Histologic Markers of Inflammation in Patients With Ulcerative Colitis in Clinical Remission

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BACKGROUND & AIMS: Mucosal healing, based on histologic analysis, is an end point of maintenance therapy for patients with ulcerative colitis (UC). There are few data on how histologic signs of inflammation correlate with endoscopic and peripheral blood measures of inflammation in these patients. We investigated patterns of histologic features of inflammation in patients with UC in clinical remission, and correlated these with endoscopic and biochemical measures of inflammation.

METHODS: We performed a prospective observational study of 103 patients with UC in clinical remission undergoing surveillance colonoscopy while receiving maintenance therapy with mesalamine or thiopurines; 2674 biopsy specimens were collected from 708 colonic segments. Each colonic segment was evaluated based on the Mayo endoscopic subscore and the Geboes histology score (range, 0–5.4). Biomarkers were measured in peripheral blood samples.

RESULTS: Histologic features of inflammation were found in 54% of patients receiving maintenance therapy; 37% had at least moderate inflammation based on histology scores. Of the 52 patients with endoscopic evidence only of left-sided colitis, 34% had histologic features of inflammation in their proximal colon. Histology scores correlated with endoscopic scores for per-segment inflammation (Spearman ρ = 0.65; P < .001). Patients with histology scores greater than 3.1 had a significantly higher mean level of C-reactive protein than those with scores less than 3.1. There were no differences among treatment groups in percentages of patients with histologic scores greater than 3.1.

CONCLUSIONS: Patients in clinical remission from UC still frequently have histologic features of inflammation, which correlate with endoscopic appearance. Patients with at least moderate levels of inflammation, based on histologic grading (score >3.1), have higher serum levels of C-reactive protein, which could be used as a surrogate marker of histologic inflammation.

Keywords: Mesalamine; Response to Therapy; Therapeutic Efficacy; Mucosal Healing; IBD.

The goal of therapy in patients with ulcerative colitis (UC) has shifted from symptom control alone to clinical remission in conjunction with mucosal healing.1 Clinical trials of drugs in patients with UC now routinely include a mucosal healing end point, and expert consensus recommends healing as an end point for optimal management in practice.2 The advantages of achieving resolution of mucosal inflammation can be seen in the reported lower rates of disease relapse, hospitalization, need for immunosuppressive therapy, and colon cancer in patients who obtain mucosal healing.3,4 Although most studies on mucosal healing focus on endoscopic scores, such as the Mayo subscore, some experts have suggested histologic inflammation may be a valuable goal of therapy.6,7 The presence of histologic inflammation is a better predictor of future clinical relapse than endoscopic appearance alone.8 A higher risk of relapse was noted in studies of patients with persistent active microscopic inflammation when compared with patients with normal histology.9–11 Histologic remission also was associated with a lower rate of hospitalization during a median 29-month follow-up evaluation in a small cohort.12 A recent abstract from Rubin et al11 reported that an increased level of histologic inflammation could predict both colectomy and hospitalization in patients with UC.

In this context, validated scoring systems for the evaluation of histologic severity in clinical trials are desirable. The Riley index, Geboes index, and Chicago index have been developed for this purpose, but none are universally used or have been

Abbreviations used in this paper: Aza, azathioprine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; 6-MP, 6-mercaptopurine; UC, ulcerative colitis.
independently validated.

Recent expert guidelines have recommended a histologic score based on clinical trials. The Geboes score was first reported in 2000 and showed good reproducibility and agreement between endoscopic grading systems in 28 patients. Its independent validation has not been reported since then.

Evaluation of the severity of histologic inflammation as an end point for drug therapy has not been part of standard practice, although persistent endoscopic and histologic inflammation in the absence of clinical symptoms is common. Patients with quiescent UC with histologic inflammation are difficult to identify because endoscopic measures of inflammation have a variable correlation with symptoms. A small prospective study, presented at abstract form, reported only modest agreement between clinical, endoscopic, and histologic measures of remission with complete agreement in just 58% of 91 patients (kappa, 0.44) and 89% agreement between endoscopy and histology. Given the potential importance of histologic healing in long-term outcomes, UC endoscopic and biochemical markers of disease activity in this setting is needed for patients in remission.

