Is histology useful for the assessment of the efficacy of immunosuppressive agents in IBD and if so, how should it be applied ?

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Abstract

Crohn's disease and Ulcerative colitis are two chronic relapsing inflammatory bowel diseases of unknown etiology. Both conditions are characterized by a considerable morbidity and have an impact upon the social and economic aspects of the patients life. At present, medical treatment is mainly aiming at the control of the inflammation. Drugs used for ulcerative colitis can induce microscopic healing of the mucosa. Similar results have been obtained recently with immunomodulatory drugs in Crohn's disease. The cost of these drugs is however high and the use of these drugs can be associated with side effects. Furthermore, many of the drugs need to be given for a long period. Therefore it is appropriate to assess the efficacy of the drugs before commercial use and even when used in routine practice. For both ulcerative colitis and Crohn's disease, clinical parameters combined in indices and endoscopy are commonly used together with some laboratory tests for the assessment of disease activity. In ulcerative colitis, histology has been used along with the other instruments for the measurement of disease activity because it was shown that the mucosal lesions could improve. More recently, histology has also been used for Crohn's disease. Routinely, disease activity when assessed with microscopy, should be divided into mild, moderate and severe. For drug trials and study purposes, more objective scoring systems should be used. Preferentially, a generally accepted score is used. This allows comparisons between different studies. Different scoring systems have been designed for ulcerative colitis and Crohn's disease. For the latter, multiple biopsies should be analysed. Most scoring systems still need validation. (Acta gastroenterol. belg., **2004**, 67, **285-289**).

Key words : inflammatory bowel disease ; ulcerative colitis, Crohn's disease ; histology ; activity ; score.

Introduction

Histology is routinely used for the diagnosis of ulcerative colitis (UC) and Crohn's disease (CD). For UC, pathologists should make not only a diagnosis but also make a distinction between quiescent disease, inactive disease and different grades of activity. Healing of mucosal inflammation has been noted to occur in UC. Because UC is a diffuse disease starting from the rectum, because rectal biopsies can be obtained easily and because it was noted already years ago that mucosal inflammation can decrease with treatment, scoring systems for the assessment of disease activity and the efficacy of drug therapy have been introduced for UC already in the 1960s (1). In contrast with UC, disease activity is not generally assessed by pathologists for CD. This attitude is mainly explained by the fact that rectal biopsies and / or surgical specimens were the only material available for pathologists until the introduction of colonoscopy. In clinical practice, even today, rectal biopsies or often the only material submitted to the pathologists, for patients with CD, making a diagnosis and certainly assessment of disease activity difficult. Crohn's disease is indeed a discontinuous disease with skip areas and the rectum is not necessarily involved. Sampling error is therefore very important, especially when only rectal biopsies are examined. Therefore, while it is generally accepted that microscopic findings are important for the diagnosis, it is not generally accepted that histology is important for the assessment of disease activity in CD. Measuring disease activity currently mainly relies on clinical indices such as the CDAI or the Harvey Bradshaw index, or on endoscopic indices such as the Crohn's disease endoscopic index of severity (CDEIS). Efficacy endpoints considered in clinical trials for CD are clinical remission, maintenance of remission, prevention of postoperative recurrence, corticosteroid sparing, closure of fistulas and endoscopic remission. It is unclear if histologic remission should be considered.

However, microscopic analysis of multiple samples from different segments of the colon and ileum could provide useful information and allow assessment of disease activity. Arguments in favour of such an assessment can be found in other diseases such as UC and Helicobacter pylori related gastritis and from clinical drug trials. In UC, it has been shown that mucosal inflammation can disappear and healing can be induced with treatment. Furthermore, histology can help to predict relapse (3-5). The effect of treatment upon Helicobacter pylori related gastritis has been studied extensively. From the data available it appears that active inflammation (neutrophils) disappears within weeks when treatment is appropriate and persists when eradication fails. Mononuclear inflammation will persist for months, even with successful eradication. Histology can thus help to evaluate the efficacy of treatment and predict relapse in chronic inflammatory diseases.

