Impact of Endoscopic and Histologic Healing on Relapse in Patients with Ulcerative Colitis-McGill University Mucosal Healing Cohort

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Abstract (English)

Background: Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease that results in chronic inflammation of the colon with potential serious complications. Symptomatic control is currently considered insufficient and endoscopic healing of the mucosa is nowadays considered a key goal. We still do not know what the role of histologic healing on disease outcome is.

Aim: To determine the role of complete remission defined as both endoscopic mucosal healing and histologic inactivity on the risk of disease relapse.

Methods: We performed a prospective cohort study with a 1-year follow-up period at the McGill University Health Center between 2013 and 2015. We included consecutive adult patients with UC diagnosed by endoscopy and histology criteria presenting to the endoscopy unit for a colonoscopy to assess disease activity or for neoplasia surveillance. Patients were required to be in clinical remission as defined by Mayo clinical score ≤ 2 with no subscore > 1, with a stable dose of medical therapy and without the use of corticosteroids for 3 months prior to endoscopy. Patients were excluded if they had previous bowel resection related to UC, were in clinical remission without disease flare for > 10 years. At the time of endoscopy, all patients had serum measurement of C reactive protein (CRP), stool level of fecal calprotectin, endoscopic evaluation with the Mayo score, and rectal biopsies for the assessment of histology with the Geboes score and documentation of basal plasmacytosis. Patients were divided into 3 groups: complete remission (group 1), endoscopic remission alone (group 2) or active endoscopic disease (group 3). Patients were followed for 1 year with visits every 3 months to document disease activity

with clinical Mayo score. Measurement of CRP and fecal calprotectin in addition to repeat endoscopies with biopsies were performed if disease relapse occurred or at the end of the follow-up period to document disease recurrence.

Results: We enrolled 100 patients in our study with median age of 49 years (interquartile range 39-59 years), 55% being male. Disease distribution was the following: 16% had proctitis, 40% had left-sided colitis and 44% had pancolitis. Medical therapy included 5-aminosalicylates in 71% of patients, thiopurines in 28% and biologics in 12%. Endoscopically, 61% had Mayo score 0, 29% score 1, 7% score 2, and 3% score 3. Geboes score ≥ 3.1 was seen in 55% of patients and basal plasmacytosis was documented in 37%. The relapse rate was similar between group 1 (24.4%), group 2 (22.2%), and group 3 (20.0%). Although inconclusive results, female sex (odds ratio (OR) = 0.45, 95% confidence interval (CI): (0.17-1.21)) and biologic therapy (OR = 0.64, 95% CI: (0.13-3.15)) were potentially associated with remission while the presence of basal plasmacytosis (OR = 2.04, 95% CI: (0.42-10.22)) or a Geboes score ≥ 3.1 (OR = 1.21, 95% CI: (0.17-8.59)) trended to predict relapse. A cutoff value for fecal calprotectin > 150 μg/g showed the most clinically relevant sensitivity (75%) and specificity (65%) to predict active endoscopic disease.

Conclusion: Although inconclusive results due to the sample size, the presence of basal plasmacytosis and active histologic disease are potential predictors of disease relapse while biologic therapy could be protective. Further studies with a larger sample size are warranted to better document these associations.

Abstract (Français)

Introduction : La colite ulcéreuse (CU) est une maladie inflammatoire idiopathique de l'intestin qui cause une inflammation chronique du colon et qui peux engendrer des complications sérieuses. L'amélioration des symptômes cliniques est maintenant considérée inadéquate et de plus en plus, la guérison de la muqueuse est devenue le but principal du traitement. De plus, nous ne savons pas encore l'importance de la guérison histologique.

Objectif : Déterminer le rôle de la guérison complète, définie par une guérison histologique en plus de la guérison de la muqueuse, sur le risque de rechute de la maladie.

Méthodologie : Nous avons complété une étude de cohorte prospective avec un suivi de 1 an qui s'est déroulé au centre universitaire de santé McGill entre 2013 et 2015. Nous avons recruté des patients adultes avec CU tel que définie par les critères diagnostiques endoscopiques et histologiques qui se sont présentés pour une coloscopie dans le contexte de l'évaluation de la maladie ou le dépistage pour le cancer colorectal. Nous avons inclus les patients qui étaient en rémission clinique tel que défini par un score Mayo ≤ 2 avec aucun sous-score > 1, les patients chez qui la dose de médicaments n'a pas changé, et les patients qui n'ont pas eu recours aux corticostéroïdes pendant une période de 3 mois avant la coloscopie. Nous avons exclu les patients avec résection de l'intestin associée à la CU ou une rémission clinique prolongée de plus de 10 ans. Au moment de la coloscopie, nous avons mesurés le niveau de la protéine C réactive (CRP) et de la calprotectine fécale en plus de l'évaluation endoscopique avec le score Mayo et le prélèvement de biopsies rectales pour documenter le score de Geboes et la présence de

plasmacytose basale. Les patients ont été divisés en trois groupes : rémission complète (groupe 1), rémission endoscopique seulement (groupe 2), et maladie active endoscopiquement (groupe 3). Les patients ont été suivis pendant un an avec une évaluation clinique avec le score Mayo à tous les 3 mois. Nous avons répété la CRP, la calprotectine fécale ainsi qu'une évaluation endoscopique et histologique si les patients ont eu une rechute ou à la fin de la période de suivi de 1 an.

Résultats : Nous avons recruté 100 patients avec médiane de 49 ans (intervalle interquartile 39-59 ans), 55% sont des hommes. La distribution de la maladie était comme suit : 16% avaient une rectite, 40% avaient colite gauche et 44% avaient pancolite. Le traitement médical inclus les 5-aminosalicylates chez 71% des patients, les thiopurines chez 28% et les produits biologiques chez 12%. À l'endoscopie, 61% avaient un score Mayo de 0, 29% un score de 1, 7% un score de 2 et 3% un score de 3. Un score Geboes ≥ 3.1 a été vu chez 55% des patients et la plasmacytose basale était documentée chez 37%. Le taux de rechute était similaire entre le groupe 1 (24,4%), le groupe 2 (22,2%), et le groupe 3 (20,0%). Bien que les résultats sont non concluantes, le sexe féminin (Rapport de cote (RC) = 0,45, intervalle de confiance (IC) 95% : (0,17-1,21)) et la thérapie biologique (RC = 0,64, IC 95%: (0,13-3,15)) ont été potentiellement associées à la rémission tandis que le présence de plasmacytose basale (RC = 2,04, IC 95%: (0,42-10,22)) ou un score Geboes ≥ 3.1 (RC = 1,21, IC 95%: (0,17-8,59)) ont eu tendance à prédire les rechutes. Une valeur pour la calprotectine fécale > 150 μg/g a démontré la meilleure utilité clinique avec une sensibilité de 75% et une spécificité de 65% pour prédire une maladie endoscopiquement active.

Conclusion : Bien que les résultats soient non concluants en raison de la taille de l'échantillon, la présence de plasmacytose basale et la maladie histologique active sont des facteurs prédictifs potentiels de rechute de la maladie tandis que la thérapie biologique pourrait avoir un effet protecteur. D'autres études avec un échantillon plus large sont nécessaires pour mieux investiguer ces associations.

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Introduction

Background

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic idiopathic inflammatory bowel diseases (IBD) characterized by the development of inflammation in the gastrointestinal tract presenting with diffuse loss of vascular pattern, erythema, and ulcerations. The disease spectrum may vary from mild symptoms with little impact on patients' quality of life to more severe symptoms that can lead to serious complications including gastrointestinal cancers, significant rectal bleeding, toxic megacolon, and perforation. Worldwide, Canada has one of the highest incidence and prevalence of IBD and more specifically UC. Based on recent data, this disease affects 194/100,000 Canadians, with 11.8/100,000 new cases per year.[1] One of the main epidemiologic features of UC is that the diagnosis is commonly made in young adults between the age of 20 and 40. There is another small speak in incidence of IBD between the age of 50 and 60. There is no gender predominance with the disease equally affecting males and females. The fact that patients are often diagnosed in early adulthood and that the disease is chronic and can lead to serious complications has significant impact on the quality of life and productivity of affected individuals. In addition, given its high prevalence, it is associated with an important economic burden to the Canadian society with an estimated $2.8 billion annually in direct and indirect cost.[2]

Mounting evidence shows that treating symptoms alone is not sufficient to prevent long-term complications and does not alter the nature of disease course.[3-7] In fact; there is a poor correlation between patient symptoms and the endoscopic appearance of the mucosa, which is more significant in CD than UC. Mucosal healing (MH), defined as healing of the mucosa

macroscopically as assessed by endoscopy, is now considered a key endpoint as it has been associated with better long-term outcomes including decreased risk for surgery.[8] This has led to discussions and a shift toward achieving MH as a therapeutic goal to prevent structural damage and disability, especially since the advent of new medical therapies including biologic agents. At present in Canada, infliximab (Remicade, Janssen), adalimumab (Humira, Abbvie), and golimumab (Simponi, Janssen) are the only anti-tumor necrosis factor alpha (TNFα) approved for use in UC. More recently this year, a new biologic agent with a novel mechanism of action, vedolizumab (Entyvio, Takeda), was approved by Health Canada for the treatment of moderate to severe UC that failed conventional therapy, providing patients and physicians with more options to treat ulcerative colitis. Vedolizumab is a blocker of the integrin α4β7 which allows it to be gut-selective.[9] It acts by blocking lymphocyte trafficking to the gut when the inflammatory cascade is activated. Population based studies have shown that standard medical therapy with corticosteroids, azathioprine or 6-mercaptopurine does not alter either the long-term outcomes or the rate of surgery in individuals with IBD over a long-term follow-up period.[10] However, most data pre-date the introduction of biologic therapy, which has the potential of healing the mucosa.[11] Little to no data is available on the effect of biologic therapy on changing the natural history of the disease course.

Definition of mucosal healing

There is a significant discrepancy in the definition of MH among physicians and a validated universal designation has yet to be developed. Several endoscopic indices (Table 1) have been used in clinical trials to assess MH which renders the comparison between rates of endoscopic remission between trials hazardous.[12-18] However in most recent studies, the Mayo UC

endoscopic index (Table 2) has been the most commonly used method to evaluate the mucosa in clinical trials and clinical practice.[16] Meanwhile, the International Organization for the Study of Inflammatory Bowel Disease (IOIBD) task force agreed on a definition for MH which includes the absence of friability, blood, erosions, and ulcers in all visualized segments of the gut mucosa.[11] This concept is crucial for many reasons. First, it is important to insure that we have a validated score and to reduce subjectivity. In addition, it is important to standardize clinical practice and clinical trial endoscopic outcomes to allow direct comparison between trials and monitoring of drug efficacy.

