UNIVERSITÀ DEGLI STUDI DI GENOVA

Dottorato di Ricerca in Emato-Oncologia e
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Reduction of fecal calprotectin levels induced by a short-course of Escherichia coli Nissle is associated with a lower likelihood of disease flares in patients with ulcerative colitis in clinical remission

Relatore:
Chiar.mo Prof. Edoardo Giovanni Giannini

Candidato:
Maria Giulia Demarzo

ABSTRACT

Background and aim: Fecal Calprotectin (FC) is a biomarker of gut inflammation, and Escherichia coli Nissle 1917 (EcN) is a probiotic strain able to reduce gut inflammation and maintain disease remission in patients with Inflammatory Bowel Disease (IBD). The aim is to assess the effects of EcN administration in patients with IBD in clinical remission and altered FC values

Methods: We prospectively included 82 patients with Ulcerative Colitis (UC) (n=49) and Crohn's Disease (CD) (n=33) in clinical remission and with FC values above 250 mcg/g (T_0) who were treated with EcN alone for 2 months. FC values were assessed at the end of EcN treatment (T_1) and clinical disease activity at 3 months (T_2).

Results: At T_1 median FC values were significantly lower compared to T_0 both in patients with CD (312 mcg/g vs 626 mcg/g; P<0.0001) and UC (100 mcg/g vs 584 mcg/g,; P<0.0001). Patients with UC who experienced disease relapse at T_2 had lesser reduction in median FC values at T_1 (-229 mcg/g, vs -397 mcg/g; P=0.049), while in patients with CD we observed no statistically significant difference (-358 mcg/g, vs -427; P=0.568). In patients with UC, a reduction of at least 532 mcg/g in FC had an accuracy of 69.7% and a positive predictive value of 65.7% in predicting maintenance of remission.

Conclusions: A short-course of EcN is associated with a reduction of FC values in patients with IBD in clinical remission and baseline altered FC values, and in patients with UC this decrease was associated with maintenance of clinical remission.

Keywords: Inflammation; relapse; probiotics; Inflammatory Bowel Disease.

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic inflammatory conditions of the gastrointestinal (GI) tract, mainly represented by Ulcerative Colitis (UC) and Crohn's disease (CD).

The pathogenesis of these diseases is not yet well understood. Although there is universal consensus that IBD arise from a complex connection among genetic predisposition, environmental factors, and gut microbiota, the mechanisms linking these pathogenic elements remain mostly unknown.

Here, experimental models in which microbes-free mouses do not develop IBD provide evidence of the central role of intestinal microbiota in IBD pathogenesis, which is also confirmed by the different microbial composition between IBD patients and healthy controls (1).

Beyond a pathogenic role, gut microbiota is capable to influence drug response through pharmacokinetic mechanism (i.e. drug availability or degradation), being somehow responsible for individual differences in IBD treatment response. (2-3).

Despite a growing pharmacological armamentarium, IBD treatment is hampered by a certain amount of primary and secondary loss of response (LOR) rate, regardless of the drug used or the patient's characteristics. In the last decades, IBD research focuses on the immunopathogenesis of these conditions not only to develop new drugs but also to understand the mechanism of therapy response in each individual to prevent LOR.

The future of IBD treatment seems to be linked to the immunopathogenesis knowledge and technological progress. Thus, a combination of molecular endoscopy, genetic research, transcriptomics, proteomics, immune analysis and gut microbiota characterization will allow to identify the best treatment tailored to the specific needs of each patient.

Gut microbiota and IBD

The microbiota in the GI tract is mostly composed of Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. Less is known about viruses, fungi and protozoa living together with bacteria in the gut. While an "healthy microbiota" is specific for each individual and stable in time (indeed environmental factors can influence the amount, but not the different types of species that colonize the intestinal (4)), IBD microbiota is characterized by a reduced alpha diversity (the total number of microbial species) and a higher bacterial instability compared to healthy people (5). Nevertheless, some studies showed an altered beta diversity (the extent of change in microbial composition) not only between IBD patients and controls, but also between CD and UC (6).