The more frequent use of immunosuppressive agents such as azathioprine and 6-mercaptopurine, methotrexate and cyclosporine A, the introduction of new immunomodulatory agents such as those directed against TNFα (infliximab, etanercept and more recently

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Table I. — **Scoring systems for ulcerative colitis**

Odze *et al.* (ref. No. 3)			
chronic inactive	not defined		
chronic active	cryptitis, crypt abscesses or surface erosion		
active	ulceration		
normal			
Keren *et al.* (ref. No. 6)			
	acute inflammation	crypt changes	chronic inflammation
normal mucosa	0	0	0
active IBD	+	+	+
inactive IBD	0	+	+ or 0
reactive mucosa	+ or 0	0	+

acute inflammation includes cryptitis, crypt abscesses and neutrophils in the lamina propria.
crypt changes include distorsion and / or atrophy.
chronic inflammation : mononuclear cells in lamina propria.

humira) have shown that also in CD, the pattern of inflammation can change. Treatment of CD with infliximab has indeed shown endoscopic and microscopic healing of the mucosa. Subsequently, histology is now also considered in studies with new agents such as HuZAF (the humanized form of a murine anti-human IFN-γ) and antegren (a recombinant humanized anti-α4 integrin antibody).

At present there are however not yet proper tools for the assessment of microscopic disease activity and definitions of microscopic improvement and remission need to be formulated. Problems which have to be solved are the design of proper scoring systems, validation of the scores, methods for analysis of the scores and identification of the number of biopsy samples, needed for adequate evaluation.

Scoring systems

Ulcerative colitis

Histology as a tool for the measurement of disease activity has been introduced first for UC. All scoring systems rely on the analysis of routinely H&E stained sections, usually of one rectal biopsy. Scoring systems can be divided largely into two groups : stepwise systems and numerical (quantitative) systems. In stepwise systems, the disease activity and / or severity is divided into different grades or phases for which different grades such as 1, 2 and 3 or names such as normal mucosa, quiescent, inactive disease, chronic persistent and active disease are used. Active disease can further be subdivided in mildly, moderately or severely active disease. In numerical systems, different variables or lesions are scored and to each of these a subjective numerical value is given. The final score is the result of the sum of the scores of different variables. Different microscopic features can be assessed. In the score of Odze *et al.* activity is defined by the presence of focal cryptitis, crypt abscesses or surface erosions (3). Biopsies were considered as normal if none of the features indicative of chronicity were present (abnormal crypt architecture ; villiform surface ; increased mixed inflammation in the lamina propria) and no features of active disease were found. Neutrophils in the lamina propria were not a sign of activity. In the score of Keren *et al.* acute inflammation without further definition is the sign of activity (6). The problem with some of these scores is the overlap of features, the fact that some grades are defined by inflammation and architectural abnormalities simultaneously, the mix between neutrophils and mononuclear cells for the identification of inflammation and the lack of a clear definition of the different grades of severity, at least in the publications (Table I). Subsequently, additional scores have been developed. These are usually more elaborate, because the different features which are important are scored separately (7,8).

