DOI:10.1046/j.1365-2125.2003.01981.x

Clinical research methods in gastroenterology, edited by M. M. Walker and B. J. R. Whittle.

Biopsy assessment of drug efficacy in the gastrointestinal tract

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When using biopsy pathology in clinical pharmacology to assess drug efficacy in the gastrointestinal tract, a number of questions must be answered: Is the biopsy necessary or more effective than macroscopic views by endoscopy? Can we extract maximal information from the specimen? Are there surrogate serum or other markers that give an overall measure of disease and/or improvement? Indeed, clinicopathological correlation is of paramount importance. If biopsy is to be used, it is important to utilize appropriate scoring systems. Many grading systems use continuous spectra, which are ordinal categorical variables and therefore a grading system of assigned 'numbers' which cannot be used in processes that require continuous variables such as linear regression. The use of grading *vs* a 'true' score with real numbers must be carefully considered, the site and number of biopsies must be precisely chosen and interobserver reproducibility of results evaluated before undertaking drug trials. Immunocytochemistry and *in situ* hybridization, however, can provide quantifiable molecular information related to mechanisms of drug action. The biopsy is of significant value as it is a true *in vivo* assessment if the above caveats are taken into account. However, further work is needed to determine sound histological criteria to assess the efficacy of drugs for use in gastrointestinal disease.

Keywords: biopsy, drug, gastrointestinal, histopathology

Introduction

In clinical practice, biopsies of the gastrointestinal tract are taken to make a diagnosis, consider progression or remission of disease and sometimes, to ascertain why, despite therapy, symptoms persist. It is given practice that any lesion that is a possible cancer must be biopsied to make or exclude this diagnosis.

In assessing drug efficacy, most biopsies are performed to establish resolution of inflammation or the consequences of the drug on the mucosa. When using a biopsy there are major issues to consider, which are: when and where it is appropriate to biopsy to obtain maximum information and how to evaluate success of a therapeutic agent using a correct method of visual assessment of the baseline and post-treatment biopsies. However, the use of histopathology as an endpoint has limitations, namely size and site (is the biopsy representative of the disease?), consistency in sampling sites (pre- and post-treatment), difficulties in handling and processing (adequate fixation, orientation, number of levels to examine), the validity of different grading systems, appropriate use of immunocytochemistry for cell function and intra- and interobserver differences of interpretation. Biopsy is also costly and hence the economic efficiency of this technique must be taken into account.

Many grading systems of inflammation are flawed in certain situations and their use should be considered carefully before use in Phase II or III studies. Whilst a specific diagnostic label is helpful, grading systems have been developed to allow clinicians to make decisions regarding prognosis and treatment of certain diseases and care should be given to choice of any system adopted in clinical trials. There may be no appreciation of the difference between real numbers and the ordinal categorical numeric labels. Many grading systems use continuous spectra, for example, in chronic inflammation, where no increase to a marked increase may be recorded and divided into discrete groups (none, mild, moderate or severe and assigned grades 0–3). These are ordinal categorical variables rather than continuous real numbers, i.e. they have a numerically labelled order but the distance between the adjacent numbers will not be the same through the whole range and there are no noninteger values [1, 2]. Thus these grades cannot be used in processes that require continuous variables such as linear

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Received 17 July 2003, accepted 5 August 2003.

regression, a technique in which a straight line is fitted to a set of data points to measure the effect of a single independent variable, the slope of the line being the measured impact of that variable. The use of grading *vs* a 'true' score with real numbers must therefore be carefully considered before undertaking drug trials. These points will be discussed in the use of the biopsy in evaluation of drug efficacy in the gastrointestinal tract and also the use of biopsy in conjunction with other indicators such as clinical, serological and other surrogate markers of disease.

Oesophagus

Biopsies of the oesophagus are principally used to gauge effectiveness of acid suppression. Healing of inflammatory lesions can be measured macroscopically by endoscopy and there is excellent correlation between microscopic and macroscopic oesophagitis, particularly if erosions are present [3]. In nonerosive gastro-oesophageal reflux disease (GORD), routine biopsies cannot be recommended for the diagnosis in patients without visible oesophageal erosions as these add little to the evaluation of the disease process [4]. To identify reflux, it was concluded that oesophageal histology does not provide additional useful information over clinical assessment by endoscopy [5].

