

## Histological healing in inflammatory bowel disease: A still unfulfilled promise

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incomplete, and somewhat conflicting data exist on this topic, especially because there is still the need to standardize both histological assessment and the severity grading of these disorders; Issues that have not been yet been resolved for clinical practice and therapeutic trials. Hopefully, with the help of an increased awareness on the clinical researchers' side, and the availability of dedicated pathologists on the other side, this matter will be effectively faced and resolved in the near future.

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### Abstract

Treatment of inflammatory bowel disease (IBD) is traditionally based on several drugs, including salicylates, corticosteroids, and antibiotics; in addition, the therapeutic armamentarium has considerably evolved with the advent of newer, effective therapeutic measures (such as the biological agents) that are able to improve in a considerable manner both the clinical and endoscopic variables. Thus, mucosal healing, at least considered from an endoscopic point of view, is today regarded as the ultimate endpoint for treatment of these conditions. However, it is also increasingly clear that endoscopic healing is not necessarily paralleled by histological healing; There are few doubts that the latter should be considered as a true, objective healing and the ultimate goal to reach when treating patients with IBD. Unfortunately, and surprisingly, only a few,

### INTRODUCTION

The medical treatment of inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is based on several different approaches relying on aminosalicylates, antibiotics, corticosteroids, and immunomodulatory agents, in addition to the newest biological agents<sup>[1]</sup>. At present, there is also a strong impulse toward treatment paradigms directed to achieve specific targets, such as a decrease in hospital admissions, the need for surgery, and mucosal healing<sup>[2]</sup>. The latter point seems to be particularly important, because reaching this goal would also produce a domino effect, resulting in less complications, hospitalization, and surgical procedures related to IBD<sup>[3]</sup>.

However, mucosal healing could be considered as an ambiguous term, because it may be related to both the endoscopic and histological aspects, and at present

a standardized definition for IBD patients does not exist<sup>[4]</sup>. Actually, by considering mucosal healing in IBD, histological remission is not recommended as primary endpoint for therapeutic trials<sup>[5]</sup>, whereas the suggested endpoint is to obtain a genuine endoscopic healing (defined as absence of friability, blood, erosions, and ulcers in all visualized segments)<sup>[5]</sup>.

Strictly speaking, the optimal treatment goal should be the complete resolution of the inflammatory process, and this is reached only when confirmed by the corresponding histological assessment. In fact, the presence of endoscopic healing does not necessarily imply that this also happens at a microscopic level<sup>[6]</sup>, as also shown (for instance) in celiac disease, in which a gluten-free diet restores the normal endoscopic aspect, but rarely does the microscopic assessment show completely normal features<sup>[7]</sup>.

Moreover, there are objective reasons that probably hamper the use of histological healing as an end point for therapeutic trials. For instance, it has been recently stated that, concerning histopathological assessment of IBD, "...bewildering variations can be observed in the terminology employed to report either individual lesions or diagnostic categories"<sup>[8]</sup>. In addition, significant differences may exist between UC and CD concerning this aspect. In fact, in UC (in which lesions are usually limited to the mucosa) mucosal/histological healing may represent the ultimate therapeutic goal, whereas in CD (a transmural process) mucosal/histological healing should be achieved as a minimum therapeutic target<sup>[9]</sup>. Thus, even considering the potentiality of the new therapeutic approaches, the importance of achieving also histological healing might add further value to future trials<sup>[10]</sup>.

A generally accepted definition of histological mucosal healing does however not exist. Histological healing could be defined as "a normal mucosa" or as a disappearance of inflammation, and hence, "a mucosa with limited architectural abnormalities but normally differentiated epithelial cells and no signs of active inflammation (presence of neutrophils) or an increased density of lymphocytes and plasma cells". Usually, histological assessment, when performed during drug trials, has focused mainly on improvement and regression of inflammatory features. The assessment is usually based on the analysis of microscopic sections stained routinely with haematoxylin and eosin. This is a cheap and simple technique which is widely available.

## ONLINE SEARCH

We made a comprehensive online search of Medline and the Science Citation Index using the keywords "inflammatory bowel disease", "ulcerative colitis", "Crohn's disease", "mesalazine", "immunosuppressant", "biologics", "infliximab", "azathioprine", "corticosteroids", "prednisolone", "beclomethasone", "endoscopy", "histologic", "mucosal", and "healing" in various combinations with the Boolean operators "and", "or", and "not".

We included only articles that related to human studies, and we performed manual cross referencing, selecting articles published in English between January 1965 and April 2012. A search of non-English language articles and journals older than 1965 was also performed in our library. We excluded letters, and we reviewed abstracts only when the full papers were unavailable.