Microbial alterations that have been frequently associated with IBD are the reduction of bacteria belonging to *Firmicutes phylum*, such as *Roseburia*, *Faecalibacterium*, *Ruminococcaceae* and *Lachnospiraceae* genera and species of the *Lactobacillus genus*, and the growth of bacteria belonging to *Proteobacteria phylum*, such as *Enterobacteriaceae* (5, 7).

The disruption of the microbial community homeostasis is called "dysbiosis". Due to the high intra- and inter-individual variability in the microbiota is very difficult to identify disease- specific bacterial patterns. Moreover, if the microbiota is altered in IBD patients, these same alterations amplify the inflammatory process and may contribute to either disease onset or progression. For example, dysbiosis promote the growth of pathobionts, commensal bacteria that show pathogenic properties only when internal or environmental factors alter the stability of the microbiota-host relationship. In a nutshell, it is an egg-chicken situation.

Since it is highly unlikely to identify a single microbial pattern shared among all IBD patients, a more targeted approach based on functional criteria seems more useful to restore balance to the gut microbiota.

Interaction between microbiota and the immune system

The gut microbiota may be considered a "living organ" which participates in the physiological activities of the host's gut. Indeed, commensal bacteria can regulate the immune and adaptive immune response through the production of metabolites and preventing the growth of pathobionts and the invasion of pathogenic bacteria.

The alteration of the bacterial mucosal and luminal community in IBD patients is associated to an up- or down-regulations of these functions, resulting in intestinal damage. The most known dysbiotic factors promoting chronic inflammation in IBD patients are (i) a decrease of short chain fatty acids (SCFAs), acetate, propionate and butyrate, capable to promote B cell differentiations and Treg activation; (ii) an increase of tryptophan level which can stimulate inflammatory cytokines production; (iii) an up-regulation of conjugated bile acids; (iv) a reduction of mucolytic commensal that contributes to the disruption of the intestinal mucosal barrier(1); (v) an increase in oxidative stress and circulating toxins (5).

Role of gut microbiota in IBD patients: from diagnosis to treatment

In the last decades, the increasing interest in the pathogenesis of IBD promoted the bloom of studies directed to the evaluation of the host-microbes interaction. Nowadays, the ethiopatogenic role of microbiota in the development of IBD is universally accepted.

The mechanism that links the microbial *flora* and the immune cells has been partly elucidated, showing how alterations in the gut microbiota can promote inflammatory processes and the development and progression of IBD. In the future, each one of these mechanisms might become a therapeutic target. For example, the development of drugs aiming to repair (or eventually to prevent) the disruption of the intestinal barrier is very promising. Moreover, beyond

pharmacocynetic interactions, it is hypothesized that the individual differences in IBD biologics response directly depend on the changes in microbial composition caused by these drugs. In the long term, the analysis of fecal microbiota could reveal if our patient will respond to that drug, suggesting dosage adjustments or even to swap drug class.

For the time being, the only microbial therapeutic approaches are represented by fecal microbial transplantation (FMT) and probiotics. However, the data are still limited and none of them is waited to enter soon in the therapeutic IBD armamentarium.

Escherichia Coli Nissle 1917

Escherichia coli Nissle 1917 (EcN) is a non-pathogenic Gram-negative bacterium belonging to the Enterobacteriaceae family. It is a well-known probiotic strain with multiple beneficial effects on intestinal homeostasis: it can stimulate the production of human beta-defensin 2, may restore a damaged epithelium through the modulation of tight junction expression, and may modulate the mucosal inflammatory response by a direct action on activated T-lymphocytes (8).

Unlike other bacteria of the family, EcN not only lacks virulence factors, but it has also a protective role for the intestinal barrier. Indeed, it can inhibit the expansion of the pathogenic "brothers" of its family and other pathogens through the secretion of microcins, peptides with antimicrobial activities, and the colonization of the intestinal epithelium with its *fimbriae* (9).