Table 1. Ulcerative colitis endoscopic scores

Index	Variable
Truelove and witts score[12]	Mucosal assessment (granularity, hyperemia)
Baron score[13]	Bleeding, vascular pattern
Powell-Tuck score[14]	Bleeding
Sutherland score[15]	Bleeding, friability
Mayo endoscopic subscore[16]	Vascular pattern, erythema, friability, erosions and ulcerations, bleeding
Rachmilewitz score[17]	Granulation, mucosal damage, vascular pattern, bleeding
Modified Baron score[18]	Vascular pattern, friability, ulcerations, bleeding

Table 2. Mayo Endoscopic Score[16]

Score	Disease activity	Endoscopic features
0	Normal or inactive	None
1	Mild	Erythema, decreased vascular pattern, mild friability
2	Moderate	Marked erythema, absent vascular pattern, friability, erosions
3	Severe	Spontaneous bleeding, ulceration

The difficulty in comparing MH is not only limited to choosing which scoring system to use but also to agree on a definition for MH for a specific index. Within the same score, there is no

consensus on the exact definition of MH. In most clinical trials, MH is defined as an endoscopic Mayo score of 0 or 1. This means that mild endoscopic disease activity is permitted and is considered by most experts as healed mucosa. This concept is debated by others who claim that mucosal healing should be strictly limited to an endoscopic Mayo score of 0. However, this strict definition has significant consequences if applied to clinical trials because the endoscopic remission rates would be less than 30% at best which is clearly inadequate for such therapy. MH was defined as either completely normal (score of 0) or mild (score of 1) mucosal disease in the ASCEND and ACT trials but < 1 in the MMX mesalamine trials. This discrepancy adds to the confusion in assessing the response and potential beneficial endoscopic effect of medical therapy. For example, in the ASCEND study, MH (score ≤ 1) was achieved in up to 80% in patients on mesalamine 4.8 g per day at week 6 of treatment.[19,20] However, when using the stricter subscore of 0 for MH, this percentage fell to approximately 30%. If the same rule of strict mucosal healing (Mayo score 0) is applied to biologic therapy, the MH rates are estimated to 10-15%. Given the potential side effect profile of biologic therapy, such low rates of MH would be at potential high cost. Table 3 describes examples of the impact of different definitions of remission threshold on clinical trials outcomes. Therefore, until better therapeutic options are available, a more liberal definition for mucosal healing is currently considered acceptable.

Table 3. Example of the impact of different definitions of remission on clinical trials outcomes

Table 3 | Impact of different definitions of remission threshold on clinical trial outcomes

Study	ASCEND I/II[5, 20, 23]			EGF study[22]		
Index	Mayo/DAI			Powell-Tuck	UC-DAI	SSS
criteria included	Stool frequency relative to normal Rectal bleeding Sigmoidoscopy PFA PGA			Bowel frequency Stool consistency Abdominal pain Rectal bleeding EIS, temperature Sigmoidoscopy	Stool frequency Rectal bleeding Mucosal appearance Physician's rating	Stool consistency Blood in stool Nocturnal defaecation
Remission score	0	≤1	≤2	≤4	0–1	0
Remission rate	22%	28%	50%	83%	33%	83%

EGF, epidermal growth factor; DAI, disease activity index; PGA, physician's global assessment; PFA, patients functional assessment; SSS, simplified symptom score.

Achieving mucosal healing

Despite the lack of agreement on a definition for MH, it is now considered an essential goal of medical therapy in UC. It is also a more objective means to assess the anti-inflammatory effect of a drug without the influence of placebo. Mucosal healing has been achieved with several drugs including 5-aminosalicylates (5-ASA), steroids, azathioprine, anti-TNFα, and anti-α4β7. In the past decade alone, seven 5-ASA studies have looked at MH as an outcome (Table 4). As mentioned above, in the ASCEND studies assessing the efficacy of different doses of oral mesalamine, MH was achieved in approximately 80% of patients with mild to moderate disease.[19,20] However, the most convincing data on MH with 5-ASA arise from the Multi Matrix System (MMX) mesalamine studies. Treatment with MMX mesalamine 2.4g or 4.8 g per day achieved complete mucosal healing (Mayo subscore of 0) rates of approximately 32% at 8 weeks.[21, 22] In addition, in the open-label extension of these studies, a 4.8 g per day dose of MMX mesalamine achieved complete MH (Mayo subscore of 0) in 45% of patients 8 weeks post

induction. Furthermore, a recent meta-analysis showed that different preparations of 5-ASA oral or topical could attain MH in approximately 50% of cases.

Table 4. Summary of 5-ASA studies in adult UC patient including MH

TABLE 1. 5-Aminosalicylate (5-ASA) Induction-of-Remission Studies in Adult Patients with Ulcerative Colitis that have Included Mucosal Healing

Author	Drug(s)	No. of Patients	Disease	Definition of Mucosal Healing
Campieri et al (1991)[12]	5-ASA enema Placebo	113	Mild to moderate, distal	Rectal mucosa repaired with appearance of a vascular pattern
Vernia et al (2003)[13]	Rectal 5-ASA plus rectal sodium butyrate Rectal 5-ASA plus placebo	51	Mild to moderate, distal, refractory to rectal 5-ASA/cortisone	Sutherland sigmoido-scopy index score of 0 (normal)
Mulder et al (1996)[14]	5-ASA enema 5-ASA enema plus beclomethasone enema Beclomethasone enema	60	Mild to moderate, distal	Endoscopic healing (normal color, vascular pattern, and friability; absent granularity, ulcers, spontaneous bleeding, and mucopurulent exudates; sharp rectal valves)
Gionchetti et al (1999)[15]	Mesalamine gel enema (ENTERASIN; Crinos S.p.A, Como, Italy) Mesalamine foam enema (ASACOL®; Procter & Gamble Pharmaceuticals, Cincinnati, OH)	103	Mild to moderate, left-sided or proctosigmoiditis	Baron sigmoidoscopy score of 0 (normal mucosa or inactive disease)
Gionchetti et al (1997)[16]	Mesalamine suppository (PENTASA®; Ferring Pharmaceuticals, Copenhagen, Denmark or Shire Pharmaceuticals Inc., Wayne, PA) Mesalamine suppository (CLAVERSAL®; Merckle GmbH, Ulm, Germany)	50	Mild to severe, proctitis or distal proctosigmoiditis	Baron sigmoidoscopy score of 0 (normal mucosa or inactive disease)
Campieri et al (1990a)[17]	Mesalamine suppository (ASACOL) Placebo	62	Mild to moderate, distal	Rectal mucosa apparently repaired
Campieri et al (1990b)[18]	Mesalamine suppository Placebo	94	Mild to moderate, distal proctitis or proctosigmoiditis	Baron sigmoidoscopy score of 0 (normal mucosa or inactive disease)
Williams et al (1990)[19]	Mesalamine suppository (ROWASA®; Solvay Pharmaceuticals, Inc., Marietta, GA) Placebo	173	Mild to moderate, proctitis	Sutherland sigmoido-scopy index score of 0 (normal)
Baron et al (1962)[20]	Oral sulfasalazine Oral salicylazosulphadimidine Placebo	50	Mild	Baron sigmoidoscopy score of 0 (normal mucosa or inactive disease)
Mansfield et al (2002)[21]	Oral sulfasalazine Oral balsalazide	50	Mild to moderate	Normal sigmoidoscopic appearance
Hanauer et al (1993)[22]	Oral mesalamine (PENTASA) Placebo	374	Mild to moderate, left sided or pancolitis	Sutherland sigmoido-scopy index score of 0 (normal)
Schroeder et al (1987)[23]	Oral mesalamine (ASACOL) Placebo	87	Mild to moderate	Mayo sigmoidoscopy score of 0

TABLE 1. (*Continued*)

Author	Drug(s)	No. of Patients	Disease	Definition of Mucosal Healing
Sninsky et al (1991)[24]	Oral mesalamine (ASACOL) Placebo	158	Mild to moderate	Mayo sigmoidoscopy score of 0
Kamm et al (2007)[25]/ Lichtenstein et al (2007)[26]/Sandborn et al (2007)[27]	Oral MMX mesalamine (LIALDA; Shire Pharmaceuticals Inc., Wayne, PA) Placebo	517	Mild to moderate	Modified Sutherland sigmoidoscopy index score of 0 (normal)
Paoluzi et al (2002)[28]	Oral mesalamine plus mesalamine enema	149	Mild to moderate	Baron sigmoidoscopy score of 0 (normal mucosa or inactive disease)
Vernia et al (2000)[29]	Oral mesalamine plus oral sodium butyrate Oral mesalamine plus placebo	30	Mild to moderate	Sutherland sigmoido-scopy index score of 0 (normal)

In the past decade, especially with the advent of biological therapy, MH was achievable in patients with more severe disease (Table 5). The mucosa of the colon is thought to be easier to heal in UC compared to CD given that in UC the ulcerations are limited to the mucosa and submucosa. In a small study, infliximab was shown to improve the morphology and function of epithelial organelles, rich mucus secretion and recovery of the chorionic components.[23] In the ACT 1 and 2 trials, infliximab improved endoscopic appearance (Mayo score 0 or 1) in up to 62% of patients with UC by 8 weeks of therapy, 50% by week 30, and 46% by week 54.[24] In the ULTRA 2 study, adalimumab achieved endoscopic remission in 41% of patients at week 8 and 25% at week 54.[25] In the PURSUIT study for Golimumab, mucosal healing was documented in 45% of patients at week 6 and 41% at week 54.[26, 27] Finally, the most recent biologic efficacious for the treatment of ulcerative colitis, vedolizumab, showed mucosal healing rates of 41% and 56% at weeks 6 and 54, respectively.[28]

Table 5. Summary of corticosteroids, thiopurines, and biologicals studies with MH in UC patients

Table 6 | Main data from the past decade regarding MH with corticosteroids, immunosuppressive and biological agents in patients with UC

