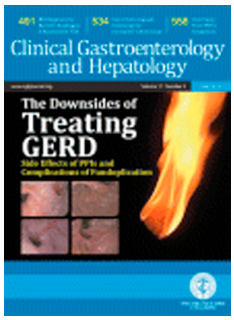
Accepted Manuscript



Histologic Normalization Occurs in Ulcerative Colitis and is Associated with Improved Clinical Outcomes

Britt Christensen, Stephen B. Hanauer, Jonathan Erlich, Olufemi Kassim, Peter R. Gibson, Jerrold R. Turner, John Hart, David T. Rubin

PII: S1542-3565(17)30196-9
DOI: 10.1016/j.cgh.2017.02.016
Reference: YJCGH 55118

To appear in: *Clinical Gastroenterology and Hepatology*
Accepted Date: 16 February 2017

Please cite this article as: Christensen B, Hanauer SB, Erlich J, Kassim O, Gibson PR, Turner JR, Hart J, Rubin DT, Histologic Normalization Occurs in Ulcerative Colitis and is Associated with Improved Clinical Outcomes, *Clinical Gastroenterology and Hepatology* (2017), doi: 10.1016/j.cgh.2017.02.016.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

TITLE: HISTOLOGIC NORMALIZATION OCCURS IN ULCERATIVE COLITIS AND IS ASSOCIATED WITH IMPROVED CLINICAL OUTCOMES

Short Title: Histologic normalization in UC

Authors: Britt Christensen (1,3), Stephen B. Hanauer (4), Jonathan Erlich (1), Olufemi Kassim (1), Peter R. Gibson (3) Jerrold R. Turner (1,2), John Hart (1,2), David T. Rubin (1)

Institutional Affiliation:

1. Inflammatory Bowel Disease Center, University of Chicago Medicine, Chicago, Il, USA
2. Department of Pathology, University of Chicago Medicine, Chicago, Il, USA
3. Department of Gastroenterology, Alfred Hospital and Monash University, Melbourne, Australia
4. Northwestern University Feinberg School of Medicine, Chicago, Il, USA

Financial/Grant Support: Funded in part by the “Digestive Disease Research Core Center of the University of Chicago (DK42086)”.

Abbreviations:

CHN: Complete Histologic Normalization MH: Mucosal Healing

SCCAI: Simple Clinical Colitis Activity Index

ACCEPTED MANUSCRIPT

Corresponding Author:

Name: David T. Rubin

Address: 5841 S. Maryland Ave. | MC4076, Room M410 | Chicago, IL 60637

Email Address: drubin@medicine.bsd.uchicago.edu

Business telephone number: 773-702-2950 Fax Number: 773-702-2182

Competing Interests/Disclosures: There are no author financial disclosures or relevant conflicts of interest for any author

Specific author contributions:

Britt Christensen: Study concept and design, acquisition of the data, analysis and interpretation of the data, drafting of the manuscript, statistical analysis

Jon Erich: Acquisition of data and drafting of the manuscript

Olufemi Kassim: Acquisition of data

Stephen B. Hanauer: Study concept and design and critical revision of the manuscript **Peter R. Gibson:** Study concept and design, drafting of the manuscript, critical revision of the manuscript and study supervision

Jerrold R. Turner: Pathology review and critical revision of the manuscript

John Hart: Pathology review and critical revision of the manuscript

David T. Rubin: Study concept and design, study supervision, drafting of the manuscript and critical revision of the manuscript

ACCEPTED MANUSCRIPT

Abstract word count: 260

Manuscript Word Count: 3997 words

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abstract:

Background & Aims: Mucosal healing, determined by histologic analysis, is a potential therapeutic target for patients with ulcerative colitis (UC). However, the histologic features of tissue normalization, as an outcome of treatment, have not been well described. We examined the prevalence and predictive values of normalization of the colonic mucosa, based on histologic analysis (histologic normalization) in patients with UC, and determined its association with risk of clinical relapse, compared to disease quiescence and mucosal healing.

Methods: We performed a retrospective study of 646 patients with confirmed UC who underwent colonoscopy at a tertiary medical center from August 2005 through October 2013. We reviewed reports from pathology analyses of random mucosal biopsies from each colon segment, and categorized them into 3 groups based on histology findings: normalization (completely normal mucosa with no features of chronicity present), quiescence (crypt atrophy or branching without signs of active inflammation including erosions, abscesses, or focal neutrophil infiltration), or active disease (epithelial infiltration by neutrophils, crypt abscesses, erosions, or ulceration). Histology findings were compared with clinical and endoscopic findings. We assessed variables associated with histology findings and calculated predictive values for clinical relapse (Simple Clinical Colitis Activity Index score ≤ 2 and sub-score of ≤ 1 for stool frequency or rectal bleeding) at follow-up evaluations 6 months later or more.

ACCEPTED MANUSCRIPT

Results: Of the 646 patients included in the study, 60% had endoscopic mucosal healing, 40% had histologic quiescence, and 10% had histologic normalization. The level of agreement between mucosal and histologic activity was moderate (agreement for 68% of samples; κ=0.50; *P*<.001). On multivariate analysis, only proctitis associated with histologic normalization (*P*=.002). Of 310 patients in clinical remission at initial review, 25% had a clinical relapse, after a median time of 16 months (inter-quartile range, 10–23 months). Histologic normalization was independently associated with increased odds of relapse-free survival compared to histologic quiescence (hazard ratio, 4.31; 95% CI, 1.48–12.46; *P*=.007) and histologic activity (HR 6.69; 95% CI, 2.16–20.62; *P*=.001); mucosal healing was not associated with increased odds of relapse-free survival compared to no mucosal healing (HR 1.02; 95% CI, 0.56–1.85, *P*=.954).