EcN is one of the first probiotics used in clinical trials for the IBD treatment and then it was chosen to be genetically engineered to produce metabolites or deliver beneficial substances with application in different medical fields.

Actually, EcN is proved to be not inferior to the established standard 5-ASA for maintenance of remission in UC (10-11) and its use is recommended by guidelines as an alternative to mesalazine for the maintenance of remission in patients with UC (8,12).

Fecal calprotectin

Fecal calprotectin (FC), a soluble protein that accounts for approximately 60% of total soluble proteins in the cytosol fraction of neutrophils, is an important biomarker in patients with IBD as it represents a readily-available and validated tool to evaluate the presence of colonic inflammation non-invasively, and proved to be able to monitor disease activity and response to treatment (13-15).

AIM OF THE STUDY

The association between active IBD and dysbiosis has been widely proved. Unlike healthy subjects, a certain bacterial instability was found in IBD patients in clinical remission.

In this study, that included patients with IBD in clinical remission alone but with FC above the limit of normal for patients with IBD (*i.e.*, 250 mcg/g), we sought to assess whether EcN administration may further reduce FC values. Moreover, we also assessed whether a decrease in FC may be associated with a lower likelihood of IBD flare in the course of follow-up.

PATIENTS AND METHODS

Patients

In this prospective study we enrolled patients with IBD who were referred to our center between March 2020 and June 2022. Inclusion criteria were: age between 18 and 85 years, presence of IBD (confirmed by endoscopic, radiologic, and histologic evaluation) in remission phase [partial Mayo score (pMS) ≤2 in patients with UC and Harvey-Bradshaw Index (HBI) ≤5 in patients with CD], calprotectin values above 250 mcg/g.

Patients were excluded in case of modification of IBD therapy during the period of the study (*i.e.*, mesalazine, azathioprine, methotrexate, or biologic therapy), disease flares treated with steroids, UC patients who underwent proctocolectomy, recent (less than 6 months) GI surgery, acute non-specific gastroenteritis and Sars-Cov2 infection. Therefore, from an initial cohort of 174 patients with IBD we included in this study 82 patients who met inclusion criteria and had not exclusion criteria (**Figure 1**).

Methods

At enrollment (T_0), patients underwent careful history taking, physical and clinical examination, and a complete blood examination, including C-reactive protein (CRP) levels and assessment of FC (Quantum Blue fCAL, Buhlmann), and were then treated with EcN alone, 2 capsules/day for the first month and 1 capsule/day for the following month. FC values were assessed at the end of EcN treatment (2 month, T_1), while clinical disease activity was evaluated at 3 month (T_2) after study inclusion.

The Montreal classification was used to assess IBD location and behavior in patients with CD and UC (15). IBD activity was defined using the pMS and the HBI for patients with UC and CD, respectively (16). FC and CRP were assessed as previously reported and normal values were considered under 250 mcg/g and under 5 mg/dL, respectively (7,8).

From a retrospective database, we included a cohort of patients with IBD who had at least a baseline and subsequent control of FC within 2 months (±2 weeks) following baseline. A propensity score matching analysis was conduced on baseline characteristics (gender, age, age at diagnosis, concomitant medication, and baseline FC.).

The study was performed according to the Declaration of Helsinki. All patients were asked to provide written informed consent before the start of the study.

Statistical analysis

Continuous data are presented as median and interquartile range (IQR), whereas categorical data are presented as absolute value and percentage. Categorical variables were compared using the Fisher's exact test. The Wilcoxon's test for paired data was used to assess clinical and biochemical disease activity index. The Mann Whitney U-test was used for the comparison between groups. Study data were evaluated in an intent-to-treat analysis. $P<0.05$ in a two-tailed test was considered statistically significant. The receiver operating characteristic curve (ROC curve) was applied to identify the ΔFC levels from T_0 to T_1 with the highest accuracy in predicting clinical relapse at T_2. Multivariate general linear fixed effect models were assessed to adjust ΔFC for variables significantly associated in univariate analysis. The PS was estimated by a logistic regression model including variables that were significantly different between the two groups: gender, age, age at diagnosis, concomitant medication, and baseline FC.