Author	Design	Drug(s)	*n*	Population	Timing of endoscopic evaluation	Endoscopic index	Definition of MH	Number of patients achieving MH (%)*
Rizello *et al.*[54] (2002)	Multicenter, randomized, double-blind, placebo-controlled study (Italy)	Beclometasone‡ dipropionate 5 mg oral once daily vs placebo	119	Active UC (UCDAI§ 3–10 points)	4 weeks	Baron index	Normalization of intestinal mucosa	18/58 (31%) vs 10/61 (16%)
Gross *et al.*[46] (2006)	Multicenter, randomized, double-blind, controlled study (Europe and Israel)	Budesonide 2 mg foam enema once daily vs budesonide 2 mg liquid enema once daily	541	Active ulcerative proctitis or proctosigmoiditis with CAI‖ of >4, and an endoscopic index of ≥4	4 weeks	Rachmilewitz endoscopic index	Endoscopic remission (Rachmilewitz index of <4)	106/204 (52%) vs 127/234 (54%)
Paoluzi *et al.*[60] (2002)	Single-center, single-blind, open-label study (Italy)	Azathioprine 2 mg/kg/day orally or methotrexate¶ 12.5 mg intramuscularly once weekly	42	Refractory active UC	6 months	Baron index and Truelove and Witts index	Absence of inflammatory changes in the mucosa	Azathioprine 22/32 (68.7%) methotrexate 6/10 (60%)
Ardizzone *et al.*[61] (2006)	Single-center, randomized, investigator-blind, controlled study (Italy)	Azathioprine 2 mg/kg/day orally vs Asacol®) delayed-released mesalazine 3.2 g three times daily	72	Steroid-dependent clinically and endoscopically active UC (Baron index of ≥2)	6 months	Baron index	Baron score of ≤1	19/36 (53%) vs 7/36 (19%)**
Rutgeerts *et al.*[76] (2005) ACT1	Multicenter, randomized, double-blind, placebo-controlled study (international)	Infliximab 5 mg/kg IV at weeks 0, 2, and 6 and then every 8 weeks vs placebo infusion at week 0, 2, and 6 and then every 8 weeks	364	Active refractory UC‡‡ (Mayo score 6–12 with endoscopic subscore ≥2) despite concurrent treatment	8 weeks 54 weeks	Mayo endoscopic subscore	Absolute subscore for endoscopy of 0 or 1	75/121 (62%) vs 41/121 (33.9%) 55/121 (45.5%) vs 22/121 (18.2%)
Barreiro§§ *et al.*[75] (2008)	Single-center, open-label study (Spain)	Infliximab 5 mg/kg at weeks 0, 2, and 6 and then every 8 weeks	17	Steroid-dependent (>10 mg/day equivalent prednisolone) active UC with resistance or intolerance to azathioprine	52 weeks	Mayo endoscopic subscore	Endoscopic score of 0 or 1	12/17 (71%)
Afif *et al.*[77] (2009)	Multicenter, open-label study (USA)	Adalimumab SC 160 mg at week 0, 80 mg at week 2 and then 40 mg every other week	20	Active refractory UC (Mayo score 6–12 with an endoscopic subscore ≥2) despite concurrent treatment	8 weeks	Mayo endoscopic subscore	Decrease in endoscopic subscore from 2 or 3 at baseline to 0 or 1	6/20 (30%)

This table summarizes main studies in the past decade that have included MH as end point for the evaluation of steroids, antimetabolites and biological agents. *Results presented correspond to MH or endoscopic remission, according to the definition in each study. ‡Patients were on concomitant 5-ASA 3.2 g/day. §Ulcerative colitis disease activity index also known as the disease activity index (DAI) according to Sutherland with modification for the endoscopic subscore. Friability corresponds to a score of 2 in the modified index. ‖Clinical activity index according to Rachmilewitz. ¶Patients included in the AZA study who were intolerant or resistant to azathioprine were switched to methotrexate. **Results correspond to complete remission (clinical and endoscopic), $P = 0.006$. ‡‡In the ACT1 trial, patients were refractory to corticosteroids alone or in combination with azathioprine. Dose of concomitant medications remained constant except for corticosteroids which were tapered after week 8 until discontinuation. $P < 0.001$ for infliximab 5 mg/kg vs placebo at week 8 and 54. §§Available only as abstract. Abbreviations: CAI, clinical activity index; IV, intravenously; MH, mucosal healing; SC, subcutaneously; UC, ulcerative colitis; UCDI, ulcerative colitis disease activity index.

Mucosal healing and clinical outcomes

In clinical practice, endoscopic assessment of the mucosa is not readily available and limited by resources. Patients might be in clinical symptomatic remission but have residual active endoscopic disease, which puts them at a higher risk of disease relapse. It has been shown that there is a significant discrepancy between symptoms and endoscopic disease activity both in UC and more so in CD. Preliminary data is showing that MH seems to have a protective effect on long-term complications in UC. In the ACT 1 and 2 trials[24], the proportion of patients who were in remission at week 30 was 4-fold greater for the patients who achieved MH at week 8 (48.3%) compared to those who did not achieve MH (9.5%). Similarly, in the same study, patients in MH had a much longer time to colectomy than patients not in MH. Furthermore, in a study by Rutter and coworkers[29], active endoscopic inflammation was associated with an increase of dysplasia and colorectal cancer. Recent data also suggest that endoscopic MH is associated with reduced risks for hospitalization and need for surgical resection[8, 30]. In a Norwegian cohort, colectomy rates were significantly lower at 1-year follow-up in patients who achieved mucosal healing.[8] Finally, MH was associated with a normalization of the perception of health and therefore the quality of life by most IBD patients independently of treatment.[31]

Role of histology on disease activity

Despite being treated with medical therapy that can achieve MH, a significant proportion of patients will relapse, with rates that vary between 15% and 40% over a 12-month period. In fact,

few studies in UC have shown that despite endoscopic MH, active histologic disease is associated with poor long-term outcomes. Riley et al. demonstrated that patients on 5-ASA with clinical and endoscopic remission but active histology, defined by the presence of acute inflammatory cell infiltrate, crypt abscesses, mucin depletion, or breaches in the surface epithelium had significantly higher relapse rates over a 12-month follow-up period.[32] However, none of these microscopic features were independent predictors of disease relapse in a study by Bitton et al.[33] In his cohort, Bitton showed that the presence of basal plasmacytosis on rectal biopsies, a histologic feature not assessed by Riley, was independently associated with a 4.5 increased risk of relapse. Furthermore, Rutter et al. showed that a higher histologic inflammation score was strongly associated with an increased risk of neoplasia in patients with UC.[29] Bryant et al.[34] also reported that an increased level of inflammation predicts colectomy and hospitalization. A one-point increase in the histologic inflammation score increased the risk of surgery by 90% and the risk of hospitalization by 52%. Recently, we retrospectively confirmed that the presence of basal plasmacytosis (figure 1) in healed mucosa was strongly predictive of disease relapse over a 12-month period (OR= 5.13, 95% CI (1.32 – 19.99)), and that the use of biologicals demonstrated a strong trend towards a protective effect (OR= 0.24, 95% CI (0.05 – 1.01)).[35]

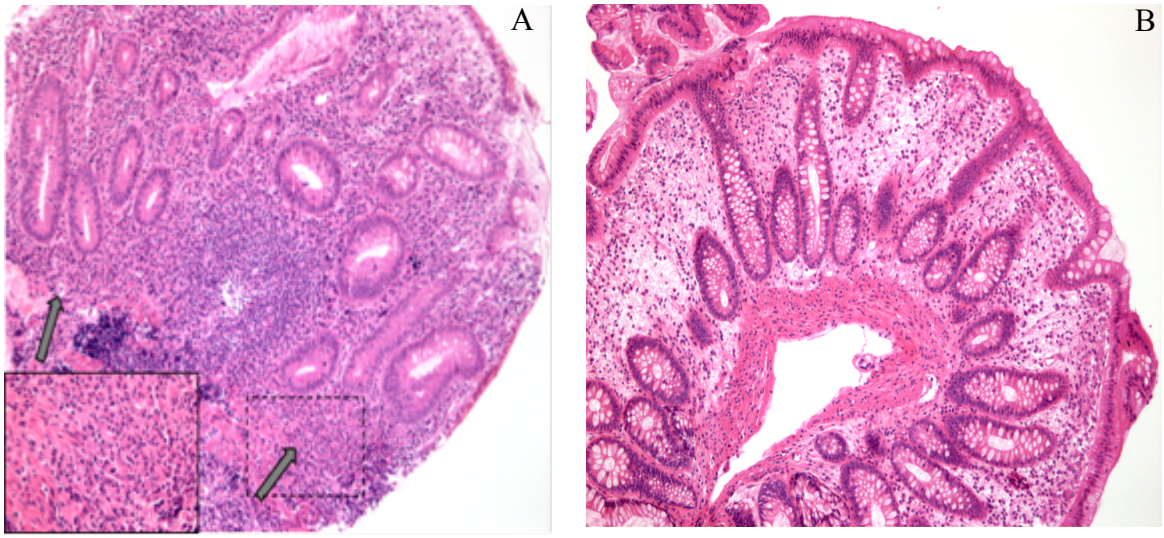

Figure 1. A) Colonic biopsy sample showing diffuse dense infiltration of plasma cells between the crypt base and the muscularis mucosa (grey arrow). B) Colonic biopsy devoid of basal plasmacytosis.

In addition, the subjective nature of the evaluation for endoscopic MH as well as the poor correlation between the endoscopic appearance and the histologic inflammatory activity renders this method of evaluating healing in IBD suboptimal. In an older study by Truelove and Richards[36], 37% of patients with normal mucosa assessed by sigmoidoscopy had mild to moderate microscopic inflammation. Our data also demonstrated that there is a poor correlation between the histologic and endoscopic mucosal assessments; in fact up to 40% of patients with normal mucosa on endoscopy demonstrated active microscopic disease as determined by a Geboes score $\geq$ 3.1 (presence of epithelial neutrophils with or without crypt destruction or erosions).[37] Hence, emerging data demonstrates the pivotal role of microscopic disease assessment, a finding that require to be validated in larger prospective studies.

Fecal calprotectin in ulcerative colitis

The assessment of the endoscopic disease activity requires patients to undergo regular sigmoidoscopies or colonoscopies. This approach is considered the gold standard but has multiple limitations. First, endoscopic procedures are costly and require many resources, which are nowadays scarce. In addition, they are invasive and therefore carry risks to the patient. Typically, the risks quoted to patients prior to the colonoscopy are complications related to the sedation, possible risk of bleeding and more rarely a risk of causing a perforation of the colon.[38] Therefore, several surrogate markers of disease activity have been developed and validated including C reactive protein (CRP), fecal calprotectin (FC) and stool lactoferrin.[39]

Fecal calprotectin is a protein found in the cytoplasm of neutrophils.[40] Neutrophils are attracted to the gastrointestinal tract when an inflammatory process is present. The calprotectin present in stool neutrophils will be shed and can be measured. Although a marker of inflammation, FC is not specific to inflammatory bowel diseases and can also be present in any condition which attracts neutrophils to the gastrointestinal tract such as acute or chronic infectious gastroenteritis, collagenous colitis, graft versus host disease and others.