Conclusion: Histologic normalization of colonic mucosa can be used as a clinical endpoint for patients with UC. We associated histologic normalization with increased odds of relapse-free survival compared to endoscopic healing or histologic quiescence. Further studies are needed to determine whether histologic normalization should be a goal of treatment for patients with UC.

KEY WORDS: histopathology, mucosal healing, inflammatory bowel disease, normalization

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease characterized by periods of disease activity alternating with periods of quiescence. The primary treatment goal in UC has been to limit these periods of activity and maintain clinical remission, traditionally defined as cessation of rectal bleeding and normalization of stool frequency.[1] In recent years endoscopic mucosal healing (MH) in UC has been associated with improved clinical outcomes compared to achieving clinical remission alone.[2-5] In fact, MH has been associated with prolonged remission, fewer hospitalizations and colectomies and an improved quality of life.[2-8]

Despite such improved outcomes associated with MH, up to 40% of patients with MH have persistent histologic inflammatory activity.[3,9-12] Therefore, there is interest in the significance of histologic remission, as a “deeper” marker of disease control compared to MH. Although more research is needed into the practicality and validity of histological assessment, it is known that reduced histologic activity is associated with decreased risks of relapse, hospitalization, corticosteroid use, colectomy and colorectal neoplasia.[3,9-18]

It has been a common understanding that, following a diagnosis of UC, histological abnormalities of the mucosa persist. This idea has been so established that when histological grading has been described in UC, normalization has not generally been defined as an outcome that is distinct from quiescence; and histologic healing is generally described simply as absence of

active inflammation.[3,9,14,19] Furthermore, in two recently described histologic indices, the focus has been on short-term treatment responsiveness and architectural abnormalities have been excluded in their assessment.[20,21]

Although there are infrequent descriptions of histologic normalization in the literature,[22] it remains incompletely defined in the context of clinical outcomes. In addition, patient, disease and treatment characteristics associated with histologic normalization have not been studied, and it had not been determined if complete histologic normalization (CHN) is associated with improved clinical outcomes compared to histologic remission or endoscopic mucosal healing alone.

The aims of this study were to examine the prevalence and predictors of histologic normalization in patients with confirmed UC, and to determine if normalization is associated with improved clinical outcomes compared to ongoing histologic activity, histologic quiescence and endoscopic mucosal healing.

METHODS

A retrospective case-control study was performed and approved by the Institutional Review Board (IRB13-1063). All patients who underwent colonoscopy at University of Chicago between August 2005 and October 2013 for UC were identified by one or more of the International Classification of Diseases, Ninth revision, Clinical Modification (ICD-9-CM) codes for UC (556.0, 556.2, 556.3, 556.5, 556.6, 556.8, 556.9). Patients were eligible for inclusion if they had

an established diagnosis of UC at the time of this "follow-up" colonoscopy and documentation of previous complete colonoscopy and segmental biopsies obtained in at least the rectum, left colon and right colon that showed chronic changes (with or without acute changes) consistent with a histologic diagnosis of UC more than one year prior. Patients who had inadequate documentation, had undergone a colectomy, or had confirmed *Clostridium difficile* infection at time of follow-up colonoscopy were excluded.

Medical Records Abstraction

Endoscopy reports were retrieved through the electronic documentation system for endoscopic reports (Provation, Minneapolis, MN). Demographic, clinical, histological and biochemical data were collected from our electronic medical record system (EPIC, Wisconsin, USA), including age of diagnosis, disease duration, smoking history and previous and current use of anti-inflammatory agents and/or immunosuppressant therapy (steroids, immunomodulators, anti-TNF agents) at time of follow-up colonoscopy.

Endoscopic and Histological Assessment

The bowel was divided into three segments as per the Montreal classification for UC[23]: proctitis (E1, rectum only), left-sided (E2, rectum to splenic flexure), or extensive colitis (E3, disease proximal to splenic flexure). Disease extent was determined using a modified Montreal classification in that, rather than endoscopic appearance, histology was used on most proximal biopsy showing

evidence of disease, whether acute or chronic inflammation or chronic architectural changes (i.e., crypt branching/shortening, decreased crypt densities and irregular mucosal surfaces). Maximal disease extent was determined at any colonoscopy performed over the patient's history. The endoscopic and histologic severity and number of biopsies taken from each segment at follow-up endoscopy were recorded.

Endoscopic Mucosal Assessment

An academic IBD expert gastroenterologist with minimum 5 years' experience performed all endoscopies, during which endoscopic photographs were obtained from each segment of bowel, with targeted photos of areas of mucosal activity. An independent reviewer classified patients into three distinct groups of endoscopic grade of inflammation using the endoscopic sub-score of the Modified Mayo Disease Activity Index (MMDAI).[24] A score of 0 (no friability, granularity and intact vascular pattern) was classified as normal, 1 (mild erythema or decreased vascular pattern) as quiescent mucosa and a score of ≥ 2 (any of moderate or marked erythema, absent vascular pattern, friability, erosions, ulceration or contact/spontaneous bleeding) as mucosal activity. Endoscopic mucosal healing was defined by either completely normal or quiescent mucosa (MMDAI endoscopic sub-score ≤ 1).

Histological Assessment

As is routine in this unit, random mucosal biopsies were obtained from each colon

segment, targeting the area of most significant mucosal disease activity. Two pathologists who specialize in gastrointestinal histology routinely assess all biopsies and report histology utilizing a standardized scale that includes histologically normal, quiescent, mild, moderate or severe disease. We reviewed these pathological reports and categorized histology specimens into three distinct groups using the modified Riley score as described by Bryant and colleagues,[16] but with the sub- categorization of histologic remission into histologic normalization and histologic quiescence based on the absence or presence of architectural changes, respectively. Based on the maximum inflammation score at each segment, patients were categorized as: 1) histologic normalization: completely normal mucosa with no features of chronicity present; 2) histologic quiescence: features of chronicity including crypt atrophy or branching but no active inflammation, such as erosions, crypt abscesses, or focal neutrophil infiltration; 3) histologic activity: presence of any epithelial infiltration by neutrophils, crypt abscesses, erosions or ulceration.