RESULTS

Baseline patient characteristics

The characteristics of the study population (UC, n=49, 59.8%; CD, n=33, 40.2%) at enrollment (T_0) are reported in **Table 1.** Approximately half of the population was male (n=48, 58.5%), median age was 50 years (IQR, 38-65), and median age at diagnosis of IBD was 34 years (IQR 19-46); almost half of patients (n = 49, 59.8%) was diagnosed before the age of 40. Baseline FC and CRP levels were 601 (413-1,205) and 2 (0-5.3), respectively. **Table 2** reports the characteristics of patients from a retrospective database before and after PS matching.

Fecal calprotectin values during the study

Median FC values at the end of EcN treatment (T_1) were significantly lower as compared to baseline (T_0) in both patients with CD (312 mcg/g, IQR 100-477 vs 626 mcg/g, IQR 432-945; P<0.0001) and UC (100 mcg/g, IQR 100-407 vs 584 mcg/g, IQR 410-1,246; P<0.0001, **Figure 2**). FC values decreased below 250 mcg/g in 16 patients with CD (48.5%) and 29 patients with UC (59.2%, P=0.372). The estimated power of FC variation was 0.88 and 0.93 in CD and UC, respectively.

Fecal calprotectin behavior subdivided according to IBD

In patients with UC, the reduction in median FC values from T_0 to T_1 (ΔFC) was significantly lower in those who experienced a clinical relapse at T_2 (-229 mcg/g, IQR -800, 1.882 vs -397 mcg/g, IQR -987-242; P=0.049), and likewise median percentage reduction (-19%, IQR -61%, 163% vs -79%, IQR -93%, -62%; P<0.0001). Furthermore, we observed a statistically significant difference in FC values at T_2 between patients supplemented with EcN (100 mcg/g, IQR 100-407) and retrospective control group (534 mcg/g, IQR 245-1123) with P=0.005.

We observed a lower reduction in median CRP median values from T_0 to T_1 in patients who relapsed as compared to patients who maintained remission at T_2 (-0.3 mg/L, IQR -2 – 0 vs -1.4 mg/L, IQR -5 – 0; P=0.021).

In patients with CD, no statistically significant difference was observed in both ΔFC (-358 mcg/g, IQR -496, -195 vs -427, IQR -576, -168, P=0.568) and ΔCRP from T_0 to T_1 between patients who maintained remission and those who experienced clinical relapse. After stratifying CD patients according to disease involvement, we did not observe any statistically significant difference in FC reduction at T_2 (ileal CD: ΔFC -336 mcg/g, IQR -537- -89,50; ileo-colonic CD: ΔFC -419, IQR-479- -336). Moreover, no significant difference was found in FC values at T_2 between patients supplemented with EcN and controls (312 mcg/g, IQR 100-477 vs 365 mcg/g, IQR 265-852, P=0.070).

Prediction of clinical remission maintenance

ROC curves performed on the whole population identified a ΔFC of 515 mcg/g from T_0 to T_1 in predicting maintenance of clinical remission at T_2 (AUC 0.675, CI 0.555 – 0.795, PPV 69.0%, NPV 35.5%, LR+ 1.15, LR-0.51, P=0.012). Considering only patients with UC, ROC curves performed on ΔFC from T_0 to T_1 identified a decrease of 532 mcg/g as the threshold that best predicted maintenance of clinical remission at T_2 (AUC 0.697, CI 0.540 – 0.854, PPV 65.7%, NPV 19.8%, LR+ 1.16, LR- 0.55, P=0.029). Considering only patients with CD, ROC curves performed on ΔFC from T_0 to T_1 were unable to identify any threshold that predicted maintenance of remission in the course of follow-up (**Figure 3**).