## HISTOPATHOLOGICAL CONSIDERATIONS

An important point is that most literature on this topic originates from only rectal sampling<sup>[6]</sup>. Thus, it is important that the pathologist receives an adequate number of biopsies correctly oriented from several sites, including the rectum and the terminal ileum. The biopsies should always be accompanied by a report including the age of the patient, clinical information, duration of disease, and type of treatment<sup>[11]</sup>.

The pathological assessment of IBD basically relies on two types of lesions, combined with each other in various ways, represented by architectural abnormalities (that include crypt branching/shortening, decreased crypt density, and irregular mucosal surface) and inflammatory features (transmucosal increase of lamina propria mononuclear cells and the presence of epithelioid granulomas)<sup>[6,12]</sup>.

Moreover, it should be always kept in mind that the histological features of IBD are variable in time, due to both the natural evolution of the disease and the different therapeutic measures. For instance, crypt distortion in UC is usually absent in early stages<sup>[13]</sup>, and the development of architectural abnormalities may take up to 2 mo to appear<sup>[14]</sup>. Also, decreased crypt density is usually not observed in the first week of the disease, and it is present in about 75% of UC patients with longstanding disease in remission<sup>[15]</sup>. Again, irregular mucosal surface (present in about 40% of IBD patients) is generally observed in subjects presenting with symptoms for > 2 wk, and it is more common in UC than in CD<sup>[6,16,17]</sup>. Thus, the absence of mucosal architectural abnormalities in the early phases may present difficulties in the differential diagnosis with acute colitis and during follow-up<sup>[18]</sup>.

Concerning inflammatory features, an increase in cellularity of the lamina propria (especially when localized in its basal third) allows us to distinguish longstanding IBD patients from normal subjects in almost 90% of cases<sup>[19]</sup>. Another useful clue is an increase of plasma cells, a population increased in IBD rectal samples compared to controls<sup>[20]</sup>; indeed, blind evaluation of IBD and control specimens has revealed that > 50% of patients displayed basal plasmacytosis<sup>[17]</sup>. However, it must always be kept in mind that inflammatory features also are not constant over time; for instance, a prospective study has demonstrated that focal basal plasmacytosis was found in 40% of IBD patients with symptoms for < 2 wk, but disappeared after 1 year follow-up in half of those without relapse<sup>[13]</sup>. Granulomas, a distinctive feature of CD, are not constantly present, and are more frequent in

**Table 1** Score for histological assessment of ulcerative colitis

|                               |   |
|-------------------------------|---|
| No significant inflammation   | Mucosa free from active inflammation; no erosions or crypt abscesses; surface and glandular epithelial cells intact; general architecture of the mucosa often disturbed; edema and fibrosis of the lamina propria with occasional foci of lymphocytes   |
| Mild to moderate inflammation | Epithelium usually intact; Glandular tubules irregularly arranged and often showing increased proliferative activity; Edema, vascular congestion, and interstitial hemorrhage presented in the lamina propria; Lymphocytes, plasma cells, and eosinophils increased in number, neutrophils often present, but less numerous than in the more severely affected specimens; Variation in intensity of inflammatory change in individual specimens giving a range of appearances from relatively quiescent to active inflammation  |
| Severe inflammation           | Mucosal surface often irregular due to edema, interstitial, hemorrhage, or inflammatory exudate in the lamina, propria; Small epithelial breaches common, sometimes with frank erosions and purulent exudate; Neutrophils and eosinophils passing through the damaged epithelium; Areas of flattened and cuboidal cells especially found near erosions; Mucosa showing heavy interstitial infiltration by lymphocytes, plasma cells, eosinophils and neutrophils; Glandular abnormalities (neutrophilic invasion of the tubules, epithelial focal degeneration and shedding of necrotic/viable cells into the glandular lumina; crypt abscesses (neutrophils, eosinophils, and epithelial debris); Sometimes, breaking down of the wall of the tubule with inflammatory exudate passing from the tubule into the lamina propria |

Adapted from Truelove *et al.*<sup>[23]</sup>, 1956.

children and in the early phases of the disease<sup>[6,21]</sup>.

It must also be stressed that in CD, being a transmural disease, mucosal healing may not be sufficient even though the mucosa may return to almost normal after treatment. Deeply situated lesions may persist, and endoscopy with biopsy can however not assess the deeper layers of the digestive tract<sup>[22]</sup>.