As previously reported, the inter-observer agreement for interpretation of UC histology between our pathologists using a 6-point scale that classifies varying severities of inflammatory activity (including normal) was very good (kappa=0.6).[13] In this study, an additional 150 samples were re-graded by one of the expert pathologists (JH) who was blinded to the prior official reads of these specimens; 50 were histologically normal, 50 quiescent and 50 had active histology. All 150 samples were interpreted correctly (kappa=1.0).

The primary outcome of *complete histologic normalization* (CHN) was defined by normalization of mucosa without histologic features of chronicity in all bowel segments on follow-up colonoscopy in a patient with previous structural changes on biopsy consistent with UC.

Assessment of Clinical Relapse-Free Survival

Patients in clinical remission at follow-up colonoscopy with ≥ 6 months of follow-up at the University of Chicago from this colonoscopy until September 2014 were included in a separate analysis of clinical relapse-free survival. At each patient clinic visit, the Simple Clinical Colitis Activity Index (SCCAI) was calculated[25]. Clinical remission was defined as SCCAI ≤ 2 and sub-score of ≤ 1 for stool frequency or rectal bleeding as determined from physician records. Clinical relapse-free survival was defined as time from colonoscopy to period of clinical relapse, with clinical relapse defined at clinical follow-up as SCCAI > 2, sub-score > 1 for stool frequency or rectal bleeding, or medication escalation for symptoms, hospitalization for UC relapse, or colectomy for refractory UC.

Statistical Analysis

Continuous variables were summarized using medians and interquartile ranges (IQR). Categorical variables were expressed as percentage and number of cohort. Cohen's kappa coefficient (κ**)** was calculated to measure agreement between mucosal and histologic activity.

ACCEPTED MANUSCRIPT

Univariate analysis of baseline characteristics was performed to identify predictive factors for CHN. The Mann-Whitney U test and analysis of variance were used to compare continuous variables, and Pearson's chi-squared test was used to compare categorical variables. Multivariate analysis to identify independent factors associated with histological outcomes was performed using logistic regression.

Kaplan-Meier analysis was performed to compare clinical relapse-free survival in those with and without CHN and MH and log-rank statistics were performed to compare sub-groups of interest. Cox proportional hazard regression analysis was performed to identify independent predictors of clinical regression.

All variables with P values of less than 0.20 on univariate analysis were retained and integrated into the multivariate models. A two-sided p-value of ≤ 0.05 was considered statistically significant. All data analyses were performed using Stata 12.0 (StataCorp, College Station, TX).

ACCEPTED MANUSCRIPT

RESULTS

Patients

646 patients fulfilled the entry criteria and were included in the analysis. Baseline characteristics are shown in table 1. Using endoscopic criteria, 40% (n=261) had endoscopic mucosal activity, 35% (n=228) mucosal quiescence and 24% (n=157) mucosal normalization on follow-up colonoscopy. Using histologic criteria, 50% (n=321) had ongoing activity, 40% (n=260) histologic quiescence while 10% (n=65) had CHN.

The level of agreement between mucosal and histologic activity was moderate (68%, κ=0.50, $p < 0.001$). 12% (19/157) of patients with mucosal normalization had histologic activity and 27% (61/228) of patients with mucosal quiescence had histologic activity. No patient (0/65) with histologic normalization had mucosal activity but 8% (20/260) of patients with histologic quiescence had mucosal activity.

Complete Histologic Normalization

10% (n=65) of patients had complete normalization of their histology in all segments that had previously shown changes (i.e., CHN). The mean number of biopsies taken at each endoscopy was 20 (SD 9.5) and the number of biopsies taken was not significantly different in patients achieving CHN and in those who did not (Supplementary Table 1).

ACCEPTED MANUSCRIPT

CHN was identified in 9% (n=35) of patients with extensive colitis, 8% (n=15) with left-sided disease and 23% (n=15) with proctitis alone. By univariate analysis, CHN was associated with less extensive disease at baseline (p=0.001), disease duration >10 years (p=0.029), and negatively associated with previous steroid (p=0.041) and anti-TNF therapy (p=0.031) (Table 2). On multivariate analysis, a diagnosis of proctitis compared to left-sided (E2) (AOR 3.63, 95% CI 1.56-8.46, p=0.003) and extensive (E3) colitis (AOR 2.81, 95% CI 1.32-5.96, p=0.007) remained significantly and independently associated with CHN. There was a trend for patients with disease duration of more than 10 years to achieve CHN (AOR 1.81, 95% CI 0.98-3.35, p=0.058).

Clinical Relapse-free Survival

310 patients who were in clinical remission at follow-up colonoscopy were assessed for clinical relapse-free survival. Baseline characteristics are shown in table 3. Using endoscopic criteria, 25% (n=80) had ongoing endoscopic mucosal activity, 41% (n=127) quiescence and 33% (n=103) mucosal normalization. Using histologic criteria, 35% (n=108) had histologic activity, 51% (n=157) quiescence and 15% (n=45) CHN.

Median follow-up was 22 (IQR 14-34) months and 25% (n=77) patients experienced clinical relapse at median time 16 (IQR 10-23) months. Patients with CHN has lower rates of clinical relapse compared to those with histologic quiescence and activity (Figure 1a) and patients with endoscopic mucosal healing

mucosal had lower rates of clinical relapse rates compared to those with mucosal activity (Figure 1b). In patients who had mucosal healing and were in clinical remission, histologic normalization remained protective against clinical relapse (Figure 1c).