In contrast, although the identification of Barrett's oesophagus is by endoscopy, biopsy is required to establish which type of metaplastic epithelium is present. Barrett's oesophagus is an acquired condition resulting from GORD [6]. Current American guidelines cite 3 cm of the lower oesophagus must be involved and that intestinal metaplasia is present in the biopsy to make the diagnosis [7]. However, a less rigid approach to diagnosis exists in Europe and moreover, the need to take multiple biopsies to establish intestinal metaplasia can be problematic [8]. New endoscopy techniques using enhanced magnification can improve the accuracy of diagnosing intestinal metaplasia and eventually may, in time, overcome the need for multiple biopsies [9].

Profound suppression of acid secretion induces partial regression of Barrett's oesophagus [10]. However, caution must be exercised in sole reliance on endoscopy to recognize regression in treatment and surveillance as residual intestinal metaplasia may underlie squamous mucosa. Endoscopy with biopsies of the treated segment is therefore recommended after reversal therapy such as acid suppression [11].

Stomach

In contrast to the oesophagus, there is little correlation between grade of gastritis at histology and endoscopy appearances. A study from Philadelphia revealed no correlation among any of the histological features or of any one histological feature with any one endoscopic feature and concluded that a tissue diagnosis was essential for the proper diagnosis of gastritis [12]. However, histology is rarely used in the assessment of gastric damage by NSAIDs in clinical trials; the endpoint is most commonly endoscopy scores of visible erosions or surrogate markers such as mucosal prostaglandin assay [13].

In assessing gastritis, the updated Sydney system provides practical guidelines for optimal biopsy sampling of the stomach, uses visual analogue scales for grading the histopathological features, and provides a formula for comprehensive standardized diagnosis [14]. Gastric sampling is dependant upon what answer the clinician needs from the biopsy: to assess grade of gastritis biopsy is paramount, however, to assess *H. pylori* eradication there are better methods, for example, ^{13}C urea breath tests [15].

In nonulcer dyspepsia (NUD), efficacy of treatment is dependent on symptomatic relief and use of the biopsy to assess gastritis is controversial, mainly as this is a disease of unknown or more likely, differing causes. The bacterium, *H. pylori* has been implicated in NUD. Two large studies that address the issue of eradication and symptomatic relief were published in the same year but have contradictory results. In one, the outcome was that there was no association between the severity of symptoms at baseline and gastritis scores on initial biopsies. However at 12 months follow up, 41/127 patients (32%) with no or mild gastritis were treatment successes (no or minimal dyspepsia) compared with 21/123 patients (17%) with moderate or severe gastritis ($P = 0.008$) [16]. In the second, there was no significant association between treatment success and histological improvement in chronic gastritis at 12 months ($P = 0.68$) and there was no evidence that curing *H. pylori* infection in patients with NUD led to relief of symptoms [17]. These conflicting results may be due to the differing scoring systems used to assess gastritis. In the first study the Sydney system [13] was used, and in the second a nonvalidated system which gave a score of up to 5 for active and chronic inflammation. The Sydney system uses ordinal categorical labels 'mild, moderate, severe' – continuous spectra, nominally 0–3. The second system also used ordinal categorical labels but on a different scale – 0–5. In both studies histology was reassessed at 12 months, but even if eradication is successful, chronic inflammation will still be present in gastric mucosa. Although neutrophils clear from the tissue soon after therapy, chronic gastritis and lymphoid aggregates persist for at least up to one year or more [18]. This illustrates the problem of relating biopsy scores to symptom relief and opting for a timely endpoint to assess healing.

In the assessment of precancerous lesions in the stomach, such as intestinal metaplasia (IM) to determine

regression after therapeutic intervention, the problem is not grading of the lesion but in accurate sampling and consideration of the malignant potential. Epidemiological studies have shown that IM in the stomach has a high cancer risk and is therefore defined as a precancerous condition – a clinical state associated with a significantly increased risk of cancer [19]. The Sydney System for grading gastritis provides practical guidelines for optimal biopsy sampling of the stomach [13] but a study from Houston showed that IM was missed in more than 50% of biopsies from 'Sydney sites' in patients with confirmed IM on multiple site sampling, It was concluded that current and future studies that use the Sydney System as a basis for detecting IM are not likely to be reliable [20]. However the extent and location of IM – along the lesser curvature (from the cardia to the prepyloric zone) may identify patients with the highest cancer risk [21].