Fecal calprotectin has gained a lot of popularity because of its high sensitivity and specificity in inflammatory bowel disease. It is a simple test that requires only 50 mg to 100 mg of stool and with a reasonable cost (estimated cost of USD$ 100 per assay). FC has been shown in multiple studies to be a very good surrogate marker of disease activity in UC. In a study of 134 patients with UC, there was a very good correlation between the Rachmilewitz endoscopic activity index

and fecal calprotectin with r = 0.834 for detection of disease activity. This correlation with FC was superior to the correlation with the Rachmilewitz clinical activity index, CRP, and blood leukocytes.[41] The sensitivity and specificity of FC for detecting endoscopic disease activity with a cut-off of 50µg/g was 93% and 71%, respectively. Determining the exact cut-off value for disease activity remains controversial. Several studies have been published and cut-off values of 150µg/g, 200µg/g, 250µg/g or even 300µg/g have been used to determine disease activity or for predicting disease relapse in inflammatory bowel diseases.[42, 43]

C-reactive protein

CRP is a marker of inflammation and is most commonly used to assess and monitor disease activity in patients with inflammatory bowel diseases. Being a simple blood test, it is widely available and very inexpensive. However, the main disadvantage of this marker is that it is non-specific to inflammation bowel disease. Any inflammatory condition whether gastrointestinal in nature or not will raise the CRP measurements and therefore can result in a false positive test. Furthermore, there is a significant heterogeneity in the production of CRP. In fact, it is estimated that 15% to 20% of normal healthy individuals do not mount a CRP response during inflammatory conditions.[44, 45] Therefore, although widely used as a surrogate marker of disease activity, CRP has multiple limitations highlighting the need for better assessment tools.

Summary and rational

Whereas mucosal healing has become a key endpoint for the treatment of UC, both MH and inactive histologic inflammation (complete remission) might be a better predictor of favorable outcomes in UC. It is still unclear whether MH or histologic inactivity should be used as the ultimate endpoint in the medical management of UC. We propose to evaluate the impact of complete remission on disease relapse.

Study findings will provide novel information on the role of macroscopic and microscopic activity in the natural history of UC as well on the predictive role of serologic, fecal, endoscopic and histologic markers on disease relapse. This insight will allow for improved identification of high-risk patients that can be used to provide closer follow-up, optimization of medical therapy, and serve as an alternative endpoint in clinical trials.

Objectives

1- To compare the predictive value of complete remission, defined as both endoscopic and histologic mucosal healing, to endoscopic MH alone and to clinical remission alone, on maintenance of remission in UC patients during a 12-months follow-up period.

2- To determine demographic, serologic, fecal, endoscopic, and histologic predictors of relapse during a 12-months follow-up period.

3- To assess the correlation between CRP, fecal calprotectin, endoscopic Mayo score, and the Geboes score (UC histologic disease activity score).

4- Determine fecal calprotectin cutoff values to predict endoscopic and histologic disease activity.

Hypothesis

Complete remission, defined as both endoscopic and histologic mucosal healing, is associated with a significant decrease in UC relapse at 1 year compared to endoscopic MH alone and to clinical remission alone.

Methods

Study site, design, and population

This prospective cohort study included a 1-year follow-up period. A research assistant approached patients treated at the various McGill IBD hospitals in Montreal, Quebec to explain the study objectives and to obtain informed consent. These clinics include two adult McGill affiliated sites (Royal Victoria Hospital and Montreal General Hospital).

Inclusion criteria:

- A confirmed diagnosis of UC based on accepted endoscopic, radiologic, and histologic criteria.
- Consecutive patients presenting to the IBD clinics or the endoscopy unit for colonoscopy for disease assessment or neoplasia surveillance.
- Adult patients that are 18 years of age and older.
- Being in clinical remission for at least 3 months prior to the colonoscopy. Clinical remission is defined as a partial Mayo score of 2 points or lower, with no individual subscore exceeding 1 point (Table 6).

Table 6. Clinical Mayo Score for Assessment of Ulcerative Colitis Clinical Disease Activity

Stool Frequency 0 = Normal number of stools 1 = 1-2 stools more than normal 2 = 3-4 stools more than normal 3 = 5 or more stools more than normal Subscore, 0 to 3
Rectal Bleeding 0 = No blood seen 1 = Streaks of blood seen less than half the time 2 = Obvious blood with stools most of the time 3 = Blood alone passed Subscore, 0 to 3
Physician global assessment 0 = Normal/inactive disease 1 = Mild disease 2 = Moderate disease 3 = Severe disease Subscore, 0 to 3

Exclusion criteria:

- Any previous surgical bowel resection associated to their ulcerative colitis.
- A diagnosis of IBD type unclassified or Crohn's disease (as per the Montreal classification).
- Prolonged disease remission without disease flare in the past 10 years.
- Use of oral or topical steroids within 90 days of study entry
- Altered dosage of mesalamine, thiopurines, or biologic therapy in the 3 months period prior to study entry.

Ethics approval was obtained from the McGill University Health Center research ethics committee (MUHC ID 12-352-BMD).

Procedure (Figure 2)

Patients were classified as having complete remission (group 1), endoscopic MH alone (group 2) or clinical remission alone (group 3). Four biopsies from the rectum were taken from all UC patients in clinical remission for at least 3 months (partial Mayo score $\leq$ 2) undergoing endoscopic assessment whether for disease activity or neoplasia surveillance colonoscopy. Biopsies are taken from the rectum because it is always affected by the disease and usually where the disease is the most severe. There are few exceptions to this rule but those situations are excessively rare. In patients on topical rectal therapy, proximal colonic biopsies were taken to avoid bias. The mucosal biopsies were fixed in 10% neutral formalin and were processed and sections stained with hematoxylin and eosin (H and E) for examination. At the time of endoscopy, all patients were required to give a blood sample that was analyzed to identify available serologic markers of inflammation. A stool sample collected the morning prior to the colonoscopy and before starting the bowel preparation was also obtained from patients to measure fecal calprotectin levels. Demographic and clinical data collection was performed by chart review and direct patient interview. Patients were seen on their regular scheduled visits every 3 months over a 12-month period and were assessed for clinical relapse using the total Mayo score. During the first year, all patients underwent endoscopic assessment of their disease by sigmoidoscopy or full colonoscopy at the time of clinical relapse or at the 12-month follow-up visit. Relapse was defined as a partial Mayo score $\geq$ 3 and endoscopic Mayo subscore of 2 or 3. Patients with established disease relapse had repeat measurement of their fecal calprotectin level as well as blood tests to document the measurement of CRP levels and remained followed for the entire length of the study.

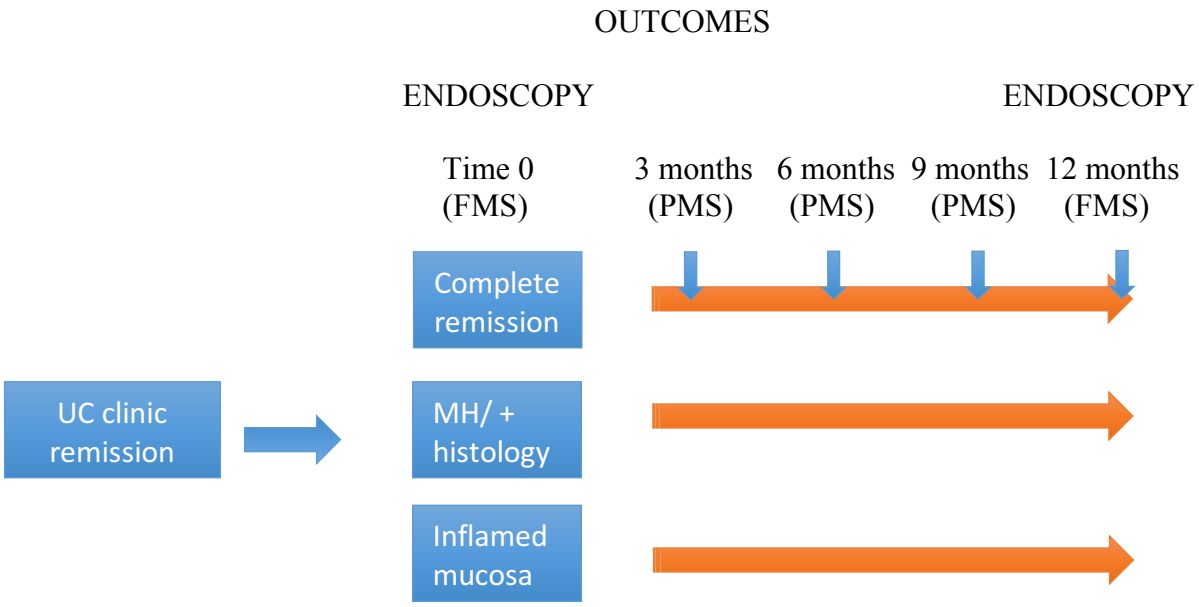

Figure 2. Study design flow chart

UC: Ulcerative colitis; MH: Mucosal healing; FMS: Full Mayo score; PMS: partial or clinical Mayo score

Patient characteristics

At the time of the baseline colonoscopy, the following demographic and clinical characteristics were collected:

- Age at time of cohort entry
- Sex
- Weight and height
- Smoking status (active or non-smoker)

- Occupation and level of education
- History of appendectomy
- Date of diagnosis of UC (duration of disease)
- Extent of disease according to the Montreal classification (E1 = proctitis, E2 = left-sided colitis, E3 = extensive colitis or pancolitis)
- Number of clinical relapses in the past 2 years prior to the colonoscopy
- Time from last relapse prior to study entry
- First-degree relatives with IBD
- Medical therapy at the time of endoscopy and at follow-up as well as previously used medical therapy and dosages

Serological variables collected at the time of endoscopy and at the time of relapse include:

- Hemoglobin concentration in g/L (normal 140-180 g/L)
- Hematocrit in L/L (normal 0.360-0.520 L/L)
- White blood cells in 10^9/L (normal 4.00-11.00 10^9/L)
- Platelets count in 10^9/L (normal 140-450 10^9/L)
- Albumin in g/L (normal 38-52 g/L)
- CRP in mg/L (normal 0.00-5.00 mg/L)