An important point to be addressed is that, to date, there is no standardized histological scoring system for the assessment of disease activity in IBD, although many scores have been proposed. A scoring system was firstly introduced many years ago for UC<sup>[23]</sup>, and in subsequent years many other histological scores have been proposed, all based on routine haematoxylin-eosin (HE) staining. As a result of the more homogeneous distribution of the lesions, most of these scores have been described in UC, many during the 1980s<sup>[24-28]</sup>, a few in the 1990s<sup>[29-32]</sup>, and only two more recently<sup>[33,34]</sup>, although some proposal for CD also exist<sup>[35-37]</sup>. However, even recent consensus conferences on CD and UC have not included a formal histological score for the pathological evaluation of these patients<sup>[38-41]</sup>.

This is partly due to the possible impact of sampling error, to the limits of routine HE staining, and to the existence of some poorly known variables. Histologically, disease activity is usually based upon the combination of the presence of neutrophils and epithelial damage, because neutrophils can be recognized reliably and it is known that they release molecules that are capable of damaging the tissue. Other cells, however, might also have deleterious effects. The activity status of cells such as macrophages and lymphocytes can however not be assessed on HE-stained sections. The influence of eosinophils is still unclear. Elevated levels of eosinophils have been observed in colonic biopsy samples from UC patients, and increased numbers of these cells and eosinophil-derived granular proteins (major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil derived neurotoxin) have been shown to correlate with morphological changes in the gastrointestinal tract, disease severity, and gastrointestinal dysfunction<sup>[42,43]</sup>.

Finally, the natural evolution of the disease and its

histological features are not well known. Inflammatory features may however decrease over time and the distribution pattern may change<sup>[44]</sup>.

## HISTOLOGICAL HEALING IN UC

### Corticosteroids

These agents were introduced early in the therapeutic armamentarium of IBD, and are still commonly used in the acute phases; moreover, their ability to induce at least macroscopic mucosal healing at endoscopy is well recognized<sup>[45]</sup>. Concerning histological healing, only a few studies are available in literature, and none recent.

The first study to tackle this topic in 40 patients with active UC investigated the effect of 1 wk treatment with rectally administered hydrocortisone hemisuccinate sodium<sup>[46]</sup>. Histological variables were analyzed by a previously described scoring system<sup>[23]</sup> (Table 1), and showed significant improvement compared to baseline and to placebo in patients treated with steroids, in whom there was a 50% decrease in severe grading and 64% in moderate grading. Moreover, 55% shifted toward mild grading after treatment. However, no normalization of histological variables was reported in this study. Interestingly, the authors of the histological scoring system stated that "...this system of grading depends to a large extent on subjective judgments"<sup>[23]</sup>.

In a study of 215 patients with UC, cell counts revealed that acute inflammation as indicated by neutrophils was decreased most notably following treatment with prednisone (and/or 6-mercaptopurine). Chronic inflammation as indicated by the presence of plasma cells was also reduced after prednisone and equally after 6-mercaptopurine and salicylazosulfapyridine. The authors further demonstrated an increase of epithelial goblet cells and lamina propria macrophages during healing<sup>[47]</sup>. Although these results are particularly interesting, the method used is time consuming and probably difficult to apply routinely.

Another 2-wk study compared the effects of aqueous hydrocortisone enema and a suspension of hydrocortisone in an inert foam base in 30 UC patients with proctosigmoiditis<sup>[48]</sup>. Histological assessment was carried

**Table 2** Modified scoring system for histological assessment of ulcerative colitis

| Inflammation grade   | Score | Intensity   | Histological criteria  |
|----------------------|-------|-------------|--|
| Active inflammation  | 0     | Normal      | Neutrophils not present in crypt or surface epithelium and no exudate, erosion or ulceration                                     |
|                      | 1     | Low grade   | Neutrophils present transmigrating through the crypt epithelium or within crypt lumina in < 20% of crypts; no erosions or ulcers |
|                      | 2     | Moderate    | Neutrophilic infiltration in > 20% of crypts or presence of erosions   |
|                      | 3     | High grade  | Presence of ulcers   |
| Chronic inflammation | 0     | No increase | Normal number of chronic inflammatory cells present primarily in the superficial lamina propria                                  |
|                      | 1     | Moderate    | Moderate number of mononuclear cells   |
| Crypt distortion     |       |             | Aggregated between crypts at the base of the lamina propria  |
|                      | 2     | Severe      | Marked increase in chronic inflammation shown by sheets of chronic cells   |
|                      | 0     | None        | Crypts had normal outlines with only artifactual irregularities  |
|                      | 1     | Mild        | Scattered or rare crypts showing irregular (bent, forked) outline  |
|                      | 2     | Moderate    | Approximately 25%-50% of crypts with an irregular outline  |
|                      | 3     | Severe      | > 50% of crypts with an irregular outline  |

Adapted from Hanauer *et al*<sup>[50]</sup>, 1998.

out by means of an active inflammation score (0-3 for each of the following three characteristics: polymorph infiltration, mucus depletion, and superficial epithelial degeneration, allocating a score of 0-9 to each biopsy). In the enema group there was a significant ( $P < 0.05$ ) improvement in active inflammation score (from 4.4 to 2.5) compared to baseline, but this was not observed in the foam group (inflammation score 5.2 *vs* 4.9). However, this apparent superiority for enema treatment was not confirmed when the two treatments were directly compared.

