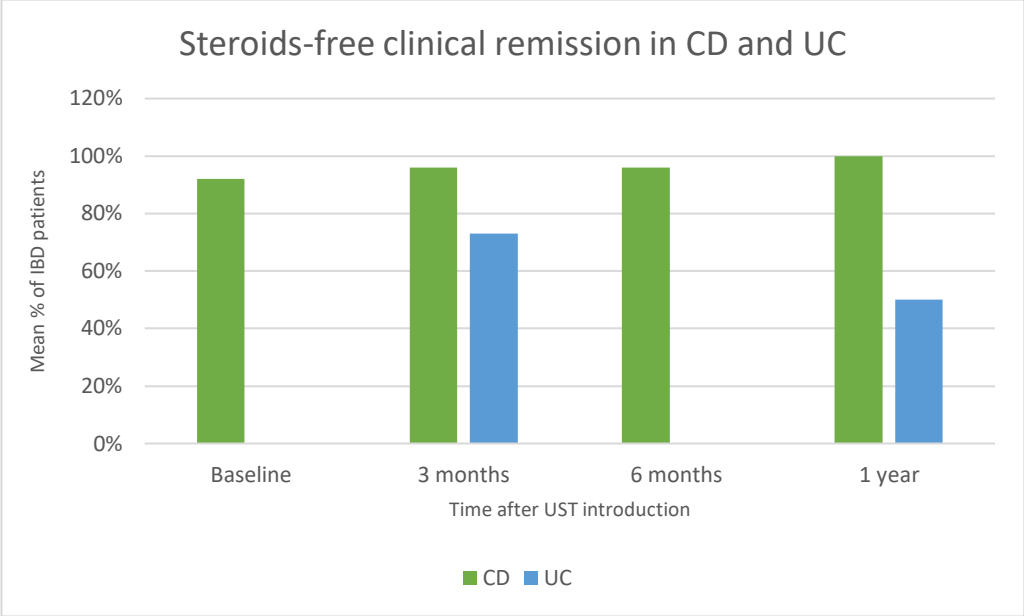


Figure 22. Comparison of steroids-free clinical remission in CD and UC patients over time from Ustekinumab introduction to end of FU.

At baseline, biochemical remission was obtained by 16% of CD and 11% of UC patients. After 3 months, it was found in 41% of both in CD and UC patients, (p= 0,952), while after 6 months, respectively in 39% of CD patients and in 42% of UC patients, (p= 0,741). After 1 year, biochemical remission was found in 52% of CD patients and in 20% of UC patients, (p= 0,028) (Table XX, Figure 23).

		0 M	**3 M**	**6 M**	**1 Y**
***Biochemical remission** (%)*	CD	16%	41%	39%	52%
	UC	11%	41%	42%	20%
p			0,952	0,741	0,028

Table XX. Biochemical remission in CD-UC patients over time from Ustekinumab introduction to end of FU.

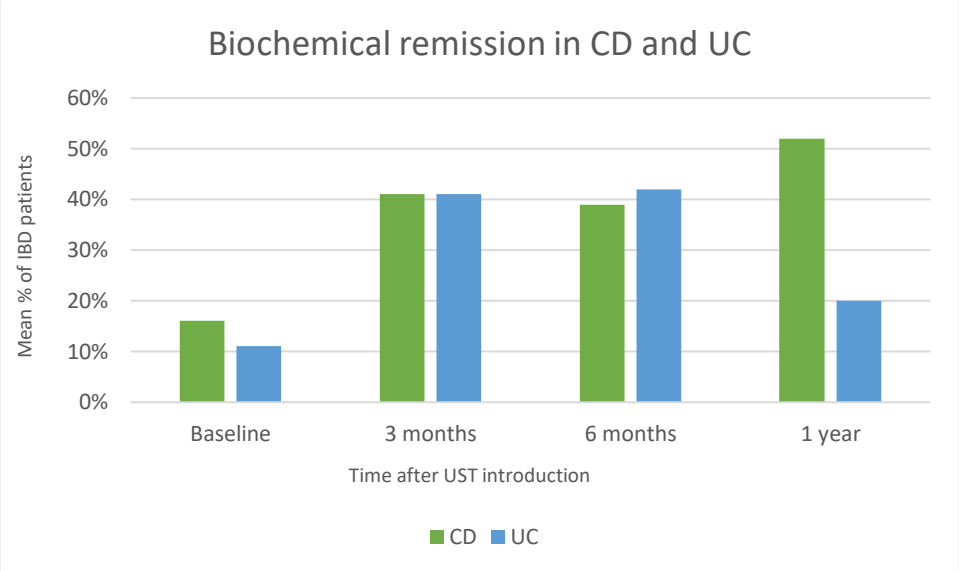


Figure 23. Comparison of biochemical remission between CD and UC patients over time from Ustekinumab introduction to end of FU.