By univariate analysis, the only factors associated with improved clinical relapse-free survival were the achievement of endoscopic mucosal healing compared to no mucosal healing, and CHN compared to both histologic quiescence and histologic activity (Table 4). By multivariate analysis, only CHN compared to histologic quiescence (HR 4.31 [1.48-12.46, p=0.007), CHN compared to histologic activity (HR 6.69 [2.16-20.62], p=0.001) and no previous exposure to cyclosporine (p=0.034) predicted clinical relapse-free survival (Table 4). Mucosal healing was not independently associated with lower rate of clinical relapse.

DISCUSSION

We have demonstrated that histologic normalization of the colon in UC is possible and is characterized by statistically superior clinical relapse-free survival. One in ten of our cohort achieved CHN.

This is the first study to describe complete normalization of histology in UC. A study by Kleer and colleagues[26] did demonstrate that areas of histologic chronic colitis became normal at some point in 22 of 41 patients (54%). However, this study only described the rate of normalization of a single point in the bowel, and

did not look at complete normalization of the bowel or examine patient characteristics that predicted this normalization.

Most descriptions of histologic normalization have been reported as 'rectal sparing'. Levine and colleagues found that, of 24 asymptomatic UC patients, 2 (8%) normalized their rectal biopsy on follow-up.[27] Odze and colleagues looked at 14 patients treated with either 5-ASA or placebo enemas and found that, in patients on 5-ASA rectal therapy, 36% of rectal biopsies normalized (defined as "complete absence" of any of the characteristic features of chronic UC).[22] However, only one patient (7%) normalized all their rectal biopsies. Finally, Bernstein and colleagues showed that 2 of 39 (5%) patients with UC have histological evidence of absolute rectal sparing at some point during their disease.[28] None of these studies looked at proximal colon histologic normalization or patient or disease characteristics associated with histologic normalization.

We found that CHN was associated with less extensive disease. It has previously been described that extensive colitis is a risk factor for more complicated disease outcomes with the rate of colectomy in these patients of about 19% at 10 years compared to 5% of those who have proctitis.[29]

Our study demonstrates that histologic normalization is associated with improved clinical outcomes when compared to both histologic quiescence and activity, and is more predictive of improved outcomes than endoscopic mucosal healing alone

or histologic quiescence alone. Several studies have now confirmed the value of histologic features of colitis predicting clinical relapse in UC.[9,11,12,16,18] Riley and colleagues[9] found in 82 UC patients that an acute inflammatory cell infiltrate, crypt abscess and mucin depletion were significantly higher in those who subsequently relapsed within 12 months. Bitton and colleagues[11] reported on 74 patients in clinical and endoscopic remission with rectal biopsy specimens and demonstrated that basal plasmacytosis was associated with UC relapse with a hazard rate of 4.5. In addition, Feagins and colleagues[18] described 51 patients in clinical remission and reported that basal lymphoplasmacytosis, erosions/ulceration of the epithelial or moderate to marked architectural distortion significantly predicted clinical flares by 6 and 12 months and was more accurate at predicting flares compared to endoscopic assessment alone. Finally, Bryant and colleagues[16] demonstrated that histologic remission predicted corticosteroid use and acute severe colitis requiring hospitalization over 6 years and, similar to our study, endoscopic mucosal healing did not. While our data confirm the importance of histologic healing in improving clinical outcomes in patients with UC over and beyond that of endoscopic mucosal healing, it is novel in that we identify histologic normalization as a stronger predictor of a decreased risk of relapse in patients with either quiescent endoscopic mucosal or histologic UC.

Despite our findings, one must be guarded in translating these associations into clinical practice. The findings cannot yet justify increasing therapy for the sole purpose of achieving histologic normalization. It is important, however, to

acknowledge that this level of “deeper remission” is associated with improved outcomes. Patients who achieve histologic normalization can be informed of their improved prognosis and may represent a cohort requiring less stringent clinical surveillance and follow-up. Furthermore, whether this is a sub-group of patients that may benefit from stable de-escalation of medical therapy or require less intensive cancer surveillance remains to be determined. Finally, similar to other recent reports,[12,16] 27% of patients in this study with mucosal quiescence had persistent microscopic inflammation. As histological assessment appears superior to endoscopic assessment in predicting clinical outcomes, this disparity between histologic and endoscopic outcomes indicates that we should consider incorporating histologic surveillance into clinical practice.

This study lends further evidence to the importance of histological assessment in prognosticating a patient’s future disease course, and has implications for the design of future clinical trials. In fact, the United States Food and Drug Administration (FDA) has pointed to the possibility of requiring documentation of histologic disease activity both at inclusion and as an outcome measure in future clinical trials.[30] Clarification of a standardized and validated reporting system for histologic disease severity is needed in UC. There are current international efforts being undertaken to develop such an index.[20-21] This study suggests any such index should include histologic normalization as a separate and independent grade.

ACCEPTED MANUSCRIPT

There are a number of notable strengths and several limitations to this study. As with any retrospective analysis, there may be inaccuracies in data collection however the extensive experience of the involved clinicians and overlapping data sources (electronic records, endoscopy and pathology reports) should minimize this limitation. The generalizability of the data is also uncertain, as this is a single-center study based in a tertiary hospital setting where experts in the area of IBD manage patients. It is unclear whether this selection issue would make normalization of histology more or less likely; although patients may be treated for their disease more aggressively, they also most likely represent a more complex range of patients with more severe disease phenotypes compared to the general community. The tertiary nature of the setting is also in part its strength, as there is standardized reporting for endoscopy and pathology at the center. In addition, although we do employ a standardized approach to sampling the mucosa in our UC patients, it is possible that these results may represent a sampling bias. We believe this limitation was minimized based on the fact that there was no significant difference in biopsy number per patient when comparing cases and controls (histologic normalization compared to no normalization). There is also minimal variability between biopsies within each colonic segment with the same percentage of intra-segment biopsies with the same histologic inflammatory activity score being 80% or greater across all segments of the large bowel.[31] Furthermore, although our histologic activity and quiescence definitions are similar to those previously described by others,[3,14,16,32] the histological scale used here to assess histologic normalization has not undergone independent

validation. Finally, given the limitations of this retrospective review, dose and duration of medication exposures were not obtained. While understanding more about therapies and how they may achieve histologic normalization is of interest, we suggest that this should be assessed in future trials, along with the potential for controlled de-escalation of therapies in patients who achieve CHN.