The goal of this study was to enroll a cohort of patients with UC in clinical remission, to determine the prevalence of histologic colitis in these patients using the Geboes grading system, and to compare the correlation between the Geboes score and endoscopic and biochemical markers of disease activity in this setting.

Materials and Methods

This was a prospective observational study performed at a single tertiary referral center. The study was approved for enrollment of human subjects by the local Institutional Review Board (protocol #2009-P-000314). All patients with a confirmed history of UC who attended the endoscopy unit for a clinically indicated surveillance colonoscopy were screened. All patients received the same bowel preparation (magnesium citrate). Clinical disease activity was determined using the Simple Clinical Colitis Activity Index, a validated score of colitis activity that has been shown to correlate well with endoscopic indexes. To be considered “in remission” for enrollment in this study, participants had to have a Simple Clinical Colitis Activity Index score less than 2.5 at the screening visit, and have had no changes in their UC medications or any steroid use in the prior month.

Each enrolled patient had baseline demographic and ulcerative colitis disease history recorded. This included disease location, duration, prior and current medication use, family history, extraintestinal disease, smoking status, and nonsteroidal anti-inflammatory drug use. During the index colonoscopy, the colon was divided into 8 segments (cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, and rectum). Endoscopic activity in each colonic segment was classified by the endoscopist using the sigmoidoscopy subscore of the Mayo activity index (Supplementary Table 1). A subset of 15 patients had their endoscopy images re-scored by a second blinded endoscopist to determine the interobserver agreement for the endoscopic score. The κ statistic was 0.7, suggesting substantial agreement between endoscopists.

The protocol for the 4 gastroenterologists who performed the colonoscopies included the recommended 4-quadrant biopsies every 10 cm. Histologic activity in all segments was classified using the Geboes scale, by a gastrointestinal pathologist (J.D.G.) blinded to the patient’s disease status and endoscopic scores. A baseline blood sample was drawn for measurement of white blood count, hematocrit (Hct), erythrocyte sedimentation rate (ESR) (in mm/h), and C-reactive protein (CRP) (in mg/L) in all patients.

Histologic Scoring

The Geboes grading system is an instrument with 6 domains: structural (architectural change), chronic inflammation, lamina propria neutrophils and eosinophils, neutrophils in epithelium, crypt destruction, and erosions or ulcers. Scores can range from 0 to 5.4, with higher scores indicating more severe histologic inflammation (Supplementary Figure 1). A total Geboes score was assigned to biopsy specimens from each colonic segment and the highest score (most inflamed segment by histology) was used as the total histology score for each patient.

Statistical Analysis

Dichotomous variables were analyzed for outcomes using the chi-square test or the Fisher exact test where appropriate, and continuous variables were analyzed using the t test if normally distributed, or the Wilcoxon test for non-normal data. Correlation between ordinal numeric scores was analyzed by the Spearman rank correlation coefficient (ρ). A κ agreement statistic was generated for assessment of dichotomous characterization of normal/not normal by endoscopic and histologic scores. Data were analyzed with JMP 8.0 (SAS Institute, Cary, NC). Post hoc power calculations were performed using the PS Power and sample size calculator (available at: http://biostat.mc.vanderbilt.edu/PowerSampleSize).

Results

A total of 147 patients scheduled for surveillance were screened, and 103 were enrolled in the study. Only 10% of the eligible population screen-failed as a result of clinically active disease at the time of colonoscopy. The baseline characteristics of these 103 patients are summarized in Table 1, and were similar to our typical surveillance population. The biopsy sampling protocol during surveillance was extensive; the mean number of biopsy specimens per colonoscopy was 26, and 2674 biopsy specimens in total were taken during the 103 colonoscopies.