At baseline, endoscopic remission was found in 22% of CD patients and in 7% of UC patients, (p= 0,029). After 1 year, endoscopic remission was observed in 27,87% of CD patients (over 61 endoscopy exams performed), while in only 20% of UC patients, (p=0,583), whereas endoscopic response was found respectively in 44% and 7% of CD and UC subjects, (p=0,011) (Table XXI, Figure 24).

		0 M	**1 Y**
***Endoscopic remission** (%)*	CD	22%	28%
	UC	7%	20%
p		0,029	0,583
***Endoscopic response** (%)*	CD		44%
	UC		7%
p			0,011

Table XXI. Endoscopic remission and response in CD-UC patients at baseline and after 1 year of Ustekinumab therapy.

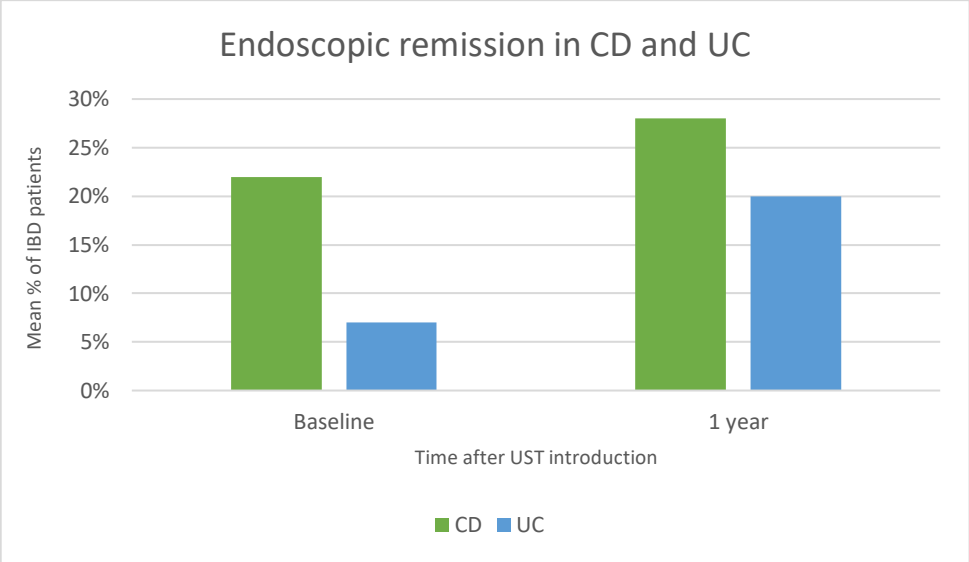


Figure 24. Comparison of endoscopic remission in CD and UC patients at baseline and after 1 year of Ustekinumab therapy.

After 1 year, a total of 15 (11,45%) adverse events occurred during follow-up in both groups, of which the majority were infectious and 4 (3,05%) were classified as serious adverse events, requiring drug suspension. Severe adverse reactions all occurred in CD patients, while in UC patients no severe adverse event took place (Table XXII).

	Adverse event	
	MILD	SEVERE
CD	• Abdominal abscess. • Herpes Zoster infection. • Erythematous phlyctene. • Infection of unknown origin. • Acrive fistula disease. • Pyelonephritis. • GI symptoms, active fistula disease reacutization.	• Active fistula disease and abdominal abscess. • Abdominal abscess. • Pyelonephritis and rectovesical fistula.
UC	• Anaemia. • Non-specific GI symptoms, menstrual cycle irregularity. • Non-specific GI symptoms, gastroduodenitis and pancolitis. • Suspected bleeding.	• None.

Table XXII. Number of mild and severe adverse reactions in CD and UC patients.

Discussion

Understanding a possible difference in terms of the efficacy of Ustekinumab in UC and CD aims to increase the knowledge of the efficacy of this new monoclonal antibody from its recent introduction. Currently there are no biomarkers that will specifically predict Ustekinumab therapy response in IBD patients, and its effects on these patients' population are heterogeneous [170] [171]. Ustekinumab has been at first introduced to manage the treatment of CD, and only more recently its use has been extended for the treatment of UC. This study has investigated the efficacy of this biological drug in a tertiary center cohort of CD and UC patients. Secondarily, it investigated Ustekinumab's safety profile in our study cohort.

Given the definitions of clinical, biochemical, and endoscopic outcomes in both IBD, it has been possible to find the following observations after 1 year from Ustekinumab's introduction.

In CD patients, the Harvey-Bradshaw Index (HBI) and its correlation with endoscopic findings have been a matter of discussion since its implementation in clinical practice. In our cohort, even though most patients were bio-experienced and almost half of the patients had undergone surgical procedures, the median HBI at baseline was 5, indicating clinical remission, and consequently, although the HBI decreased during therapy, there was not a statistically significant improvement in the clinical remission outcome (HBI $\leq$ 5), due to low baseline HBI. The clinical response was instead meaningful. In CD patients, considerable improvements were also found in biochemical remission, endoscopic remission, and endoscopic response. In addition, steroids use significantly decreased. In UC patients neither clinical remission nor clinical response were meaningfully improved. In addition, biochemical remission, despite an initial increase, was statistically insignificant. On the other hand, when endoscopic remission and response were evaluated over time, it was found a significant improvement. Moreover, steroids use decreased significantly. In general, Ustekinumab was effective in both IBD; however, CD patients showed better clinical, biochemical, and endoscopic remission, together with more effective steroids-free clinical remission. In UC patients, steroids-free clinical remission frequencies at baseline and after 6 months reached the frequency

of 0%. This outcome was probably due to the lack of data in the steroids use in UC patients in these specific times.