The major features which are considered include architectural distortion, chronic inflammation defined as the presence of lymphocytes and plasma cells, the presence of neutrophils, the presence of eosinophils and increasing degrees of epithelial damage : crypt abscess formation ; crypt destruction ; erosion and ulceration (Table II). Architectural distortion is the result of the relapsing nature of the disease and the damage of epithelial cells, probably especially of the proliferative compartment in the basal part of the crypts. Chronic inflammation is the expression of the immunologic response. Lymphocytes, plasma cells and monocyte cells are the only cells considered. The duration of an immunologic reaction is usually longer than a reaction mediated by granulocytes. This is certainly noted in non relapsing infectious colitis (9). The extent of epithelial and tissue damage, due to lymphocytes, monocyte cells and plasma cells is more difficult to assess. Eosinophils may be important for tissue damage but the exact role of these cells is unclear. They are common in UC where they could be part of the disease process but they could also reflect an allergic reaction towards some drugs. Cryptitis, crypt abscesses, crypt destruction, erosions and ulcerations are major features of disease activity. “Active inflammation” is defined as an injury characterised by unequivocal damage of the epithelium typically in conjunction with neutrophils. Several arguments support this view. Neutrophils can reliably and reproducibly be detected in routinely, Haematoxylin and eosin (H&E) stained sections (10). The choice of neutrophils as indicator for disease activity is in agreement with studies using leukocyte scanning and showing that active inflammation in UC is dominated by neutrophils infiltrating the tissue (11-13). The survival of neutrophils in the tissue, outside blood vessels is limited in time because of a short half-life. Their presence in the tissue therefore indicates recent recruitment and hence persistent aggression. Neutrophils can induce tissue damage through their metabolites and enzymes such as gelatinase B (MMP-9), while the role of immune complex-mediated cell damage in IBD is limited.

Table II. — **Scoring systems for the assessment of severity in Ulcerative colitis**

Danielsson *et al.* (ref. No 7)	
Grade 1	normal mucosa
Grade 2	slight inflammation : isolated inflammatory cells or cell aggregates of either lymphoplasmocytic cells or eosinophils
Grade 3	intermediate inflammation : marked increase of inflammatory cells with some changes in secretory cells ; mild atrophy
Grade 4	severe inflammation : crypt abscesses ; lymphoid follicles in deeper cell layers ; massive increase in inflammatory cells and pus ; marked atrophy
Grade 5	fuminant inflammation : ulcerations with pus ; crypt abscesses ; atrophy ; deep follicles
Geboes *et al.* (ref. No 8)	
Subgrades are defined for each grade. Examples are given for grade 0 & 1	
Grade 0 :	**Structural (architectural change)**
Subgrades :	0.0 No abnormality ; 0.1 Mild abnormality ; 0.2 Mild or moderate diffuse or multifocal abnormalities ; 0.3 Severe diffuse or multifocal abnormalities
Grade 1 :	**Chronic inflammatory infiltrate**
Subgrades :	1.0 No increase ; 1.1 Mild but unequivocal increase 1.2 Moderate increase ; 1.3 Marked increase
Grade 2 :	**Lamina propria neutrophils and eosinophils**
2A : Eosinophils	
2B : Neutrophils	
Grade 3 :	**Neutrophils in epithelium**
Grade 4 :	**Crypt destruction**
Grade 5 :	**Erosion or ulceration**

Crypt abscesses and crypt destruction are separated from erosions because the latter are a lesion of the mucosal surface. Such a lesion could improve more rapidly with topical therapy. Overall a scoring with clear separation of the features and precise definitions of the lesions allows a more reliable and reproducible use of the score (8).

Crohn's disease

Different scoring systems have been developed for CD.

Scores implying rectal biopsies

Analysis of different features, usually with cell counts.

Scores implying multiple biopsies of different segments of the colon

A scoring system making a distinction between 5 grades (0-4) : 0 = normal ; 1 = mild oedema and inflammation in lamina propria ; 2 = crypt abscess and inflammation in lamina propria ; 3 = more severe inflammation with destructive crypt abscesses plus or minus granulomata ; 4 = more severe inflammation with active ulceration was designed for the colon alone. In this score, each biopsy from the different segments of the colon was graded separately and a total score was achieved by adding the individual scores of the six biopsies together (maximum 24). This system is comparable with another score used for the colon and based on the assessment of one to two biopsies obtained from each of the six colorectal segments. In this score a distinction is made between 4 grades : normal (0 = normal or sparsely distributed mononuclear cells in the stroma), mild (1 = granulocytes in the stroma), moderate (2 = granulocytes in the surface and / or crypt epithelium and / or presence of crypt abscesses) and severe (3 = ulcerations or fissures) (14,15). The latter score showed a significant correlation with colonoscopic assessment of inflammatory activity ($r = 0.632$, $p < 0.0001$).