Of the 103 colonoscopies, 54% of patients had at least one biopsy specimen with evidence of any histologic inflammation, and 37% had biopsy specimens that met the Geboes criteria for abnormal histologic inflammation (score ≥ 3.1). Eleven patients (11%) had a colonoscopy that showed histologic inflammation in the right colon (score > 0) in the absence of endoscopic inflammation, and 6 of these patients had scores of 3.1 or greater. Of the 52 patients with endoscopic evidence only of left-sided colitis, 34% had histologic inflammation in their right colon. Among all colonic segments (n = 708), 20% had histologic evidence of inflammation, and 21% had an abnormal endo-
scopic appearance. In colonic segments with endoscopic inflammation (Mayo score, ≥1), 57% of biopsy specimens from these segments had a Geboes score less than 3.1, and 43% had a score of 3.1 or greater. In colonic segments with a Mayo score of 0 (normal endoscopic appearance), only 6% had underlying histologic evidence of inflammation on biopsy.

To determine the correlation between the Geboes histologic score and the Mayo endoscopic score, the score for each colonic segment was plotted on an x-y plot. There was a good correlation between histologic and endoscopic scores (Spearman ρ = 0.65; P < .001; Figure 1). When mean endoscopy scores for each segment were grouped according to Geboes grade (0–5), only segments with histologic inflammation scores greater than 3 had an endoscopic score greater than 1 (Figure 2). If endoscopic and histologic scores were dichotomized (0, >0), the agreement statistic (κ) was 0.62 for the presence of normal histology in patients with normal endoscopic appearance.

Because peripheral blood biomarkers have been associated with intestinal inflammation, we next sought to assess the blood levels of CRP and ESR in patients with histologic inflammation. As can be seen in Figure 3, mean CRP levels were predominantly in the normal range (<5 mg/L) for patients whose highest histologic score was less than 3.1, but a wider distribution of levels was apparent in those with greater histologic inflammation (P = .008 by analysis of variance). When Geboes scores were dichotomized (<3.1 or ≥3.1), the mean CRP was higher in patients with a histologic score of 3.1 or greater than those with normal histologic appearance (mean, 5.4 vs 2.3 mg/L; P = .001 by t test) (Figure 4). The performance of CRP as a test for histologic inflammation of 3.1 or greater in patients in clinical remission was modest; the area under the receiver-operator characteristic curve was 0.67. A CRP cut-off value of 2 mg/L had the highest accuracy (sensitivity, 66%; specificity, 63%) in this cohort. For correctly identifying patients with histologic scores of 3.1 or greater, a CRP level greater than 10 mg/L had an 86% positive predictive value, whereas levels
less than 0.5 mg/L had an 88% negative predictive value. ESR was not significantly different between the 2 groups.

Finally, we examined whether there were differences in histologic appearance according to maintenance therapy. Of the 18 patients taking thiopurines, all had been prescribed for longer than 3 months, and 52% were prescribed the recommended weight-based dose at the time of enrollment (6-mercaptopurine [6-MP] 1.5 mg/kg, azathioprine [Aza] 2.5 mg/kg). Of the 78 patients taking mesalamine, 80% were taking 2.4 g/d or more. As can be seen in Figure 5, the patients’ overall mean histologic level of inflammation was similar across treatment groups (mean scores, 1.9, 1.1, and 1.2 for mesalamine, Aza/MP, and mesalamine and Aza/MP, respectively). The proportion of patients taking mesalamine or Aza/MP who had a histologic score of 3.1 or greater also was similar (33% and 42%, respectively). Although we did not measure adherence to these medications directly, prior studies from our patient population have reported greater than 70% adherence rates.21

Discussion

The goal of treating patients with inflammatory bowel disease has evolved to focus on achieving mucosal healing, which has been associated with a reduced risk of long-term complications.22 Histologic assessment of underlying healing may be important in predicting future relapse and complications.11 The main limitations to the use of this end point are the incomplete validation of the available histologic scoring systems, and a lack of data on the patterns of histologic healing when patients achieve clinical remission.