In conclusion, we demonstrate that histologic normalization is an outcome in patients with UC and have found that it occurs more often in association with less extensive disease. We demonstrate for the first time that complete histologic normalization in UC is associated with improved clinical outcomes and provide further evidence that, despite the introduction and search for other predictive biomarkers in IBD, traditional histopathology may well be the most reliable. We propose that histologic assessment of disease activity should be part of endoscopic assessment in IBD. In addition, future standardized and validated histologic indices include histologicl normalization as a unique outcome and encourage these findings to be incorporated into future clinical trials and clinical practice.

ACCEPTED MANUSCRIPT

REFERENCES

1. D'Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology 2007;132:763-86.
2. Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. Gut 2012;61:1619-35.
3. Truelove SC, Richards WC. Biopsy studies in ulcerative colitis. Br Med J 1956;1:1315-8.
4. Froslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population- based cohort. Gastroenterology 2007;133:412-22.
5. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology 2011;141:1194-201.
6. Sandborn WJ, Rutgeerts P, Feagan BG, et al. Colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. Gastroenterology 2009;137:1250-1260.
7. Feagan BG, Reinisch W, Rutgeerts P, et al. The effects of infliximab therapy on health-related quality of life in ulcerative colitis patients. Am J Gastroenterol 2007;102:794-802.
8. Peyrin-Biroulet L, Sandborn W, Sands BE, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol 2015;110:1324-28.

ACCEPTED MANUSCRIPT

9. Riley SA, Mani V, Goodman MJ, et al. Microscopic activity in ulcerative colitis: what does it mean? Gut 1991;32:174-8.

10. Bessissow T, Lemmens B, Ferrante M, et al. Prognostic value of serologic and histologic markers on clinical relapse in ulcerative colitis patients with mucosal healing. Am J Gastroenterol 2012;107:1684-92.

11. Bitton A, Peppercorn MA, Antonioli DA, et al. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. Gastroenterology 2001;120:13-20.

12. Park S, Abdi T, Gentry M, et al. Histological Disease Activity as a Predictor of Clinical Relapse Among Patients With Ulcerative Colitis: Systematic Review and Meta-Analysis. Am J Gastroenterol. 2016;111:1692-701.

13. Rubin DT, Huo D, Kinnucan JA, et al. Inflammation is an independent risk factor for colonic neoplasia in patients with ulcerative colitis: a case-control study. Clin Gastroenterol Hepatol 2013;11:1601-8.

14. Gupta RB, Harpaz N, Itzkowitz S, et al. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. Gastroenterology 2007;133:1099-1105.

15. Hefti MM, Chessin DB, Harpaz NH, et al. Severity of inflammation as a predictor of colectomy in patients with chronic ulcerative colitis. Dis Colon Rectum 2009;52:193-7.

16. Bryant RV, Burger DC, Delo J, et al. Beyond endoscopic mucosal healing in UC: histological remission better predicts corticosteroid use and hospitalisation over 6 years of follow-up. Gut. 2016;65(3):408-14.

ACCEPTED MANUSCRIPT

17. Peyrin–Biroulet L, Bressenot A, Kampman W. Histologic remission: the ultimate therapeutic goal in ulcerative colitis? Clin Gastroenterol Hepatol 2014;12:929-934.

18. Feagins LA, Melton SD, Iqbal R et al. Clinical implications of histologic abnormalities in colonic biopsy specimens from patients with ulcerative colitis in clinical remission. Inflamm Bowel Dis. 2013;18:1477-82.

19. Geboes K, Riddell R, Öst A, et al. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 2000;47:404-409.

20. Marchal-Bressenot A, Salleron J, Boulagnon-Rombi C, et al. Development and validation of the Nancy histological index for UC. Gut. 2015. doi:10.1136/gutjnl-2015-310187.

21. Mosli MH, Feagan BG, Zou G, et al. Development and validation of a histological index for UC. Gut. 2015. doi:10.1136/gutjnl-2015-310393

22. Odze R, Antonioli D, Peppercorn M, et al. Effect of topical 5- aminosalicylic acid (5-ASA) therapy on rectal mucosal biopsy morphology in chronic ulcerative colitis. Am J Surg Pathol 1993;17:869-75.

23. Satsangi J, Silverberg M, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006;55:749-753.

24. Scherl EJ, Pruitt R, Gordon GL, et al. Safety and efficacy of a new 3.3 g bid tablet formulation in patients with mild-to-moderately-active ulcerative colitis: a multicenter, randomized, double-blind, placebo- controlled study. Am J Gastroenterol 2009;104:1452-1459.

ACCEPTED MANUSCRIPT

25. Walmsley RS, Ayres RC, Pounder RE, et al. A simple clinical colitis activity index. Gut 1998;43:29-32

26. Kleer CG, Appelman HD. Ulcerative colitis: patterns of involvement in colorectal biopsies and changes with time. Am J Surg Pathol 1998;22:983-9.

27. Levine TS, Tzardi M, Mitchell S, et al. Diagnostic difficulty arising from rectal recovery in ulcerative colitis. J Clin Pathol 1996;49:319-23.