DISCUSSION

FC is an antimicrobial protein secreted by neutrophils that is mainly used, in clinical practice, as a non-invasive tool able to discriminate which patients with chronic intestinal symptoms should be the subject of a more thorough investigation, including colonoscopy, due to a greater likelihood of organic disease such as IBD (12). Furthermore, in patients with IBD, FC values can be used as a marker of disease activity due to their correlation with endoscopic activity, and it has been suggested that FC values may be used also to predict relapse in patients with quiescent IBD (16-18).

EcN is a non-pathogenic gram-negative strain that can be used in patients with IBD to reduce gut inflammation and maintain disease remission (8); in fact IBD patients suffer from several changes in the composition and function of the gut microbiota and specific bacterial strains have been suggested to play a protective role. EcN in one of the most studied and has been demonstrated to have positive effects on IBD, to the point of being considered an alternative as effective and safe as mesalazine in the maintenance of remission in patients with UC (5,10).

In this prospective study, we observed that in patients with IBD in remission but with altered FC values, administration of EcN for 2 months led to a significant decrease in median FC values, that was sustained up to one months after EcN discontinuation.

FC value, alone, has an established role as a predictor of IBD relapse, as reported in a meta-analytic review of prospective studies (19). In these studies, FC had no differential predictive value for disease relapse in patients with CD or UC. In our study we observed that patients with UC who maintained disease remission following EcN treatment, showed a significantly greater decline in FC values at the end of treatment, while this was not observed in patients with CD. Moreover, we observed that in patients with UC a decrease of at least 500 mcg/g in FC values was associated with maintenance of remission with acceptable accuracy. These results are of direct clinical impact, as

monitoring FC at the end of a short course of EcN may help manage treatment and schedule follow-up patients evaluations. As a fact, patients with UC in remission are often scheduled for 6-monthly visits at our Unit, but the evidence of no reduction in FC values following a short course of EcN treatment may allow physicians to modify the intensity of visits and likely be pro-active in terms of therapeutic management.

On the other hand, we observed that the decrease in FC values in patients with CD was not predictive of maintenance of disease remission. This result may be explained taking into consideration that FC has high accuracy in the assessment of colonic inflammation and response to therapy being less sensitive in patients with CD, a disease characterised by digiunal and ileal involvement (15). Likely, the more proximal location and smaller surface of affected mucosa may impact on FC values as it has been demonstrated that large ileal ulcerations (>5 mm) were significantly associated with lower FC concentrations than colonic lesions (20). A definite explanation to this finding could not be provided even after stratification for disease localization, as patients with ileal alone and those with ileo-colonic involvement had similar, negative behavior, and the lack of patients with purely colonic CD involvement in our series does not allow us to draw definite conclusions on this issue. Another possible explanation is related to differences between CD and UC gut microbiota. Sankarasubramanian j. et al. showed an higher beta diversity between CD and UC group of patients and a relatively low beta diversity between UC patients and healthy controls (HC) compared to CD patients and HC (6). Later, Vestergaard M.V. et al. confirmed these results and described 38 novel genera associations in CD and 28 in UC. We may speculate that, considered the gut microbial diversity in CD vs UC, disease-specific probiotics course is needed to restore balance (7).

Despite the inclusion criteria were designed to consider only patients in clinical remission, we also investigated the CRP concentration in our patients with UC and CD treated with EcN for two

months. CRP median values, as expected, had a greater reduction in patients who maintained remission at three months compared to patients who experienced a clinical relapse. Overall, CRP values at baseline were slightly above the upper limit of normal, and therefore in these patients and under these conditions being less prone to further reduction, and eventually with a less meaningful behavior than FC.

This study had some limitation: first, this is a single center study, and the sample size was therefore limited; second, the study included no prospective control group, and therefore we were not able to assess the behaviour of FC values over time in a similar, untreated group of patients; lastly, we did not systematically investigate endoscopic status in the study patients, as ethical concerns may be raised in performing endoscopic examination in patients in clinical remission. Despite these limitations, we feel that our study has some points of strength such as the identification of a definite FC cut-off able to predict remission, and the evaluation of longitudinal modifications in FC after treatment. The use of an historic cohort of patients confirms the longitudinal analysis and provides a support to our results. Finally, the estimated power of main analysis was adequate to confirm the results.