Lee *et al*<sup>[49]</sup> subsequently compared the effect of mesalazine foam enema (2 g) and prednisolone foam enema (20 mg) in 295 patients with relapsing distal UC. After 4 wk treatment, histological remission (score 0), using the above scoring system<sup>[48]</sup> was obtained in 27% of patients in the mesalazine group and 21% of patients in the prednisolone group.

In another study, 233 patients with active distal UC/proctitis were randomized to either a placebo enema or budesonide enema at a dose of 0.5 mg/100 mL, 2.0 mg/100 mL, or 8.0 mg/100 mL<sup>[50]</sup>. Biopsy specimens were histologically graded for active inflammation, chronic inflammation, and crypt distortion, and graded according to a modification of the Truelove and Richards scoring system<sup>[23]</sup> (Table 2). After treatment, total histopathological score (defined as the sum of the three above components) significantly improved in the 2.0 and 8.0 mg groups, compared to placebo, whereas no differences were found for the 0.5 mg dose. However, remission rate in this study did not include histological remission.

A study conducted on 17 patients with severe UC compared the effect on unfractionated heparin and corticosteroids, and demonstrated an improvement of histopathological grading (that, however, was not formally described), not significantly different between the two treatments (63% in the heparin group and 50% in those treated with steroids) at the end of the study<sup>[51]</sup>.

Rizzello *et al*<sup>[52]</sup> have evaluated the effect of oral 5-aminosalicylic acid (5-ASA) (3.2 g/d) compared to oral

beclometasone dipropionate (5 mg/d) and to placebo in 118 patients with extensive or left-sided mild to moderate UC. After 4 wk of treatment, the histological score<sup>[23]</sup> was significantly improved compared to baseline, but no differences between treatment groups were observed and no specific histological description was given.

In a more recent study, two budesonide formulations (foam and enema) were compared in 449 patients with distal UC/proctitis<sup>[53]</sup>. Histological assessment was conducted according to the score proposed by Riley *et al*<sup>[29]</sup>, which takes into account six features [acute inflammatory cell infiltrate (neutrophils in the lamina propria), crypt abscesses, mucin depletion, surface epithelial integrity, chronic inflammatory cell infiltrate (round cells in the lamina propria), and crypt architectural irregularities], each graded on a 4-point scale (0 = none; 1 = mild; 2 = moderate; 3 = severe). At the end of treatment, a similar histological improvement was obtained in both groups (51% foam, 57% enema). Again, no specific histological description was given.

Overall, by considering the above studies, although steroid treatment seems to improve histological abnormalities in UC patients, true remission rates are probably quite low, and no more than 30%.

### Salicylates

Overall, not many studies are available in the literature that have assessed the effect of these drugs on histological healing in UC, and it should be stressed that these studies included different subtypes of patients and used different pharmacological formulations and modes of administration. The study by Sommers *et al*<sup>[47]</sup> mentioned earlier did show some beneficial effects on inflammatory cell counts in the lamina propria.

The first study to assess this topic was that of Rao *et al*<sup>[54]</sup>, who compared the effects of olsalazine (2 g/d) and sulfasalazine (3 g/d) in 37 patients with mild to moderate active distal UC. The degree of inflammation in rectal biopsies was graded as absent (without inflammation), mild (chronic glandular damage with definite increase in inflammatory cells), moderate (mild inflam-



mation plus small foci of ulceration), or severe (moderate inflammation in the presence of crypt abscesses and widespread ulceration). In both groups, a similar histological improvement (44% for olsalazine and 46% for sulfasalazine) was seen after 1 mo, even though this was not as impressive as the improvement of endoscopic appearance or clinical response. However, no histological details were given in this study, apart from generic statements (improved, unchanged, worsened), nor it was stated whether histological remission was achieved.

In a 12-wk multicenter double-blind parallel group study, the effects of balsalazide (6.75 g/d) or sulfasalazine (3 g/d) were compared in 57 UC patients, stratified for disease severity; topical and/or oral steroids were administered if clinically needed<sup>[55]</sup>. Histological assessment (only graded as 0, normal; 1, mild UC; 2, moderate UC; 3, severe UC) showed a similar improvement in both groups at the end of treatment. No histological details were given.