Previous studies demonstrated Ustekinumab's efficacy within the singular disease, CD or UC [172] [173]. From our study it emerged that Ustekinumab is more effective in CD rather than in UC, both from clinical and biochemical and endoscopic outcomes. This can probably be related to the younger age at the introduction of the biological drug in CD patients and to the more significant use of immunosuppressors at baseline in UC patients. Better steroids-free clinical remission in CD patients can relate to the more important steroids use at baseline in UC patients, in which the steroids use did not decrease as significantly as in CD patients. These baseline features were similar to the characteristics of the population reported in the clinical trials conducted to reach the approval of the biologic drug [174] [171] [175].

Adverse events occurred in 15 cases (11,45%), characterized mainly by mild-to-moderate infections. Only 4 cases (3,05%) were considered severe and required drug suspensions. Severe adverse reactions all occurred in CD patients, while in UC patients no severe adverse event took place. Adverse reactions were in line with the ones reported in previous studies in literature, such as non-specific gastrointestinal symptoms, fever and infections [170] [173].

The efficacy and safety of Ustekinumab for the treatment of CD have emerged from accumulating evidence in both randomized clinical trials and real-life experiences[173] . Previous studies have shown similar results in CD, proving the biological drug to be highly efficient, with the maintenance of clinical remission through 5 years [173] [176]. According to the Sustain study (2022), it was demonstrated to be effective in real-world use in the short and the long run [176]. Indeed, according to the ECCO guidelines, Ustekinumab is currently approved in patients with moderate-severe CD as first- or second-line therapy, in particular in patients refractory to anti-TNFs and without other treatment options [177] [178]. However, the use of Ustekinumab in the other form of IBD, UC, has only recently entered specific guidelines and has not been investigated enough compared to CD. In line with our results, similar improvements in clinical outcomes have previously been reported in little previous real-life studies, which include one that assessed the short-term

effectiveness of the drug in 103 patients[179] [180], one conducted with data from the ENEIDA registry[181], and the study by Chiapetta and coauthors, which reported improvements in the partial Mayo score and steroid use in a cohort of 68 Italian patients treated with Ustekinumab [182].

Some limitations of the study included the known and expected disadvantages of retrospective studies, such as inferior level of evidence compared with prospective studies. Controls are often recruited by convenience sampling; thus, they are not representative of the general population and prone to selection bias prone to recall bias or misclassification bias subject to confounding (other risk factors may be present that were not measured). In addition, retrospective studies cannot determine causation, but only association; they need large sample cohort sizes. Moreover, if outcomes are rare some key statistics cannot be measured, and temporal relationships are often difficult to assess.
Another limitation of our study was missing data, particularly regarding UC endoscopic findings at 1 year due to the shorter mean follow-up of UC patients. In addition, the lack of a standardized follow-up approach in terms of endoscopic assessment and the use of different laboratories for the assessment of biochemical findings contributed as limitation for the study. Moreover, the short follow interval (year) to provide definitive results and the need to exclude some patients for too many missing data, especially in the long term, restricted the value of the study. However, all these limitations occur quite frequently in clinical practice, and therefore we believe that our results can be generalized to other population samples with the same characteristics at baseline.

On the other hand, there are some points of strength to underline. This study was one of the first national real-life experiences on the short-, medium-, and long-term efficacy and safety of Ustekinumab (Stelara®) for the treatment of both CD and UC, especially in case of refractoriness to standard therapies and anti-TNFα. In the future, it is suggested to expand the case history of the two populations enrolled. Moreover, the impact of this study consists in helping to define the positioning of next-generation biological drugs in the event of failure to a first anti-TNF-a, to customize as possible the therapy of the patient to the characteristics of the individual patient. Since the little population of our study and the short follow-up

period of the patients involved, it is not possible to confirm the safety of the biological drug. However, in our little study Ustekinumab's safety outcomes reflect results reported previously in literature.

Conclusions

Ustekinumab was an effective therapy in both patients with CD and UC. However, CD patients have shown better clinical, biochemical, and endoscopic outcomes than UC patients. In particular, in UC patients, clinical, biochemical, and endoscopic remission after 1 year of Ustekinumab were not statistically significant as compared to baseline. Steroids use decreased in both diseases; however, CD patients obtained better steroids-free clinical remission.

Ustekinumab was safe and well-tolerated in the short and the long term, with only 4 severe adverse reactions out of 131 patients in both groups. Severe adverse events occurred only in CD patients. Infections were the most frequent adverse events. Similar studies should be conducted in the future on a broader IBD population base, to obtain more accurate outcomes.

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