Endoscopic evaluation was based on the Mayo endoscopic subscore of 0-3 (0: normal; 1: erythema, decreased vascular pattern, mild friability; 2: marked erythema, absent vascular pattern, friability, erosion; 3: spontaneous bleeding, ulcerations).[16] Mucosal healing was defined according to the international organization for the study of inflammatory bowel disease (IOIBD) definition of MH; absence of friability, blood, erosions, and ulcers in all visualized segments of

the gut mucosa, which is equivalent to the endoscopic Mayo score of 0 or 1.[11] The three endoscopists participating in the study who are all co-investigators were requested to describe the mucosal findings as well as completing a full Mayo score. Relapse was defined as a total Mayo score of ≥ 3 with endoscopic Mayo subscore of 2 or 3. Clinical relapse was defined as partial Mayo score of ≥ 3. An expert gastrointestinal pathologist, independent of the three endoscopists and blinded to clinical information, evaluated the microscopic disease activity using the Geboes score (Table 7)[37] and documented the presence of basal plasmacytosis (focal/diffuse pattern). Basal plasmacytosis is defined as dense infiltrate of plasma cells around the deep part of the lamina propria or between the base of the crypts and the muscularis mucosa.[46] The Geboes score is a non-additive score where the highest grade is retained in the total score. A higher score indicates greater inflammation. Grades and subgrades provide the basis for evaluating disease activity in UC as follows: grade 0, structural and architectural changes; grade 1, chronic inflammatory infiltrate; grade 2, lamina propria neutrophils and eosinophils; grade 3, neutrophils in the epithelium; grade 4, crypt destruction; grade 5, erosions or ulcerations. We defined active histologic disease as a Geboes score ≥ 3.1 (presence of epithelial neutrophils with or without crypt destruction or erosions). This cutoff is arbitrary and was chosen to be concordant with previous studies that used the same cutoff including our retrospective study.[35]

Table 7. Geboes Score for Assessment of Ulcerative Colitis Histologic Disease Activity[37]

Grade 0	**Structural (architectural changes)**
Subgrades	
0.0	No abnormality
0.1	Mild abnormality
0.2	Mild or moderate diffuse or multifocal abnormalities
0.3	Severe diffuse or multifocal abnormalities
Grade 1	**Chronic inflammatory infiltrate**
Subgrades	
1.0	No increase
1.1	Mild but unequivocal increase
1.2	Moderate increase
1.3	Marked increase
Grade 2	**Lamina propria neutrophils and eosinophils**
2A Eosinophils	
2A.0	No increase
2A.1	Mild but unequivocal increase
2A.2	Moderate increase
2A.3	Marked increase
2B Neutrophils	
2B.0	No increase
2B.1	Mild but unequivocal increase
2B.2	Moderate increase
2B.3	Marked increase
Grade 3	**Neutrophils in epithelium**
Subgrades	
3.0	None
3.1	<5% crypts involved
3.2	<50% crypts involved
3.3	>50% crypts involved
Grade 4	**Crypt destruction**
Subgrades	
4.0	None
4.1	Probable- local excess of neutrophils in part of crypt
4.2	Probable- marked attenuation
4.3	Unequivocal crypt destruction
Grade 5	**Erosion or ulceration**
Subgrades	
5.0	No erosion, ulceration, or granulation tissue
5.1	Recovering epithelium + adjacent inflammation
5.2	Probable erosion-focally stripped
5.3	Unequivocal erosion
5.4	Ulcer or granulation tissue

Testing for Calprotectin

Fecal calprotectin has been shown to correlate well with clinical and endoscopic disease activity in UC. However, its role as a predictor of clinical relapse in patients with mucosal healing has not been fully assessed. In addition, it is still unclear which cutoff value of fecal calprotectin should be used to document endoscopic mucosal healing and histologically inactive disease. It was measured at the time of mucosal healing as assessed by endoscopy and also when clinical relapse occurs. Patients were asked to bring a sample of the first morning bowel movement prior to starting the bowel preparation for the colonoscopy. Fecal calprotectin was measured by standard ELISA which was done by a blinded laboratory technician at the Montreal General Hospital and Hôpital Maisonneuve-Rosemont using Quantom Blue ® Calprotectin high (100-1800 μg/g) range Rapid tests by Bühlmann.[47]

Sample size

Approximately 200 patients were required to participate in the study. Numbers provided are estimates of reality since no data is available and the purpose of this study is to help elucidate this issue. This total is expected to be subdivided, respectively, into sizes of 50 (25%), 90 (45%), and 60 (30%) for the non-mucosal healing, mucosal healing histology negative and mucosal healing histology positive groups, respectively. In the non-mucosal healing group, we expect a one-year relapse rate of 35%, for which a sample size of 50 will result in a 95% confidence interval width of ± 0.1325. Similarly, in the mucosal healing histology negative group, we expect a one-year relapse rate of 15%, for which a sample size of 90 will result in a 95%

confidence interval width of ± 0.074. Finally, for the mucosal healing histology positive group, we expect a one-year relapse rate of 25%, for which a sample size of 60 will result in a 95% confidence interval width of ± 0.11. To compare the mucosal healing histology positive and negative groups, with sample sizes of 90 and 60, respectively, we expect a confidence interval width of ± 0.1265, which could be insufficient to find the estimated 10% difference, but will inform future studies. To compare the difference between the mucosal healing histology positive group and the non-mucosal healing group, sample sizes of 60 and 50, respectively, will lead to a confidence interval width of ± 0.175, which could not be sufficient to find the estimated 10% difference, although again, the information will enlighten future studies. The expected difference in relapse rates of 20% between the non-mucosal healing and mucosal histology negative is much larger and will likely be detected with the given sample sizes, the expected confidence interval width being +/- 0.15.

Data analysis

Forms for data collection were developed (see figures 3 & 4) and the data was entered in Excel (Microsoft Office). The data was password protected for confidentiality. Data was analyzed using statistics software R statistics version 3.2.2 and SAS version 9.4. The analyses were planned as follows:

1) The rate of relapse in the 3 groups of patients. Rates were expressed as crude rates where numerators are the total number of relapsers in a given group divided by the total number of patients in the same given group (denominator).

2) Potential demographic, serologic, stool, and histologic predictors of clinical disease relapse were assessed through univariate and multivariate logistic regression models. Depending on the level of measurement of the characteristics considered, association tests (e.g. Chi-square, Fisher's exact, or t-test) were performed. When an association is identified, its time-dependent role on clinical relapse was assessed by Kaplan-Meier survival curves or Cox regression analysis.
3) We assessed the association between endoscopy, histology, CRP, and fecal calprotectin using Spearman's rank correlation coefficient.
4) Receiver operating characteristic (ROC) curves were performed to determine cutoff values for fecal calprotectin to predict endoscopic and histologic healing.

P values < 0.05 were considered significant. On the univariate analysis, p values < 0.1 were considered as a trend toward significance and were included in the multivariate analysis. Variables known to be associated with the outcome but not found statistically significant on univariate analysis was forced in the multivariate analysis. For the Spearman's rank correlation coefficient values, a value for r between 0.00 and 0.19 was considered very weak, between 0.20 and 0.39 as weak, between 0.40 and 0.59 as moderate, between 0.60 and 0.79 as strong, and between 0.80 and 1.00 as very strong.

SOURCE DOCUMENT

Inclusion Criteria (Must all be Yes)

Adults 18 years of age and older ..... Yes No

Confirmed Diagnosis of ulcerative colitis
Accepted endoscopic, radiologic & histologic.....Yes No

Clinical remission for at least 3 months
Seen on endoscopy will be eligible .....Yes No

* Clinical remission defined as a partial mayo score of 2 points or lower, with no individual subscore exceeding 1 point

Exclusion Criteria: (Must all be No)

Previous Surgical resection.....Yes No

A Diagnosis of IBD type unclassified (Mtl Classification) .....Yes No

Prolonged disease Remission over 10 years.....Yes No

Use of Topical or Oral Steroids within 90days (3 months).....Yes No

Altered IBD Medication Dosage in the last 3 months prior
To study entry (mesalamine, thiopurines, or Anti-TNF.....Yes No

Baseline

Patients Name:_____ Date of Diagnosis:_____

Age:_____ Sex:_____

Weight:_____Kg Height:_____cm

Samples for future research.....Yes No

Consent Signed.....Yes No

NB#_____ ☐ Whole blood ☐ Serum ☐ Biopsy _____ (dd/mmm/yyyy)

☐ Stool _____ (dd/mmm/yyyy)

Figure 3. Data collection form 1

Smoking Status: Smoker_____

Ex-Smoker_____

Never Smoked_____

Occupation:_____

Years of Education:_____

History of Appendectomy:_____

Extent of Disease :_____ Cm
(Proctitis, Left-sided colitis, extensive colitis)

Number of Relapses in past 2 yrs:_____

Time From Last Relapse: _____ First degree Relative with IBD:_____

Hgb:_____ Hematocrit:_____ WBC:_____ Platelets:_____

Albumin:_____Bili Total:_____ Amylase:_____ AST:_____ ALT:_____

Alk Phos:_____ Creat:_____ Urea:_____

Medical Therapy and Dosages:

At Time of Endoscopy

_____

_____

_____

_____

_____

Previously Used Medication

_____

_____

_____

_____

Figure 4. Data collection form 2

Results

Study participants

We report on 100 patients with UC and their baseline characteristics (Table 8). Given the slow recruitment and long follow-up period, we performed an interim analysis. In our cohort, the median age was 49 years (interquartile range IQR 39-59 years) with 55% of patients being male. The majority of our patients (60%) had university education and 12% were active smokers at study entry. The Montreal classification distribution of our cohort was the following: 16% had proctitis (E1), 40% had left-sided colitis (E2) and 44% had extensive colitis or pancolitis (E3). When evaluating the medications that patients were taking at the time of study entry with a stable dose for at least 3 months (non-exclusive categories), 71% of patients were on 5-aminosalicylates, 28% on thiopurines (either azathioprine or 6-mercaptopurine) and 12% on biologic therapy. Patients on 5-aminosalicylates could be on the oral or topical formulation or a combination of both. Patients on biologic therapy were all on anti-TNF therapy with the vast majority being on infliximab (10/12 or 83%) and the remainders were on adalimumab (2/12 or 17%).

Given the fact that our cohort of patients was in clinical remission, we expected to have a cohort with mild or no active disease. Indeed, the mean full Mayo score was 0.81, which is clearly below the cutoff of 3 required for clinical remission. In addition, biomarkers of disease activity confirmed the low-grade clinical disease activity with a mean CRP of 4.68mg/L (normal < 5.00 mg/L) and a mean fecal calprotectin of 196 μg/g. The exact cutoff for fecal calprotectin to

predict clinical remission is still controversial but most studies use a value of < 200 μg/g or < 250 μg/g.

The baseline endoscopic and histologic examination was indeed very interesting. As expected, the majority of patients (61%) had no active endoscopic disease with a Mayo score of 0 while 29% had mild disease with a Mayo score of 1. As mentioned above, it is generally accepted that endoscopic Mayo scores of 0 or 1 are considered as endoscopic healing. It is interesting to note that despite being clinically in remission, 10% of patients had moderate to severe disease endoscopically. This highlights the discrepancy that can occur between the symptoms and endoscopic findings. When examining the histology, 55% of patients in this cohort had active disease with 37% having basal plasmacytosis.