This study provides a number of novel pieces of information for the gastroenterology community. First, more than half of patients in clinical remission undergoing surveillance colonoscopy had histologic evidence of inflammation, and a third had at least moderate histologic inflammation. This was independent of the type of maintenance therapy they were receiving, but was associated with higher mean serum CRP levels in our study. Baars et al14 reported that 63% of their cohort of 98 UC patients in remission had histologic inflammation, and 20% had at least moderate inflammation, using a nonvalidated scale. They did not correlate histologic scores with endoscopic scores, or CRP. Cumulatively, these data provide some useful natural history information of histologic healing in the era of widespread use of mesalamine and thiopurines as maintenance agents. Whether persistent histologic inflammation is associated independently with a worse prognosis over time is unconfirmed, but raises interesting questions about how achievable histologic healing is as a goal for maintenance therapy in practice.11,14

Second, a small proportion of patients with left-sided endoscopic disease have right-sided histologic inflammation, which may have implications for their colon cancer risk.23 Patients with distal colitis can have isolated areas of inflammation in more proximal regions, usually perianal-pedunculated patches in the cecum.24 Its clinical significance in terms of disease activity is unknown, although a small study reported a similar overall disease severity when compared with those without right-sided inflammation.25 These findings support the role of complete colonic endoscopic surveillance with biopsies, even in patients with a history of only left-sided disease. Of interest, the proportion of patients with histologic inflammation was similar, regardless of which maintenance agent patients were taking (Figure 5).

We have confirmed independently that the Geboes histologic scoring scale correlates with endoscopic appearance, showing good agreement between endoscopic and histologic scales of inflammation, and determination of a healed colon in patients with UC in remission. Osada et al,26 in their study with 54 UC patients with both active and inactive disease, reported a similar degree of correlation between the Mayo endoscopic score and histologic scores, although they did not use the Geboes scale, and only 20 patients were in clinical remission. The reported threshold for moderate histologic inflammation of 3.1 on the Geboes scale was supported in our study by higher endoscopic scores, and higher CRP levels, in these patients.20

Finally, our findings that mean CRP level was higher in patients with a Geboes histologic score of 3.1 or greater than those with minimal histologic changes suggests that CRP level could be used to screen patients in the clinic for unresolved histologic inflammation. As can be seen in Figure 3, the majority of patients with histologically normal or mildly inflamed
colons had CRP levels less than 5 mg/L. In contrast to its use as a marker in Crohn’s disease, CRP has been described as a less reliable correlate of disease activity in patients with UC, except perhaps for severe extensive colitis. A small study of 34 patients with UC did not find an association between a CRP level greater than 8 mg/L and histologic inflammation, although most of the patients had only mild histologic inflammation. Our finding of an association between CRP levels and histologic inflammation warrants further validation because many factors independent of colitis can lead to false-positive or false-negative CRP levels.

Limitations of this study included the small proportion of patients with moderate to severe histologic inflammation of a total of 103 patients enrolled, so variables that occur at small frequencies but differ between groups may have been subject to a type II error. A post hoc power calculation predicted one would need to enroll a cohort of approximately 700 surveillance patients to detect a difference between variables that occur at frequencies of less than 20%. However, the study was strengthened by the prospective enrollment and standardized scoring of all patients with comprehensive clinical phenotypes. This was a large study that prospectively described histologic patterns, and correlated the Geboes histologic scale with endoscopic and biochemical markers in patients in remission.

In conclusion, this study highlights that many patients with UC in clinical remission have underlying histologic inflammation, and this correlates with endoscopic appearances and serum CRP levels. In practice, these findings should prompt clinicians to consider using CRP to screen patients with UC in clinical remission for underlying ongoing histologic inflammation. Future work will determine the significance of this microscopic inflammation in medium- and long-term outcomes in patients, and may provide insights into the benefits of aiming for histologic healing in this patient population.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2013.02.030.

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Conflicts of interest
These authors disclose the following: Alan Moss has served on Advisory Boards for Janssen, Abbott, and UCB, and has received research funding from Shire and Salix; and Adam Cheifetz has served on Advisory Boards for Janssen, Abbott, and UCB. The remaining authors disclose no conflicts.

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