To assess risk of development of cancer in IM it has been considered that the histochemical profile of mucins may be important. However, a recent study has shown a high prevalence of type III IM in the general population (4%) and indicates that its role as a precursor of gastric carcinoma may have been overemphasized [22]. Critical reviews have found many exceptions to given types of IM as precursor lesions of cancer [23]. There is a need to separate 'dangerous' precursors in many precancerous lesions, and it is likely that molecular markers will eventually be useful. At present no validated studies have proven anything as yet as useful than simply noting the presence and extent of IM. The accuracy of endoscopic diagnosis in IM was shown to be 71.3% in a study from Taipei [24]. Another endoscopic method of evaluation is dye-endoscopy using methylene blue (methylthioninium chloride). This technique though described is not in widespread use. A Japanese study showed it was valuable in assessing regression of IM [25], however, time constraints of endoscopists may restrict this detailed type of examination.

Intervention studies in the prevention of gastric cancer in Columbia by eradication of *H. pylori* have evaluated gastric atrophy by conventional and morphometric measurements of on biopsy. No differences were noted between the different methods [26]. Atrophy can also be assessed by surrogate methods. The new serum markers amidated gastrin-17 (S-G17) and pepsinogen I (PGI) can identify atrophy: low serum levels of G-17 and PGI indicate atrophic antral and corpus gastritis, respectively. Thus a low S-G-17 is a sign of the multifocal or antrum-limited atrophic gastritis in patients infected with *H. pylori*. If this is coupled with serological markers of *H. pylori* infection, a single sample can identify gastric cancer risk [27, 28].

Whilst it is reassuring that a study looking at the performance of routine Sydney protocol biopsies in which there was no visible lesion at endoscopy failed to identify any neoplastic lesions [29], it is important to regularly biopsy gastric ulcers until healed to exclude malignancy. In an endoscopy study of 265 gastric ulcers, 37 proved to be malignant (14%), but there is a tendency to over-diagnose malignancy by endoscopy alone and repeated endoscopy and biopsy of all gastric ulcers until they are completely healed is advised [30]. In another study this advice was reinforced, showing that prior treatment with histamine H_2-receptor antagonists can mask cancer by promoting superficial and misleading mucosal healing [31].

Duodenum

Biopsy of the duodenum in routine practice is performed in the assessment of iron deficiency anaemia to exclude coeliac disease. There is good correlation (89%) with the appearance of endoscopic peptic duodenitis and histology [32], so biopsy is rarely warranted in the assessment of acid-related disease. In patients with nonulcer dyspepsia with symptoms of ulcer like pain, the correlation of histological and endoscopic active antroduodenitis has also been described [33]. Peptic duodenitis and gastric metaplasia (GM) occur in the bulb but are rare in the second part [34, 35]. However if a biopsy is considered necessary then it should be remembered that duodenitis is a stage of duodenal ulcer disease which may be focal and can be missed on one biopsy only. Two are a minimum requirement, the anterior wall and roof, and must be biopsied more than 10 mm distally from the pylorus to avoid sampling errors. This will detect GM in 95% of cases. Histological assessment of duodenitis should include a systematic examination of the biopsy for surface erosions, regeneration, intra-epithelial lymphocytes, neutrophils and pathogens. Gastric metaplasia is restricted to the surface epithelium, and is most easily detected using a PAS (periodic acid Schiff) stain to show mucin. In the lamina propria chronic inflammation is seen as a definitive increase in lymphocytes and neutrophils are abnormal [36].

The assessment of coeliac disease (CD) requires a biopsy for the initial diagnosis and biopsies must be taken from the distal duodenum and repeated if there is any doubt as to the diagnosis. Recent guidelines emphasize this practice [37]. The characteristic features are well-known, but latent disease can be a diagnostic problem. The histological features of fully established/untreated/relapsed CD are easily appreciated using histology; established villous atrophy can be recognized without difficulty and with crypt hyperplasia, is an indicator of CD [38]. More subtle and early changes are difficult to diagnose with confidence. There is a wide variation in villus height in the normal population and age has no effect

on this microscopic feature of CD [39]. The normal villous height : crypt depth ratio is 3 : 5 and the surface enterocyte height is normally 29–34 μm. These features are undisputed but an increase in intraepithelial lymphocytes (IELs) has raised considerable controversy. Normal ranges in the UK are cited as 10–30 IELs/100 enterocytes [38] whilst the quoted values in Europe are 40IELs/100 enterocytes [40]. However, a recent study [41] has defined the normal IEL count in the human duodenum as 25 IELs/100 epithelial cells. The significance of a raised IEL count without villous atrophy (not an uncommon finding) has been discussed [42] and it is suggested that this is a manifestation of latent coeliac disease [43] particularly if the IELs are γδ? T cells, which are important in inflammatory and autoimmune disease in the gut [44].