Table 8. Baseline patient characteristics

	UC cohort n = 100
Median age, years	49 (IQR: 39-59)
Male sex %	55
University education %	60
Active smoker %	12
Montreal classification disease extend % - **Proctitis (E1)** - **Left-sided colitis (E2)** - **Extensive colitis (E3)**	16 40 44
Appendectomy %	4
Medication % - **None** - **5-Aminosalicylate** - **Thiopurine** - **Biologics**	 8 71 28 12
Mean CRP (mg/L)	4.68 (SD 14.0)
Mean Fecal calprotectin (µg/g)	196 (SD 238)
Mean baseline full Mayo score	0.81
Endoscopic Mayo score % - **0** - **1** - **2** - **3**	 61 29 7 3
Geboes score ≥ 3.1 %	55
Basal plasmacytosis %	37

IQR: interquartile range; SD: standard deviation; CRP: C reactive protein (normal < 5.0 mg/L);

Normal fecal calprotectin < 250 µg/g.

Cohort relapse rate

Over the 12-month period of follow-up, 23% of patients had disease relapse with a mean full Mayo score of 6.65 (Table 9). As per protocol, patients were requested to undergo an endoscopy to document relapse. Out of 23 patients, 7 (30.4%) refused the repeat endoscopy therefore we used the clinical Mayo score > 2 to classify these patients as part of the relapse category. In the remaining patients, endoscopy confirmed relapse of disease using a Mayo score of 2 or 3. The mean relapse CRP was 5.54 mg/L (standard deviation: 7.29 mg/L) and median of 2.38 mg/L (IQR: 0.38-7.93 mg/L). The mean relapse fecal calprotectin was 711 µg/g (standard deviation 542 µg/g) and medial of 887 µg/g (IQR: 86-1035 µg/g).

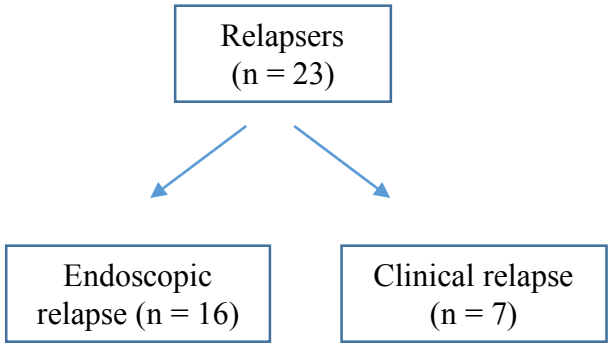

Figure 5. Flow sheet of relapsers

Table 9. 12-month relapse characteristics

	Cohort n = 100
Relapses	23
Mean relapse full Mayo score	6.65
Mean relapse CRP (mg/L)	5.54
Mean relapse fecal calprotectin (µg/g)	711

CRP: C reactive protein (normal < 5.0 mg/L), Normal fecal calprotectin < 250 µg/g

Comparison of relapse rate among the 3 groups

The main study outcome was to compare the rate of disease relapse during a 12-month follow-up period in the 3 groups of patients described below (Table 10). The disease relapse was similar in the complete remission group (group 1), the endoscopic remission but active histology group (group 2) and in the patients with active endoscopic disease (group 3), with rate of relapse of 24.2%, 22.2%, and 20.0%, respectively. The between group comparison results were not statistically significant with confidence interval values crossing 1 (Table 11).

Table 10. Relapse rate per group

Relapse group	# relapse	# patients in group	Relapse rate (%)	95% CI
Group 1	11	45	24.4%	12.9-39.5%
Group 2	10	45	22.2%	11.2-37.1%
Group 3	2	10	20.0%	2.52-55.6%

CI: confidence interval

Table 11. Comparison between relapse groups

Relapse groups	% difference	95% CI
Group 1 vs 2	-2.2%	-19.7-15.3%
Group 3 vs 1	-4.4%	-32.2-23.3%
Group 3 vs 2	-2.2%	-29.8-25.4%

CI: confidence interval

Predictors of relapse

On univariate logistic regression analysis, none of the demographic, medication, serologic, fecal marker, endoscopic or histologic features were significantly associated with relapse with confidence interval crossing 1 for each as seen in table 12.

Table 12. Univariate logistic regression analysis on relapse

variables	Odds ratio	95% confidence interval
Age	0.98	0.95-1.02
Sex	0.45	0-17-1.21
Smoking	1.13	0.28-4.59
5-aminosalicylate therapy	2.28	0.70-7.43
Thiopurine therapy	1.17	0.42-3.24
Biologic therapy	0.64	0.13-3.15
Fecal calprotectin	1.001	0.999-1.003
C reactive protein	0.99	0.97-1.03
Geboes score ≥ 3.1	0.77	0.14-4.20

A Wald mutlivariate logistic regression analysis showed no statistically significant associations between known characteristics influencing our primary outcome (see Table 13).

Table 13. Multivariable logistic regression analysis on relapse

Variable	Odds ratio	95% confidence interval
Fecal calprotectin	1.000	0.999-1.003
C reactive protein	0.99	0.97-1.03
Geboes score ≥ 3.1	1.21	0.17-8.59
Basal plasmacytosis	2.04	0.42-10.22

Predictors of time to relapse

When evaluating factors that could be predictive of time to relapse (Table 14), active endoscopy with Mayo score 2 or 3 (HR = 1.28, 95% CI (0.25-6.52)), presence of basal plasmacytosis on biopsies (HR = 5.11, 95% CI (0.76-34.3)), and an increase of 1 unit of fecal calprotectin (HR = 1.00, 95% CI (1.00-1.01)) were not associated with shorter time to relapse. Meanwhile, active histologic disease with Geboes score ≥ 3.1 (HR = 0.12, 95% CI (0.02-0.79)) was associated with longer time to relapse.

Table 14. Cox regression to predict time to relapse

Variable	Hazard ratio (HR)	95% Hazard ratio CI
Mayo score 2 or 3	1.28	0.25-6.52
Basal plasmacytosis	5.11	0.76-34.3
CRP > 5 mg/L	0.99	0.97-1.03
Fecal calprotectin	1.00	1.00-1.01
GS ≥ 3.1	0.12	0.02-0.79

CI: confidence interval; CRP: C reactive protein; GS: Geboes score

Correlation between biomarkers, endoscopy, and histology

Correlations between the biomarkers, endoscopy and histology are presented in table 15. CRP correlated very weakly with the Geboes score (r = 0.16) and weak with fecal calprotectin (r = 0.26) and endoscopic Mayo score (r = 0.34). The correlation between fecal calprotectin and the Geboes score was very weak (r = 0.13) and weak with the endoscopic Mayo score (r = 0.35). The correlation between the Geboes score and the endoscopic Mayo score was moderate with an r = 0.48.

Table 15. Correlation between CRP, fecal calprotectin, endoscopy, and histology

	CRP	Fecal calprotectin	Endoscopic Mayo score	Geboes score
CRP	1.00	0.26	0.34	0.16
Fecal calprotectin		1.00	0.35	0.13
Endoscopic Mayo score			1.00	0.48
Geboes score				1.00

Fecal calprotectin to predict endoscopic and histologic disease activity

We performed a ROC in an attempt to identify cutoff limits for fecal calprotectin to predict active endoscopic or histologic disease (figures 6 & 7). Fecal calprotectin tends to better predict active endoscopic disease with Mayo score 2 or 3 than active histologic disease with Geboes score ≥ 3.1 with area under the curve (AUC) = 0.69, 95% CI (0.47-0.90) and AUC = 0.54, 95% CI (0.41-0.66), respectively. When defining MH as Mayo 0 vs 0 and 1, the correlation was similar (results not presented)

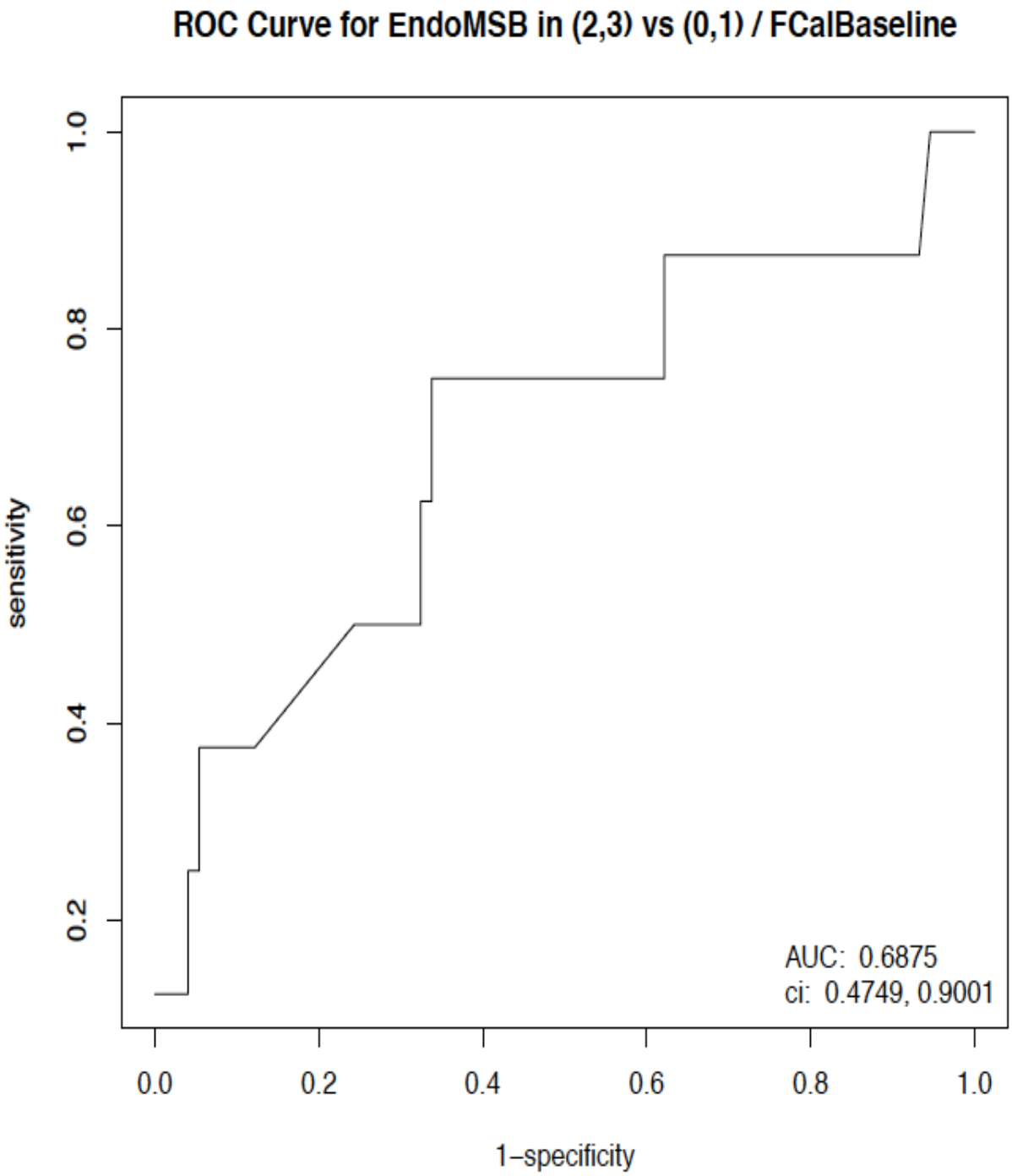

Figure 6. Receiver operating characteristic (ROC) curve for fecal calprotectin on endoscopic Mayo score 2 or 3 to determine cutoff values for endoscopic disease.