28. Bernstein CN, Shanahan F, Anton PA, et al. Patchiness of mucosal inflammation in treated ulcerative colitis: a prospective study. Gastrointest Endosc 1995;42:232-7.

29. Solberg IC, Lygren I, Jahnsen J, et al. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort. Scand J Gastroenterol 2009;44:431-440.

30. U.S. Department of Health and Human Services Food and Drug Adminstration Center for Drug Evaluation and Research (CDER). Ulcerative Colitis: Clinical Trial Endpoints Guidance for Industry. Available: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm515143.pdf [Accessed 4th September 2016]

31. Mikolajczyk AE, Watson S, Ackerman MT, et al. Assessment of the Degree of Variation of Histologic Inflammation in Ulcerative Colitis. Gastroenterology 2014;146:S-232.

32. Hanauer S, Schwartz J, Robinson M, et al. Mesalamine capsules for treatment of active ulcerative colitis: results of a controlled trial. Am J Gastroenterol 1993;88:1188-97.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

FIGURE LEGENDS:

Figure 1: Kaplan Meier analysis of affect of endoscopic mucosal and histologic activity on clinical relapse-free survival. A) Clinical relapse- free survival vs. histologic healing B) Clinical relapse-free survival vs. endoscopic mucosal healing C) Clinical relapse-free survival vs. histologic healing in patients with endoscopic mucosal healing.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1: Clinical Characteristics at Baseline

Baseline characteristics (n = 646)	Median (IQR) or Percentage (n)	
Age at diagnosis of UC (years)	29 (22-41)	
Gender (male)	50.2% (n=324)	
Greatest disease extent seen:		
Proctitis	10.1% (n=65)	
Left-Sided	30.5% (n=197)	
Pancolitis	59.4% (n=384)	
Duration of disease (years)	13 (7-22)	
Smoking status (current smoker)	6.6% (n=41)	
Mucosal Activity on follow up:		
Mucosal normalization	157 (24.3%)	Mucosal healing: 385 (59.6%)
Mucosal quiescence	228 (35.3%)	
Mucosal activity	261 (40.4%)	
Histological Activity on follow up:		
Histological normalization	65 (10.1%)	Histological healing: 325 (50.3%)
Histological quiescence	260 (40.2%)	
Histological activity	321 (49.7%)	
Medications, n(%)		
Oral steroid exposure	394 (66.8%)	
Current oral steroid	54 (98.6%)	
5-ASA exposure	637 (99.5%)	
Current 5-ASA	517 (81.6%)	
Immunomodulator exposure	294 (48.1%)	
Current immunomodulator	190 (30.1%)	
Previous cyclosporine salvage	25 (4.2%)	
Anti-TNF exposure	109 (18.1%)	
Current anti-TNF	82 (13.0%)	

ACCEPTED MANUSCRIPT

Table 2: Univariate Analysis of Predictors of Complete Histological Healing in UC

Characteristic **(n= 646)**	***CHN (n=65)***	***No CHN (n=581)***	***P value***
Age, y, n (%)			
≤ 16 y	11 (17%)	49 (9%)	0.071*
17-39 y	39 (61%)	357 (63%)	
≥ 40 y	14 (22%)	160 (28%)	
Gender, m, n(%)	40 (46%)	294 (51%)	0.496
Current smoker, n (%)	5 (8%)	36 (6%)	0.666
Disease extent at baseline, n (%)			
E1	15 (23%)	50 (9%)	0.001**
E2	15 (23%)	182 (31%)	
E3	35 (54%)	349 (60%)	
Disease > 10 y, n (%)	47 (73%)	336 (59%)	0.029**
Oral steroid Exposure, n (%)	33 (55%)	361 (68%)	0.041**
5-ASA monotherapy, n (%)	40 (63%)	291 (51%)	0.082
Previous immunomodulator, n (%)	22 (36%)	272 (50%)	0.033**
Current immunomodulator, n (%)	15 (23%)	175 (31%)	0.220
Previous cyclosporine salvage, n (%)	4 (7%)	21 (4%)	0.340
Previous anti-TNF, n (%)	5 (8%)	104 (19%)	0.031**
Current anti-TNF Rx, n (%)	5 (8%)	77 (14%)	0.192*
Current immunomodulator and anti-TNF, n (%)	4 (6%)	26 (5%)	0.555

* Incorporated into multivariate analysis as p-value < 0.2

** Significant p-value < 0.05

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 3*:* Baseline Characteristics of Those in Remission at Baseline

Patients	**N=310**
Sex, n (%), male	159 (51.3%)
Age, median (IQR), y	48.4 y (36.6-58.8)
Age of diagnosis, median (IQR), y	29 y (22-41)
Active smoking status, n (%)	21 (6.8%)
Duration of disease, median (IQR), y	14 y (1-52 or 8-49)
Disease extent, n (%)	
E1	34 (11.0%)
E2	89 (28.7%)
E3	187 (60.3%)
Mucosal healing, n (%)	
Endoscopic mucosal activity	80 (25.0%)
Endoscopic mucosal quiescence	127 (41.0%)
Endoscopic mucosal normalization	103 (33.2%)
Histological healing, n (%)	
Histological activity	108 (34.8%)
Histological quiescence	157 (50.7%)
Complete histological normalization	45 (14.5%)
Medications, n (%)	
Past oral steroid exposure	185 (66.3%)
Current oral steroids	15 (4.9%)
Past 5-ASA exposure	303 (99.3%)
Current 5-ASA therapy	242 (79.3%)
Past IMM therapy exposure	156 (52.9%)
Current Immunomodulator (IMM) therapy	110 (36.2%)
Past anti-TNF therapy exposure	57 (19.5%)
Current Anti-TNF therapy	49 (16.2%)
Past cyclosporine salvage therapy	12 (4.1%)