Scores implying biopsies of ileum and different segments of the colon

1 : Paired assessment as worse, no change, improvement and resolution of inflammation was used in one study.

2 : A score with four grades : 0 = no inflammation ; 1 = chronic +/- slight active inflammation ; 2 = more severe active inflammation with crypt distortion or crypt abscess formation ; 3 = severe active inflammation with ulceration. The presence of granulomas did not influence the score (16,17).

3 : A scoring system using different topics, scored independently (18) (see Table III).

The different scoring systems have however not yet been validated. Furthermore, definitions for improvement and remission are still unclear.

Analysis of scoring systems

Many of the features used in scoring systems are continuous spectra, e.g. chronic inflammation assessed from no increase to marked increase, but are divided into discrete groups. While they have a numerically labelled

Table III. — **Scoring system according to D'Haens *et al.* for Crohn's disease (18)**

Histologic variable	grading
1 : epithelial damage	0 = normal ; 1 = focal ; 2 = extensive
2 : architectural changes	0 = normal ; 1 = moderate (> 50%) 2 = severe (> 50%)
3 : mononuclear cells in lamina propria	0 = normal ; 1 = moderate increase 2 = severe increase
4 : polymorphonuclear cells in lamina propria	0 = normal ; 1 = moderate increase 2 = severe increase
5 : neutrophils in epithelium	1 = surface epithelium ; 2 = cryptitis 3 = crypt abscess
6 : erosion or ulceration	0 = no ; 1 = yes
7 : granuloma	0 = no ; 1 = yes
8 : number of biopsies affected (total : n = 6 or more	0 = none ; 1 = > 33% 2 = 33-66% ; 3 = > 66%

Each variable is scored independently. The total score is the sum of all individual scores (max = 16).

order, the distance between adjacent numbers will not be the same through the whole range. The consequence is that these grades should not be assessed in processes which require continuous variables. The most appropriate way for an analysis seems pairwise comparisons (before and after treatment) or by means of histograms (19).

Number of biopsies

The appropriate number of biopsies which need to be examined has not been established. For UC, usually one single rectal biopsy is examined. We propose to study two samples because it has been shown that the inflammation in UC can become patchy (20).

For CD, obviously there is a need for multiple samples, preferentially from different sites including the ileum.

Results

Using scoring systems, it has been shown that treatment with immunosuppressive / immunomodulatory agents can induce a decrease of microscopic disease activity and even mucosal healing in both UC and CD. In UC, intravenous cyclosporine 4 mg/kg/day can induce endoscopic and histologic improvement which parallels clinical improvement.

In patients with adult CD and using a score with a maximum of 16, a decrease was observed of a mean of 13 at entry to 7 at completion using azathioprine after several months and a drop from 8.8 +/- 1.7 (range 2-10) to 2.7 +/- 1.7 (range 0-8) at 4 weeks using infliximab. Variable results have been observed with other newly introduced or tested drugs.

Medical treatment and other markers

Several studies have shown that increased expression of HLA-DR, MHC class II molecules in UC is reduced or disappears following different types of treatment such as corticosteroids, 5-ASA (21). Similar results are noted in CD following treatment with cyclosporine, antibodies directed against CD4 lymphocytes and antibodies against TNFalfa (21,22). In general treatment with infliximab induces a profound down regulation of the inflammation in Crohn's ileocolitis, including a reduction of the expression of ICAM-1 on endothelial cells and of ICAM-1 and LFA-1 on immune competent cells (22).

We have also shown that infliximab decreases mucosal lymphocyte proliferation in CD, whereas epithelial cell proliferation remains largely unchanged (23,24).

Conclusion

Histology can be used for the assessment of disease activity in IBD because it has been clearly shown that medical treatment has an influence upon the microscopic features, characteristic for both UC and CD. It implies the use of multiple biopsy samples (two in UC ? ; multiple from different segments in CD). It implies also the use of scoring systems. These should be validated. For clinicians and pathologists it is important to realize that drugs have an effect upon histology. This effect can be used for assessment of disease activity but which can also confuse diagnosis (25).

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