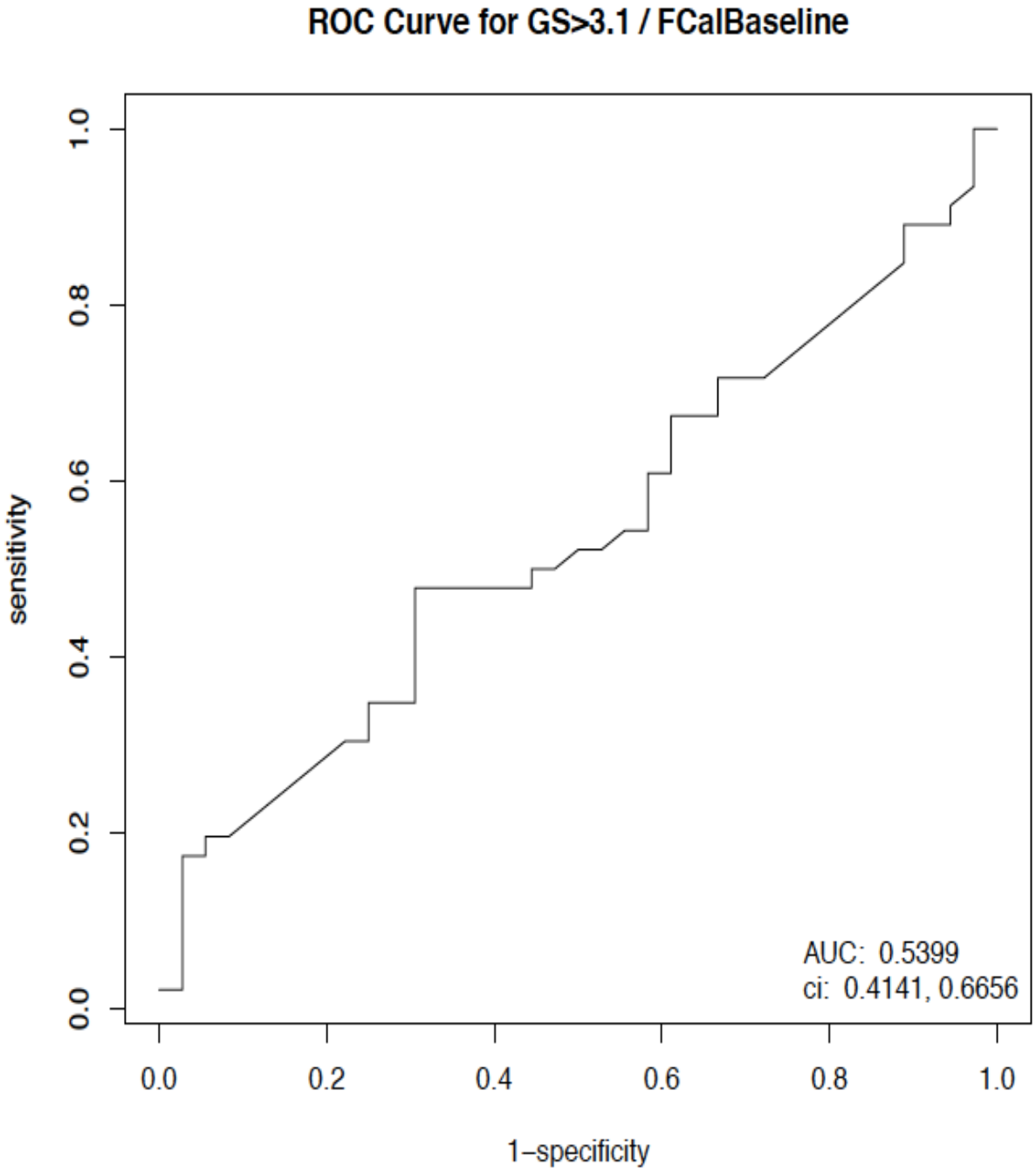

Figure 7. Receiver operating characteristic (ROC) curve for fecal calprotectin on Geboes score ≥ 3.1 to determine cutoff values for histologic disease.

Sensitivity and specificity to predict active endoscopic disease defined as endoscopic Mayo score of 2 or 3 and active histologic disease defined as Geboes score ≥ 3.1 for several fecal calprotectin cutoff values have been calculated (Table 16). To predict an endoscopic Mayo score of 2 or 3, the sensitivity and specificity for fecal calprotectin > 50 (µg/g) were 75.0% and 37.8%, for fecal calprotectin > 104 (µg/g) they were 75.0% and 51.4%, for fecal calprotectin > 150 (µg/g) they were 75.0% and 64.9%, for fecal calprotectin > 205 (µg/g) they were 62.5% and 66.2% and for fecal calprotectin > 247 (µg/g) they were 50.5% and 71.6%. To predict Geboes score ≥ 3.1, using the same cutoff values for fecal calprotectin, the sensitivities and specificities were 65.2% and 38.8%, 52.2% and 50.0%, 48.6% and 69.4%, 41.3% and 69.4%, 34.8% and 75.0% respectively.

Table 16. Sensitivity and specificity for fecal calprotectin to predict endoscopic and histologic disease activity

Fecal calprotectin	Endoscopic Mayo score 2 & 3		Geboes score ≥ 3.1	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
> 50 (µg/g)	75.0%	37.8%	65.2%	38.8%
> 104 (µg/g)	75.0%	51.4%	52.2%	50.0%
> 150 (µg/g)	75.0%	64.9%	48.6%	69.4%
> 205 (µg/g)	62.5%	66.2%	41.3%	69.4%
> 247 (µg/g)	50.0%	71.6%	34.8%	75.0%

Discussion

Our study is one of the first and unique studies looking prospectively at determining the role of endoscopic and histologic healing in disease relapse in patients with ulcerative colitis. For decades, the main objective of treatment was symptomatic improvement but more recently this has been demonstrated to be insufficient. There are many reasons why symptomatic improvement alone is not recommended. First, data demonstrated that there is a poor correlation between symptoms and the endoscopic appearance.[24] Symptomatic assessments have been based on the partial or clinical Mayo endoscopic score which is very subjective and patients often get used to a new threshold for symptomatic complaints which allowed to have mild symptoms such as stool urgency or increased number of bowel movements. Data failed to show a long-term benefit without change in the disease course.[3-7] In addition, data demonstrated that patients with active endoscopic disease are at an increased risk of disease relapse, hospitalization, and neoplasia. [8,30]

Baseline cohort characteristics

The median age was 49 years (interquartile range: 39-59 years) which is representative of the target population given that ulcerative colitis is a chronic disease that is commonly diagnosed between 20 and 40 years of age. There is a balanced sex distribution with male patients representing 55% of the cohort. Only 12% were smokers which is something we expect in ulcerative colitis because smoking tends to have a protective effect on the development of the disease. We used the Montreal classification for disease distribution which showed that 16% of

patients have proctitis, 40% left-sided colitis and 44% pancolitis. This demonstrates a normal distribution of disease for a tertiary care center with the majority of patients having left-sided colitis or pancolitis. Pancolitis has been shown to be a predictor of worse disease outcome therefore almost half of the cohort is at increased risk of worse prognosis.[48] History of appendectomy was shown to be protective from developing UC, therefore, it is not surprising that the rate is very low at 4% in our cohort. When inspecting the medical treatment patients were receiving at enrollment, 8% were on no treatment at all, 71% on 5-aminosalicylates, 28% on thiopurines (either azathioprine or 6-mercaptopurine), and 12% on biologic treatment (10% on infliximab and 2% on adalimumab). Combination therapy with 5-aminosalicylates and thiopurines was seen in 13%, 5-aminosalisates and biologics in 3%, thiopurines and biologics in 3% and 5-aminosalicylates, thiopurines, and biologics in 2%.

At study entry, we included patients that were in clinical remission as defined by a partial Mayo score of < 3. This is consistent with the mean baseline full Mayo score of 0.81 (remission < 3), a mean CRP of 4.68 mg/L (standard deviation = 14.0 mg/L) where the normal is < 5 mg/L and a mean fecal calprotectin of 196 μg/g (standard deviation = 238 μg/g) where the estimated cutoff value for disease activity is > 200 μg/g or > 250 μg/g depending on the source used. Interestingly 10% of patients in clinical remission had an endoscopic Mayo score of 2 or 3 consistent with moderate to severe endoscopic disease. This finding adds to the previously published data demonstrating that there is a significant discrepancy between endoscopic and symptomatic disease activity. The assessment of the histologic disease is very interesting where 55% of patients in clinical remission showed active histologic disease. These results are consistent with previous data that showed a rate up to 40-50% for histologic disease activity in patient with

clinically inactive disease.[35] Basal plasmacytosis, a previously identified independent predictor of disease relapse, was documented in 37% of patients. This rate is slightly higher than a previous study where the rate was 21%.[35]

Disease relapse per group

The rate of disease relapse in the entire cohort was 23%. In previous studies, the 1-year rate of clinical relapse was up to 33%.[49] Therefore, our rate of relapse is slightly lower than some of the published data and consistent with some more recent data.[35] As per protocol, disease relapse was defined as a partial Mayo score ≥ 3 and endoscopic Mayo subscore of 2 or 3. Upon symptomatic relapse, 7 out of the 23 patients (30.4%) refused to have endoscopic assessment (see figure 5). Therefore, we opted to use clinical remission as an indicator for disease relapse. We believe that this is a fair estimate of the rate of disease relapse because in our cohort, upon clinical relapse, every patient with endoscopic Mayo score of 2 or 3 had a clinical Mayo score of ≥ 3 and vice versa. In addition, the mean relapse full Mayo score was 6.65, which is consistent with moderate disease. The mean relapse values for CRP and fecal calprotectin were 5.54 mg/L and 711 µg/g respectively, both consistent with active endoscopic disease. Of note, the value for mean relapse for CRP is only slightly above normal. This is a common finding, as CRP tends to be marginally elevated in UC because it is a superficial mucosal disease. In a cohort study by Henriksen et al., only 23% of patient had an elevated CRP during active endoscopic disease.[50] In addition, it is estimated that 15% of normal healthy individuals do not mount CRP in response to active inflammation.[44, 45] This can be explained by different genetic polymorphism resulting in the non-production of CRP.