In conclusion, in patients with IBD in clinical remission and altered FC at baseline, we observed that EcN administered for two months is associated with a significant decrease in FC values. Moreover, in patients with UC, a greater reduction in FC values is associated with a lower likelihood of disease flares one month after the end of treatment, and this finding may have clinical relevance. Future studies, in larger populations and with a control group are needed to confirm these promising, preliminary findings.

Table 1. Baseline demographic and clinical features of the 82 patients with Inflammatory Bowel Disease enrolled in this study.

Parameter	Values
Gender, male [n (%)]	48 (58.5%)
Age [years]	50 (38 – 65)
Body Mass Index [Kg/m^2]	22.7 (21.0 – 25.6)
Age at diagnosis [years]	34 (19 – 46)
Disease duration [years]	10 (5 – 19)
Extra-intestinal manifestation [n (%)]	21 (25.6%)
Surgery [n (%)]	23 (28.0%)
Concomitant medication [n (%)]	
Biological therapy	36 (43.9%)
Steroids	0 (0%)
Immunosuppressors	10 (12.2%)
Crohn's disease [n (%)]	33 (40.2%)
Montreal age at diagnosis [n (%)]	
A1: <17 years	7 (3.2%)
A2: 17-40 years	13 (5.9%)
A3: >40 years	13 (5.9%)
Montreal behavior [n (%)]	
B1: non-stricturing, non-penetrating	15 (6.8%)
B2: stricturing	15 (6.8%)
B3: penetrating	3 (1.4%)
Montreal localization [n (%)]	
L1: terminal ileal	16 (7.3%)
L3: ileocolon	16 (7.3%)
L3 + L4: ileocolon + upper gastrointestinal tract	1 (0.5%)
Perianal disease [n (%)]	7 (21.2%)
Ulcerative colitis [n (%)]	49 (59.8%)
Montreal Ulcerative Colitis	
E1: Ulcerative proctitis	3 (1.4%)
E2: left-sided UC	27 (12.3%)
E3: exstensive	19 (8.6%)

Continuous data are median and IQR and nominal data are number (% patients)

Table 2. Baseline demographic and clinical features of patients with IBD extracted from a retrospective database before and after propensity score matching analysis.

Patients with Ulcerative Colitis	**Unmatched controls n = 276**	**PS-matched controls n = 45**	**EcN patients n = 49**	**p**
Gender, male [n (%)]	158 (57%)	28 (62%)	26 (50%)	0.370
Age [years]	51 (33-65)	52 (39-65)	50 (38-66)	0.871
Age at diagnosis [years]	33 (22-51)	31 (20-53)	36 (25-45)	0.658
Concomitant medication [n (%)]				
Biological therapy	66 (20%)	9 (20%)	15 (31%)	0.238
Steroids	67 (20%)	2 (4%)	2 (4%)	0.931
Immunosuppressors	31 (10%)	3 (6%)	4 (8%)	0.782
Montreal Ulcerative Colitis				
E1: Ulcerative proctitis	27 (10%)	2 (4%)	3 (6%)	
E2: left-sided UC	114 (41%)	18 (40%)	27 (55%)	0.265
E3: extensive	135 (49%)	25 (56%)	19 (39%)	
Baseline fecal calprotectin	250 (61-1,095)	624 (327-1,110)	584 (410-1,246)	0.669
Patients with Crohn's Disease	**Unmatched controls n = 186**	**PS-matched controls n = 35**	**EcN patients n = 33**	**p**
Gender, male [n (%)]	64%	22 (63%)	22 (68%)	0.743
Age [years]	55 (42-66)	53 (41-65)	52 (40-62)	0.288
Age at diagnosis [years]	36 (25-53)	34 (21-55)	33 (16-54)	0.397
Concomitant medication [n (%)]				
Biological therapy	102 (55%)	24 (69%)	21 (64%)	0.667
Steroids	65 (35%)	9 (26%)	4 (12%)	0.154
Immunosuppressors	19 (10%)	5 (14%)	6 (18%)	0.663
Montreal behavior [n (%)]				
B1: non-stricturing, non-penetrating	95 (51%)	17 (49%)	15 (46%)	0.967
B2: stricturing	78 (42%)	15 (43%)	15 (46%)	
B3: penetrating	13 (7%)	3 (8%)	3 (8%)	
Montreal localization [n (%)]				
L1: terminal ileal	73 (39%)	16 (46%)	16 (49%)	
L2: colonic	30 (16%)	4 (11%)	0	
L3: ileocolonic	80 (43%)	15 (43%)	16 (49%)	0.131
L3 + L4: ileocolonic + upper gastrointestinal tract	3 (2%)	0	1 (1%)	
Perianal disease [n (%)]	28 (15%)	4 (11%)	7 (21.2%)	0.274
Baseline fecal calprotectin	195 (52-982)	452 (273-853)	626 (432-945)	0.070