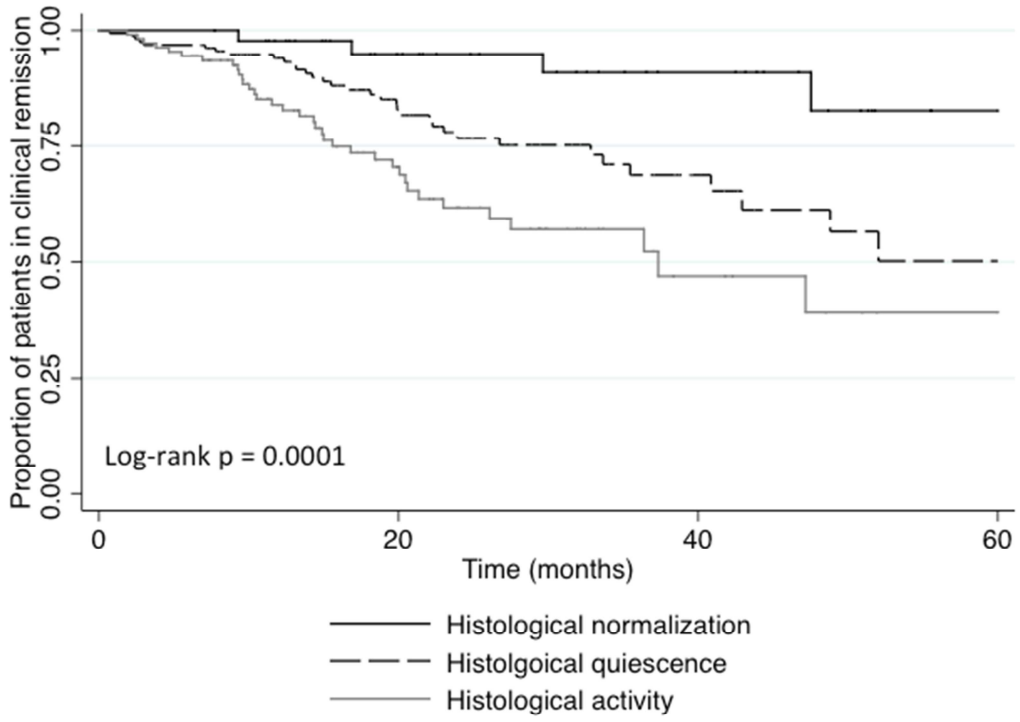
ACCEPTED MANUSCRIPT

Table 4: Univariate and Multivariate Analysis For Factors Associated With Clinical-Relapse Free Survival

Risk Factor	*Univariate analysis: HR[a] (95% CI)*	*P value[b]*	*Multivariate analysis: HR[a] (95% CI)*	*P value[b]*
Sex (Female)	1.15 (0.74-1.81)	0.533		
Older age at colonoscopy	0.99 (0.97-1.00)	0.152*	0.99 (0.98-1.01)	0.408
Older age of diagnosis	0.99 (0.98-1.01)	0.436		
Current smoker	1.19 (0.51 – 2.75)	0.687		
Longer duration of disease	0.99 (0.96-1.01)	0.301		
Disease extent:				
E2 vs E1	1.33 (0.59-2.98)	0.487		
E3 vs E1	1.41 (0.66-3.01)	0.369		
Endoscopic Mucosal Healing:				
-Quiescent disease vs Normalization	1.56 (0.87-2.76)	0.131		
-Active disease vs Normalization	2.44 (1.37-4.36)	0.002		
No endoscopic mucosal healing	1.93 (1.21 – 3.07)	0.006**	1.02 [0.56-1.85]	0.954
Histological healing:				
-Quiescent disease vs Normalization	3.79 (1.34 – 10.68)	0.012**	4.31 [1.48-12.46]	0.007**
-Active disease vs Normalization	6.76 (2.39 – 19.14)	< 0.001**	6.69 [2.16-20.62]	0.001**
Steroids	0.72 (0.23-2.30)	0.582		
5-ASA therapy	1.19 (0.67-2.14)	0.555		
Anti-TNF therapy	0.88 (0.44-1.78)	0.724		
Duel therapy (IMM + anti-TNF)	1.42 (0.61-3.27)	0.416		
IMM therapy	1.37 (0.86-2.17)	0.183*	1.21 (0.72-2.04)	0.469
Past anti-TNF therapy exposure	0.81 (0.42-1.54)	0.522		
Past IMM therapy exposure	1.32 (0.83-2.09)	0.236		
Past cyclosporine salvage therapy	2.34 (0.99-5.52)	0.052*	2.71 [1.07-6.82]	0.034**
Past oral steroid exposure	1.08 (0.67-1.74)	0.749		