The same authors carried out another study with the same drug regimens (but without concomitant use of steroids) in 50 patients with mild to moderate UC<sup>[56]</sup>. Pathological assessment was used to identify patients in whom clinical remission was also associated with histological remission. Rectal biopsies, obtained at baseline and after 8 wk) were graded on a 4-point scale (0, normal; 1, minimal inflammation but not active disease; 2, moderate inflammation; 3, severe inflammation); all patients included in the study had at least mild inflammation at baseline, with 58% displaying severe inflammation. After treatment, histological grades improvement was observed in both groups, but only 34% of patients were free from inflammation. No other histological details were given.

In another study, 264 patients with distal active UC were treated with 5-ASA foam or liquid enema<sup>[57]</sup>. Histological assessment (no details were given), performed after 4 wk, showed similar improvement in both groups (46% generically defined remission in the foam group and 50% in the enema group).

An 8-wk double-blind multicenter trial compared three doses of 5-ASA (1.5, 3 and 4.5 g/d) in 321 patients with active UC<sup>[58]</sup>. Colonic biopsies were obtained at baseline and at the end of the study, and were assessed according to a previously described scoring system<sup>[29]</sup>, considering histological improvement as reduction of at least one point of the histological activity index. At the end of study period, histological improvement was documented in 42% patients in the 1.5 g group, 65% of the 3 g group, and 63% of the 4.5 g group. Once again, no details on histological assessment were given.

Other authors compared the effects of slow-release (MMx) mesalazine with topical 5-ASA in 79 patients with active left-side UC<sup>[59]</sup>. The tissue inflammatory response was evaluated according a previously described score<sup>[26]</sup>, and graded as follows: grade 1, normal mucosa; grade 2, enhanced glands with intraepithelial granulocytes. In the stroma, enhancement beyond normal of lymphocytes,

plasma cells, or eosinophils (slight inflammation); grade 3, goblet cells depletion, loss of tubular parallelism, reduced mucin production in some glands, intraepithelial granulocytes, marked increase of inflammatory cells in the stroma (intermediate inflammation); grade 4, marked gland and mucosal atrophy, evident crypt abscesses and pus on the surface, massive increase of acute inflammatory cells and follicle formation in deeper cell layers (severe inflammation); and grade 5, ulceration with pus, gland and mucosal atrophy, crypt abscesses, extensive stromal inflammation, and deep follicles (fulminant inflammation). Histological remission (defined generically according to the above score) was obtained in only 15% patients of the MMx group and in 8.0% of those receiving enemas. No other histological details were given.

A more recent study compared two different scheduling dosages (3 g *oid* or 1 g *tid*) of mesalazine granules in 380 UC patients<sup>[60]</sup>. Biopsy samples were obtained at the start and the end of the trial, and were scored according to previously published criteria<sup>[29]</sup>, with the total histological index based on the more severely affected colonic segment. Histological remission (grade 0), observed in 35% patients in the *oid* group and in 41% of the *tid* group, did not show significant differences between the two regimens.

Overall, the available evidence for salicylates in histological healing of UC patients suggests that an improvement may be obtained in 30%-60% of patients (obviously depending on the formulation and the dose scheduling), but the actual healing rate is lower (10%-30%).

### Immunomodulators

There are still only a few data on histological healing with immunomodulatory agents. A study conducted in 32 patients with refractory active UC<sup>[61]</sup> showed that after 6 mo azathioprine treatment, 78% of patients in remission were free of histological inflammation graded according to previously described criteria<sup>[23]</sup>. However, after a median of 4 years follow-up, histological relapse was found in almost 90% of these patients. No histological details were given.

Another study compared the effects of intravenous cyclosporine A to those of methylprednisolone in 30 patients with severe UC<sup>[62]</sup>. Histological assessment, carried out according to a previously described scoring system<sup>[33]</sup> (Table 3), was done at baseline, and after 7 and 30 d therapy; after 1 wk no effects were seen, and only after 1 mo therapy was there a significant decrease in inflammatory cells, and severity of epithelial damage was similarly observed in both groups, although only small changes in the architectural mucosal disturbances were seen. Overall, it seems however that these agents have a beneficial effect on mucosal histology.