Continuous data are median and IQR and nominal data are number (% patients)

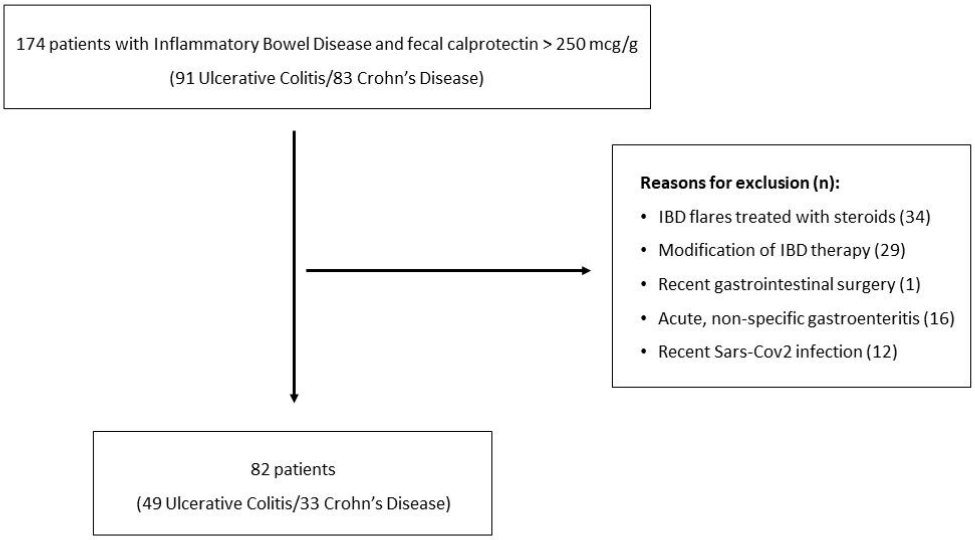
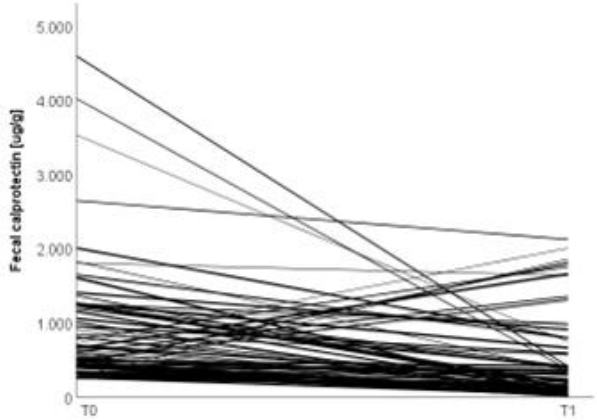
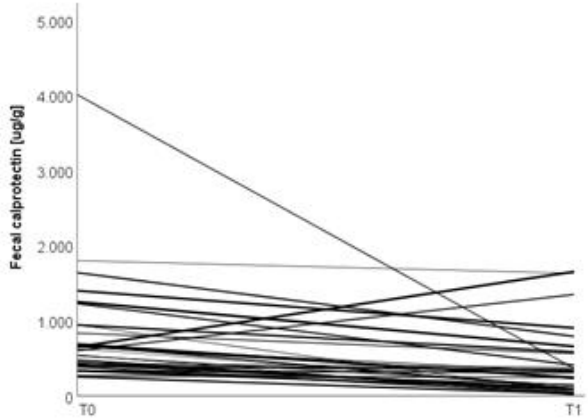