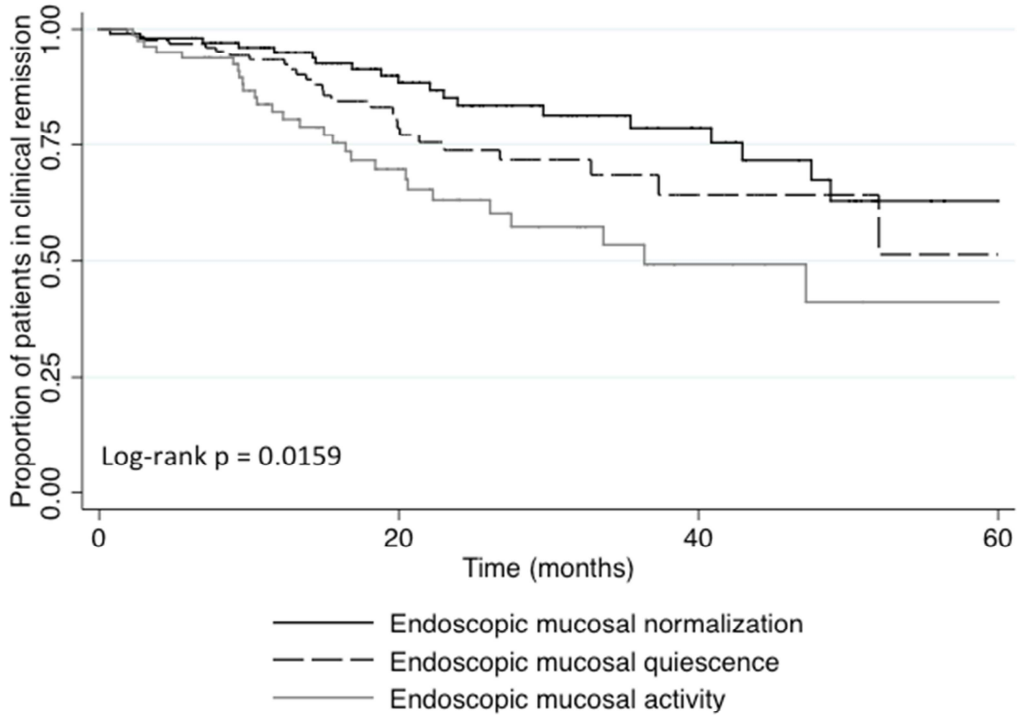
[a]Hazard ratio for each risk factor in Cox model (estimate and 95% CI)
[b]Significance level (**significant P-value < 0.05)
* Incorporated into multivariate analysis as P-value < 0.2
CI, confidence interval

A



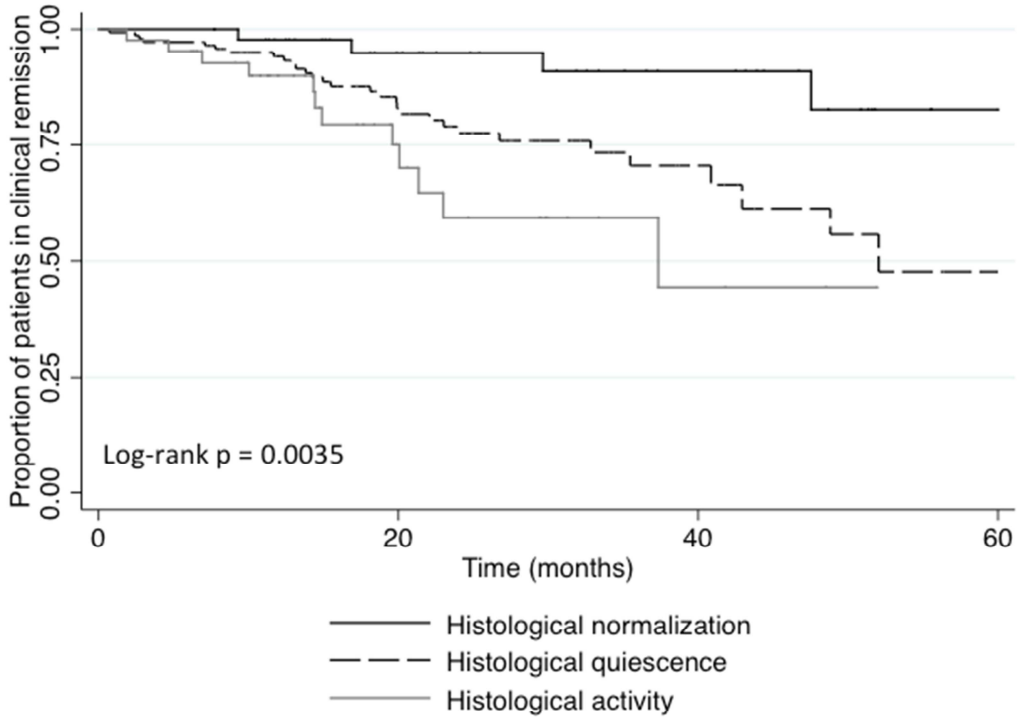
ACCEPTED MANUSCRIPT

B

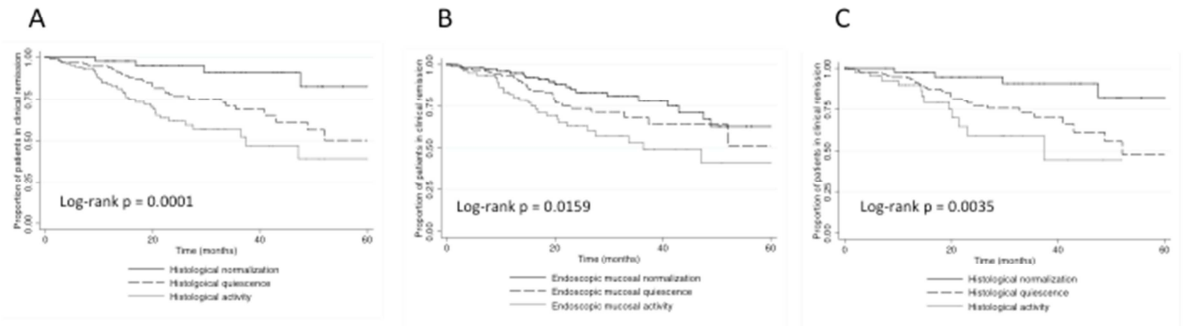


ACCEPTED MANUSCRIPT

C



ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary Table 1: Biopsies in Each Segment of the Colon by Healing Status

	CHN	***No CHN***	
	Median biopsy # (IQR)		***P value***
# right colon	6 (4-10)	7 (4-11)	0.237
# left colon	6 (4-9)	7 (4-10)	0.412
# rectum	3 (2-4)	4 (2-5)	0.089

ACCEPTED MANUSCRIPT