Other causes of a raised IEL count which are not related to coeliac disease are cows milk protein sensitivity [45], giardiasis (often the biopsy is normal) [46] IgA deficiency [47], tropical sprue [48] and postinfective malabsorption [49]. In coeliac patients with noncompliance to diet, the IEL count may rise [50]. Dermatitis herpetiformis is also another recognized disease with a raised IEL count as the first sign of intestinal damage in gluten challenge [51]. Thus in assessing duodenal biopsies for immune disorders, the IEL count itself is an important parameter.

Colon

In the evaluation of drug efficacy in the colon, endoscopically obtained biopsies have been used to determine drug effectiveness and mechanisms of action of new therapies, principally in inflammatory bowel disease (IBD). These can be assessed by clinicopathological scoring systems to assess pre- and post-treatment inflammation. Several systems are in use on both sides of the Atlantic. It is important to recognize the differing inflammatory conditions in the colon, as effective management depends on accurate disease classification.

The difficulties in diagnosis of IBD have been emphasized in a large series from Sheffield. Biopsy is good at distinguishing normal mucosa from inflamed mucosa but there are longstanding difficulties in distinguishing Crohn's disease and ulcerative colitis [52]. The British Society of Gastroenterology has published useful guidelines on biopsy diagnosis [53], while a practical review of histological patterns with clinical implications from the USA [54] emphasizes the differential diagnoses encountered in IBD. A review of morphological features of mucosal inflammation in IBD and treatment emphasizes the difficulties in monitoring Crohn's disease activity. The tissue involvement is typically patchy and sampling errors pose a problem. Multiple stepwise biopsies should be used and the score based on either the mean value or the worst lesion present. The key feature of activity for IBD is neutrophil infiltration in the epithelium and this can resolve rapidly on treatment. However, the overall celluarity in the lamina propria can take a considerable time to resolve. This review by Geboes & Dalle concludes that it is still not clear that histological healing is an appropriate endpoint in clinical trials [55].

There is therefore a need to develop other methods to assess the efficacy of treatment in IBD. Immunohistochemistry can delineate the type of inflammatory cell infiltrate in the mucosa and *in situ* hybridization can be used to assay expression of mRNA; these methodologies can also be used to study production of mucosal cytokines and adhesion molecules. Such an approach has, for example, been used in patients with Crohn's disease to evaluate the actions of the tumour necrosis factor α monoclonal antibody, infliximab. Severity of inflammation, mechanism of drug action and drug response have been assessed by both histological score and immunohistochemical staining with antibodies against HLA-DR, CD68, tumour necrosis factor α, intercellular adhesion molecule 1, lymphocyte function-associated antigen, CD4, CD8 and interleukins [56]. Biopsy can also demonstrate failure of drug action. Determining the nuclear concentrations of NFκ B p65 in colonic mucosal biopsy samples may reflect the response to infliximab. However, whilst treatment improved clinical symptoms in 88% of patients with Crohn's disease after 1 week, the response in some patients was short lived. Reactivation of the mucosal and the systemic immune system preceded clinical relapse and was predicted by the immunological response *in vitro* [57].

In addition to biopsy, there are surrogate markers that can predict relapse in IBD. Calprotectin is a calcium binding protein secreted by neutrophils and thus faecal excretion of this protein can be used as a substitute activity index to predict clinical relapse of disease activity in patients with Crohn's disease and ulcerative colitis [58]. This has also been used to distinguish organic and nonorganic disease in the colon. Thus, faecal calprotectin in conjunction with intestinal permeability and positive Rome I criteria, was shown to provide a safe and noninvasive means of helping differentiate between patients with organic and nonorganic intestinal disease [59].

A review of the activity indices and therapeutic endpoints used in clinical trials in Crohn's disease concluded that important progress has been made especially with activity indices. The shortfall was the definition of endoscopic and histological endpoints in complicated disease and the measurement of quality of life improvements. This review stresses the need for collaboration by academia and the pharmaceutical industry in this area [60].

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