174 patients with Inflammatory Bowel Disease and fecal calprotectin > 250 mcg/g
(91 Ulcerative Colitis/83 Crohn's Disease)
Reasons for exclusion (n):
• IBD flares treated with steroids (34)
• Modification of IBD therapy (29)
• Recent gastrointestinal surgery (1)
• Acute, non-specific gastroenteritis (16)
• Recent Sars-Cov2 infection (12)
82 patients
(49 Ulcerative Colitis/33 Crohn's Disease)

Figure 1

A) All patients



B) Crohn's Disease



C) Ulcerative Colitis

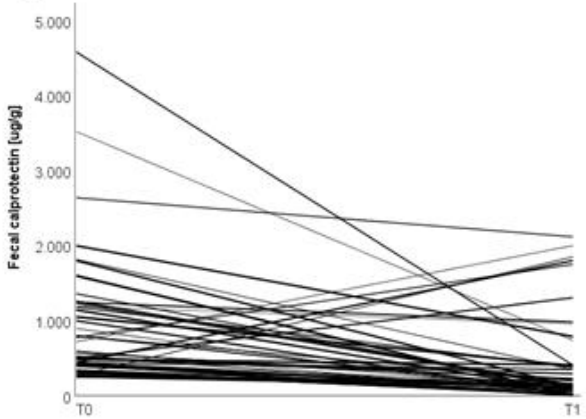
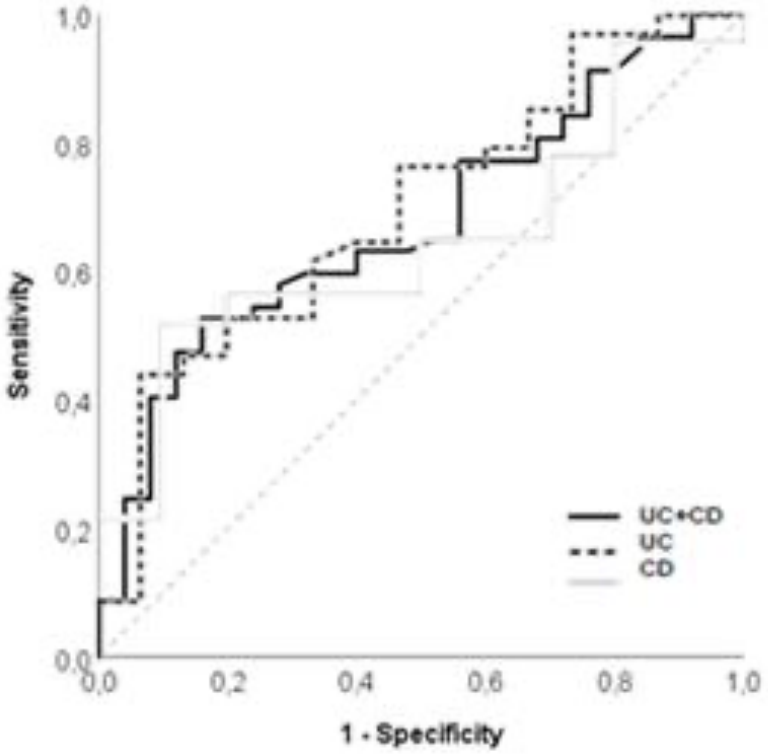


Figure 2


1,0
0,8
0,6
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0,0
Sensitivity
0,0
0,2
0,4
0,6
0,8
1,0
1 - Specificity
UC+CD
UC
CD

Figure 3

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