### Biological agents

Concerning biological therapy, although anti-tumor necrosis factor (TNF)- $\alpha$  therapy can lead to endoscopically assessed mucosal healing in patients with UC<sup>[63,64]</sup>,

**Table 3** Histological scoring system for ulcerative colitis and Crohn's disease

|   |   |
|---|---|
| Ulcerative colitis  |   |
| Grade 0   | Structural (architectural change)                     |
| 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                       |
| 1   | 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  | None  |
| 2B.1  | Mild but unequivocal increase                         |
| 2B.2  | Moderate increase                                     |
| 2B.3  | Marked increase                                       |
| Grade 3   | Neutrophils in epithelium                             |
| 3   | None  |
| 3.1   | < 5% crypt involved                                   |
| 3.2   | < 50% crypt involved                                  |
| 3.3   | > 50% crypt involved                                  |
| Grade 4   | Crypt destruction                                     |
| 4   | 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                                 |
| 5   | No erosion, ulceration, or granulation tissue         |
| 5.1   | Recovering epithelium plus adjacent inflammation      |
| 5.2   | Probable erosion-focally stripped                     |
| 5.3   | Unequivocal erosion                                   |
| 5.4   | Ulcer or granulation tissue                           |
| Crohn's disease   |   |
| Epithelial damage   |   |
| 0   | Normal  |
| 1   | Focal pathology                                       |
| 2   | Extensive pathology                                   |
| Architectural changes   |   |
| 0   | Normal  |
| 1   | Moderately disturbed (< 50%)                          |
| 2   | Severely disturbed (> 50%)                            |
| Infiltration of mononuclear cells in the lamina propria       |   |
| 0   | Normal  |
| 1   | Moderate increase                                     |
| 2   | Severe increase                                       |
| Infiltration of polymorphonuclear cells in the lamina propria |   |
| 0   | Normal  |
| 1   | Moderate increase                                     |
| 2   | Severe increase                                       |
| Polymorphonuclear cells in the epithelium                     |   |
| 1   | In surface epithelium                                 |
| 2   | Cryptitis   |
| 3   | Crypt abscess   |
| Presence of erosions and/or ulcers                            |   |
| 0   | No  |
| 1   | Yes   |
| Presence of granuloma   |   |
| 0   | No  |
| 1   | Yes   |
| No. of biopsy specimens affected                              |   |
| 0   | None (0 of 6)   |
| 1   | < 33% (1 or 2 of 6)                                   |
| 2   | 33%-66% (3 or 4 of 6)                                 |
| 3   | > 66% (5 or 6 of 6)                                   |

Each topic scored independently. Moderate increase, up to twice the number of cells that can normally be expected; severe increase, more than twice the normal number of cells.

histological data are still scarce. A 10-wk study conducted on nine moderate to severe UC patients<sup>[65]</sup>, using a score that included polymorphonuclear infiltration of the epithelium and lamina propria, crypt abscesses, loss of glandular parallelism, crypt shortening and/or ramification, mucus epithelial depletion, involvement of muscularis mucosae and/or submucosa. Each histological variable was scored from 0 (normal) to 1 (mild), 2 (moderate) and 3 (severe). In addition, the total number of neutrophils, lymphocytes, and plasma cells in the lamina propria was counted in five high-power fields. At week 10, histological score significantly decreased only in responders (67% of patients), but normal architecture was observed only in 33% of these patients. The histological improvement was mainly due to a decrease in neutrophils associated with restoration of normal crypt architecture and mucus content in epithelial cells. No significant reduction of mononuclear cell infiltration was observed in responders and non-responders.

Another study took into account the ultrastructural features of the colon in seven patients with UC refractory to standard treatment, before and after 2 wk treatment with infliximab<sup>[66]</sup>. Before treatment, severe alterations of the epithelium were present, such as microvilli depletion, shattering of epithelial junctions, cytoplasmic vacuolization, dilatation of the endoplasmic reticulum, pycnotic nuclei, altered structure of mitochondria and Golgi complexes, in addition to rarefaction of the goblet cells with abnormal mucus formation and secretion. The chorion showed structural alteration of component cells, obstructed capillaries, erythrocyte extravasation, and many plasmocytes and neutrophils. After infliximab, there was improvement in morphology and function of the epithelial organelles, rich mucus secretion, and recovery of the chorionic components.

### Miscellaneous

D'Ovidio *et al*<sup>[67]</sup> have evaluated the effects of granulocyte-monocyte apheresis in 12 patients with mild to moderate UC refractory to therapy/steroid dependent. After 6 wk, complete histological remission, defined as grade 1 according to the score of Florén *et al*<sup>[26]</sup>, was obtained in six (50%) patients, and histological improvement in five (42%) patients. Once again, no further details on histology were given. In general, it seems that various drugs can lead to microscopic healing and a normal mucosa in a minority of patients (with a maximum of approximately 30%).