Similar relapse rates were demonstrated in the complete remission group (group 1), the endoscopic remission but active histology group (group 2) and in the patients with active endoscopic disease (group 3), with rates of relapse of 24.2%, 22.2%, and 20.0%, respectively. The percent difference in rates of remission was very small with corresponding wide 95% confidence intervals. The results are inconclusive due to the small sample size and do not show a trend toward lower disease relapse rate in either groups. With these preliminary data, we are unable to prove that the addition of histologic healing to endoscopic healing is beneficial but further studies with larger numbers are required to better evaluate this association.

Predictors of disease relapse

Our analysis of the predictors of disease relapse showed mainly inconclusive results as shown by wide 95% confidence intervals that includes 1. This is most likely due to the small sample size and thus an underpowered study. We will therefore look at the results and draw some conclusions from the trends. In the univariate analysis, female sex could potentially be protective from disease relapse with an odds ratio (OR) = 0.45, 95% CI (0.17-1.21). In addition, the use of biologic therapy was also potentially protective from disease relapse with OR = 0.64, 95% CI (0.13-3.15). This finding was also identified in the study by Bessissow et al. showing that biologic therapy barely missed significance favoring remission (OR = 0.24, 95% CI (0.05-1.01), P=0.052).[35] This finding is clinically sound since biologic treatment demonstrates the highest rates of mucosal healing which should prevent relapse. When examining the other variables, no trend was identifiable.

In the multivariate logistic regression analysis, the results were also inconclusive with very large 95% confidence intervals. CRP and fecal calprotectin had an OR very close to 1 which is clearly not significant and does not have an effect favoring relapse or remission. From a clinical standpoint, we would expect CRP to be neutral because of the fact that it is only abnormal in minority of UC patients however; we would have expected fecal calprotectin to be associated with disease relapse. In fact, De Surray et al. demonstrated that fecal calprotectin level start increasing 4-6 months prior to clinical relapse.[51] Both Geboes score $\geq$ 3.1 and presence of basal plasmacytosis show trends favoring disease relapse with OR = 1.21, 95% CI (0.17-8.59) and OR = 2.04, 95% CI (0.42-10.22), respectively. Once again, this was demonstrated by Bessissow et al.[35] in their retrospective study and it is clinically plausible that active histologic inflammation may be associated with increased risk of disease relapse.

We then performed a Cox regression analysis to determine whether any of the captured variables are predictive of time to relapse. Once again, CRP and fecal calprotectin had a neutral hazard ratio (HR). No studies to our knowledge identified any of these variables to be associated with shorter time to relapse. In a cohort study, an elevated CRP was predictive of increase risk surgery but not disease relapse therefore an elevated CRP seems to be present only in patient with very severe disease.[50] Meanwhile, a Mayo endoscopic subscore of 2 or 3 and the presence of basal plasmacytosis showed a potential trend toward shorter time to relapse with HR = 1.22, 95% CI (0.25-6.52) and HR = 5.11, 95% CI (0.76-34.3). To our knowledge, basal plasmacytosis was associated with shorter time to relapse in only one prospective study by Bitton et al. [33] Surprisingly, the presence of active histologic disease with Geboes score $\geq$ 3.1 was associated with longer time to relapse (HR = 0.12, 95% CI (0.02-0.79)). This is a difficult result to explain

as we would have expected active histologic disease to be associated with shorter time to relapse. Perhaps this represents a statistical error and will need to be further assessed in a larger study.

Correlation between biomarkers, endoscopy, and histology

Given the high resource requirements and the invasive nature of colonoscopy, we are in need of biomarkers with very good to excellent correlation with endoscopic disease activity and ideally histologic disease activity. Currently, CRP and fecal calprotectin are the most often used clinical biomarkers. Given what was discussed above on CRP, it is no surprise that CRP correlates poorly with fecal calprotectin, endoscopy and histology with R values of 0.26, 0.36, and 0.16, respectively. In fact, Mosli have shown that with a CRP cutoff of 5 mg/L, the sensitivity and specificity to predict endoscopic disease in UC was only 0.49 (0.34-0.64) and 0.92 (0.72-0.98).[39] Fecal calprotectin correlates also poorly with endoscopy with r = 0.35 and very poorly with histology (r = 0.13). Although the initial data has shown a good correlation between endoscopy and fecal calprotectin, more recent data from randomized controlled trials from the vedolizumab and tofacitinib drugs trials is showing that the correlation is not as good as predicted with the accuracy being only fair to good.[28, 52] In addition, there is a lot of variation in the reference values of calprotectin within individual patients.

The correlation between endoscopy and histology was moderate at r = 0.48. These results raise many issues. One would wonder why the correlation is not stronger and could it be related to the definition of what is endoscopic remission. As stated previously, mucosal healing is commonly defined as endoscopic Mayo score of 0 or 1. The endoscopic Mayo score of 1 allows mild

endoscopic activity which could in part explain the results. In this cohort, 29% of patient had an endoscopic Mayo score of 1 and 20 out of 29 patients or 69% had a Geboes score ≥ 3.1. Amongst the cohort of patients with endoscopic Mayo score of 0, 25 out of 61 patients or 41% had a Geboes score ≥ 3.1. This percent different is statistically significant ($p < 0.001$). Therefore, significantly more patients with Mayo endoscopic score of 1 have active histologic disease than in patient with a score of 0. In addition, we have to recall that the endoscopic scoring is subjective. It is imperative to question our ability to better score the mucosal appearance. The implication of a score of 0 vs 1 has an impact on the clinical symptoms where patients with a score of 1 will have residual symptoms of urgency and occasional rectal bleeding. In addition, some preliminary data demonstrated that the risk of relapse is significantly increased in patients with endoscopic score 1 vs 0 (36.6% vs 9.4%, $p<0.001$).[53]

Fecal calprotectin to predict disease prediction

Fecal calprotectin has been identified as a promising biomarker. It is non-invasive, relatively easy to perform, and cheap. The only drawback is that patients often refuse to bring stool samples. In our cohort, 17% of patients refused to bring a stool sample. Nevertheless, given its convenience, it is worth investigating the cutoff values that could predict endoscopic and histologic disease activity. Multiple small studies have been published and more recently a meta-analysis was performed to assess this matter.[39] There was a lot of variation among the studies with sensitivities ranging between 60% and 100% and the same applies for specificities ranging from 7% to 100% and this for different cutoff values anywhere between 50 μg/g and 250 μg/g. In this same meta-analysis, Mosli et al. determined that the optimal cutoff value for fecal

calprotectin to detect endoscopic disease was 50 µg/g with sensitivity of 88% and specificity of 79%.[38] In our cohort, to predict an endoscopic Mayo score of 2 or 3, the sensitivity and specificity for fecal calprotectin > 50 (µg/g) were 75.0% and 37.8%, for fecal calprotectin > 104 (µg/g) they were 75.0% and 51.4%, for fecal calprotectin > 150 (µg/g) they were 75.0% and 64.9%, for fecal calprotectin > 205 (µg/g) they were 62.5% and 66.2% and for fecal calprotectin > 247 (µg/g) they were 50.5% and 71.6%. We acknowledge that the area under the curve (AUC) is fair at best with AUC = 0.69. The use of fecal calprotectin in this context would be to identify with precision which patients are in endoscopic remission therefore we would need a high specificity. According to our data, fecal calprotectin value > 150 µg/g have the best sensitivity and specificity to predict active endoscopic disease among those patients with Mayo score of 0 or 1.

No data on histologic cutoff values for fecal calprotectin was presented in the study by Mosli et al. The data is still very limited and a cutoff value of 150 µg/g has been proposed by Guardiola et al.[54] In our study, to predict Geboes score ≥ 3.1, using the same cutoff values for fecal calprotectin as for endoscopic disease, the sensitivities and specificities were 65.2% and 38.8%, 52.2% and 50.0%, 48.6% and 69.4%, 41.3% and 69.4%, 34.8% and 75.0% respectively. Again here, we acknowledge that the AUC is poor at 0.54 which is barely better than a flip of a coin. From a clinical point of view, a high specificity is important because we need to identify with high certainty patients in histologic remission. Our data does not allow us to find the optimal cutoff value. Further studies with larger cohorts are required to better answer this question.

Limitations

Our cohort study has many strengths. First, this is a prospective study with a well defined cohort. Regular follow-ups every 3 months with Mayo score was documented. All patients had clinical, endoscopic, histologic assessments and biomarkers data was collected. Remission and relapse are both confirmed endoscopically. However, there were also many limitations. First and most important is that our study is underpowered as per our sample size calculation. This might explain why some of the data is at this point inconclusive however it is also possible that there is simply no difference between the groups. Second, 7/23 or 30.4% of patients refused to do an endoscopy to confirm relapse and we needed to use the clinical Mayo score to define relapse. Although we were reassured in seeing that all patients that had endoscopy with Mayo score 2 or 3 at the time of relapse also had a clinical Mayo score > 3 and vice versa. Thirdly, we had 17% of patients that did not bring stool samples for fecal calprotectin. This might have had an impact on the correlation and sensitivity and specificity results. We are currently continuing recruitment to be able to reach the objective of 200 patients in order to obtain adequate power for our main analysis. Conclusions may thus differ from this preliminary analysis of the data.

Conclusion

The prospective nature of our study makes it unique but is limited by a small sample size and thus, an underpowered study. Therefore, the results at this point are inconclusive but we can still draw some hypothetical conclusions. There was no difference in the rate of relapse among patients with complete remission or endoscopic remission alone. Female sex and biologic therapy might favor remission. The presence of active histologic disease with Geboes score ≥ 3.1 and basal plasmacytosis could predict disease relapse and shorter time to relapse. Finally, it appears that a fecal calprotectin value of > 150 μg/g could predict active endoscopic disease. Further studies with larger sample sizes are warranted to better define the role of endoscopy and histology in the development of disease relapse.

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