## HISTOLOGICAL HEALING IN CD

### Corticosteroids

These agents are frequently employed for treatment of disease flares; however, clinical and endoscopic data demonstrate that mucosal healing in CD is unlikely (less than one third of treated patients) after steroid treatment<sup>[68]</sup>. Moreover, there have been few studies evaluating histological healing in CD after steroid treatment.

In a study comparing 1 year treatment with budeso-

nide or azathioprine in 77 CD patients, histological assessment was carried out with a previously developed score<sup>[37]</sup> (Table 3). At the end of the study, the histological score fell significantly versus baseline only in the azathioprine group and was significantly lower than in the budesonide group at the end of the study<sup>[69]</sup>. This improvement was observed for all acute parameters (epithelial damage, acute lamina propria inflammatory cell infiltration, erosions and/or ulcers) irrespective of disease location, whereas glandular architecture and the presence (but not the degree) of chronic inflammation were not influenced by azathioprine treatment.

A 2-wk regimen of 20 mg oral prednisone showed, in individual biopsies of 30 CD patients undergoing surveillance colonoscopy, a decrease in the overall histological activity (assessed by a previously reported scoring system<sup>[33]</sup>) compared to placebo, whereas no significant differences were found with respect to the overall severity of inflammation<sup>[70]</sup>. No other details on histological assessment were given.

### Immunomodulators

These agent represent to date the cornerstone of treatment in CD, thus researchers' interest has given rise to a few studies aimed at evaluating their effects on histological healing as well.

**Azathioprine:** A first study assessed the effect of 6 mo of therapy in 19 patients with severe postoperative recurrent CD<sup>[71]</sup>. Histological evaluation was done in seven patients (four with complete mucosal healing and three with near-complete healing at endoscopy). Only in patients with complete healing did comparison of the biopsy specimens taken before and after azathioprine show persistent mucosal architectural changes with complete disappearance of the inflammatory infiltrate. The same authors then reported pre/post-therapy data on 20 CD patients with colitis-ileocolitis taking azathioprine for at least 9 mo<sup>[72]</sup>, using a histological disease severity score<sup>[37]</sup>. A decrease in histological score paralleled endoscopic healing, even though it was not as complete as shown by endoscopy. In patients with endoscopic complete healing, the colon showed a global decrease in histological score from 10 to 3 and inflammatory score from 5 to 2; in the ileum, global histological score decreased from 7 to 2 and inflammatory score decreased from 2 to 0. No significant changes were documented in patients without endoscopic healing while taking azathioprine. The study of Mantzaris *et al.*<sup>[69]</sup>, comparing azathioprine with budesonide, has been discussed above.

**Methotrexate:** Methotrexate has been even less frequently investigated in this setting. A pilot study evaluated the effects of adding this agent to standard treatment (steroids, mesalazine) in 14 CD patients with refractory disease<sup>[73]</sup>; four patients (28.5%) were reported to have normal histology at 12 wk. Another study compared the effects of methotrexate with those of azathioprine and

infliximab in 40 patients with CD<sup>[74]</sup>. After 3 mo treatment, the scoring of microscopic activity<sup>[37]</sup> was similar in the three groups. No further histological data or data on histological healing were given.

### Biological agents

A few studies have been carried out on biological agents. D'Haens *et al.*<sup>[75]</sup> investigated histological healing in 30 CD patients treated with infliximab in a placebo-controlled trial. After 4 wk intravenous administration of 5, 10 or 20 mg/kg infliximab or placebo as a single infusion, inflammatory infiltrate disappeared only in infliximab-treated patients but architectural changes persisted in most of them. Another study from the same group evaluated the effects of a single infusion of infliximab in 15 patients with steroid-refractory CD<sup>[76]</sup>. Histological activity scores, assessed 4 wk after infusion showed, compared to placebo, a significant decrease mainly due to the reduction of inflammatory activity. Immunohistochemical assessment also revealed a decrease in inflammatory features due to global reduction of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes and CD68<sup>+</sup> mononuclear cells.

A follow-up study conducted in CD patients evaluated histological improvement by a previously described scoring system<sup>[37]</sup> after 54 wk of treatment with infliximab, and showed that histological mucosal healing was associated with a consistent decrease in the expression of inflammatory markers<sup>[77]</sup>. The use of etanercept (another anti-TNF- $\alpha$  agent) in 10 patients with refractory active CD did not result in any significant histological improvement after 2 wk of therapy<sup>[78]</sup>.

### Miscellaneous

Yamamoto *et al.*<sup>[79]</sup> evaluated 28 patients with active CD, treated with an elemental diet for 4 wk. Histology was evaluated by a previously described scoring system<sup>[80]</sup>, taking into consideration enterocyte appearance, extent of crypt inflammation, and the intensity of mononuclear and neutrophilic lamina propria inflammation. Each feature was given a score of 0-3, depending on the severity of the abnormality. The scores were added, and a total score was calculated. Grade 0 (no inflammation) was assigned a total score of 0 or 1, grade 1 (mild) was scored 2-4, grade 2 (moderate) was scored 5-8, and grade 3 (severe) was scored 9-12. Histological healing was defined as grade 0, and histological improvement (including healing) was defined as a decrease of at least 1 grade. At the end of treatment, histological healing and improvement rates were 19% and 54% in the terminal ileum and 20% and 55% in the large bowel, respectively.

A retrospective study carried out in 39 patients with refractory severe colonic CD evaluated the effect of anti-*Mycobacterium avium* ss *paratuberculosis* therapy<sup>[81]</sup>. Histological acute and chronic ileitis or colitis were recorded as the most severe inflammation found in available biopsies, and were generically reported by a pathologist as ranging from inactive chronic inflammation to mild, moderate, and severe active inflammation. After an aver-



age 3 years of therapy, 15/39 (38.5%) patients showed a marked reduction of acute and chronic inflammatory infiltrates; however, only in six (15.4%) patients was normalization of histological variables observed. No other histological variables or details were given.

## HISTOLOGICAL HEALING IN IBD: WHERE ARE WE, AND WHERE ARE WE GOING?

Although mucosal healing has been associated with positive outcomes in IBD, most of the supporting data are retrospective and largely based on endoscopic assessment; in addition, it is not clear whether complete mucosal healing produces better outcomes than partial healing<sup>[82]</sup>. It has been stated that, in clinical practice, evaluation of mucosal healing (again, by an endoscopic point of view) should be considered in patients with persistent symptoms despite adequate therapy and when treatment discontinuation is being considered<sup>[83]</sup>.

However, it must be stressed that histological assessment of mucosal healing, the only way to establish in an objective manner that the mucosa has reverted to a normal state, is at present not recommended as a primary end point for therapeutic trials because of the lack of a standardized approach<sup>[5]</sup>. Thus, it is unknown whether patients who achieve and maintain deep remission may stop treatment without having further/future problems<sup>[84]</sup>.

As reported above, true histological healing may be grossly obtained in only about 30% of treated patients; this may justify the high (20%-40%) loss of response to even newer therapeutic approaches<sup>[85]</sup>. A recent study has shown that adding histological and mucosal gene panel assessment may predict the lack of response to infliximab (directed against TNF- $\alpha$ ) treatment in patients with UC or Crohn's colitis, whereas no such predictive gene set could be identified for ileal disease<sup>[86,87]</sup>.

Therefore, adding histological healing as an endpoint, at least in clinical trials, would substantially improve the evaluation of the actual effectiveness of a given treatment. Histological assessment is cheap and widely available. The features of disease activity are known. They include the presence of neutrophils in the lamina propria and the epithelium, epithelial cell damage (loss of cells, mucin depletion, cryptitis, crypt abscesses, erosion), and an increase in lymphocytes and plasma cells. Some of these features such as the presence of neutrophils can reliably be evaluated with good interobserver agreement. In addition, histology could help the management of the patient. Some histological variables are indeed associated with relapse. They include persistence of neutrophils, basal plasmacytosis and persistence of eosinophils in the lamina propria<sup>[29,88,89]</sup>. Furthermore, microscopic activity may be related to the development of dysplasia<sup>[90]</sup>.

We realize that adding data on histological healing to investigations and trials on IBD patients might be a relatively difficult goal to achieve, and that such an approach would increase the investigators' burden. Furthermore, there are several unresolved issues. It has been shown that UC may become a discontinuous inflammatory pro-

cess during its natural course or following medical treatment. This means that several biopsies would be needed for an adequate microscopic evaluation; the optimal number of samples has however not yet been determined. Although routine HE staining is a good tool for identification of neutrophils, epithelial damage and even eosinophils as markers of active disease, it is less appropriate for the evaluation of lymphocytes. The presence of lymphocytes can be determined adequately but the activation status is beyond the scope of routine histology and requires immunohistochemical staining. Yet, the application of this technique and the markers needed are not yet well determined.

## CONCLUSION

In conclusion, it appears to us that histological healing should be considered as an end point in the treatment of IBD patients, especially for UC. Therefore, additional studies looking for optimal sampling, standardizing scores and features should be performed.

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