

# Oesophageal histology in reflux oesophagitis

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**SUMMARY** Multiple specimens taken at oesophageal suction biopsy were obtained from 56 patients, of whom 44 had symptoms of gastro-oesophageal reflux and 24 had endoscopic evidence of erosive oesophagitis. Biopsies were examined independently by two histopathologists for the following criteria for reflux: epithelial hyperplasia, vascular dilatation and congestion, neutrophil infiltration, and eosinophil infiltration. The incidence of these criteria in patients with and without endoscopic evidence of oesophagitis or symptoms of reflux was investigated. It was concluded that vascular dilatation and epithelial hyperplasia, defined as basal zone thickness  $\geq 15\%$  and papillary elongation  $\geq 66\%$ , can be detected most reliably, but their diagnostic accuracy is limited unless multiple biopsies are examined.

Accurate assessment of excessive gastro-oesophageal reflux has proved difficult as symptoms of reflux may be absent or atypical and the endoscopic appearance of the oesophageal mucosa may be normal.<sup>1-3</sup> Recent reports have focused on the diagnostic value of prolonged monitoring of oesophageal pH, but this technique is technically difficult and time consuming.<sup>4,5</sup> An alternative approach has been to examine oesophageal mucosal biopsies for histological markers of abnormal reflux.

Different histological criteria have been proposed as indicators of abnormal reflux, and these include neutrophil<sup>6</sup> and eosinophil<sup>7</sup> infiltration, vascular dilatation,<sup>8</sup> and epithelial hyperplasia.<sup>9-11</sup> Gras biopsies taken during endoscopy are often crushed and tangential, so that histological assessment is difficult. The use of a Quinton suction or hydraulic tube biopsy instrument, however, has been recommended to obtain well orientated tissue,<sup>12</sup> and this technique was used to establish the criteria of epithelial hyperplasia for diagnosing abnormal gastro-oesophageal reflux.<sup>9-11</sup>

Few studies have assessed the practical value of these histological criteria in the routine assessment of patients with suspected reflux oesophagitis. We therefore documented the incidence of the different criteria in oesophageal suction biopsies from patients with reflux symptoms. Furthermore, to assess the reproducibility of a histological diagnosis we examined the agreement in diagnosis between two histopathologists when each was asked to assess

the oesophageal biopsies independently and without clinical information.

## Material and methods

Fifty six patients including 23 men (age range 18-75 years) were studied. Forty four presented with heartburn associated with regurgitation or dysphagia, or both, as their major complaints. Symptoms were graded according to the criteria of Demeester *et al*<sup>4</sup> before endoscopic examination or histological assessment was performed. The highest obtainable score was 9, representing a patient with heartburn that interfered with daily activities, episodes of pulmonary aspiration secondary to regurgitation, and dysphagia requiring admission to hospital for relief of meat impaction (Table 1). Patients were included in the study if they had a symptom score of 3 or more and at least two symptoms of reflux.

The remaining 12 patients presented with epigastric or midabdominal pain and denied any symptoms of reflux. None had any endoscopic abnormality of the oesophagus or any gastroduodenal abnormality other than mild gastritis. A record of cigarette and alcohol consumption was obtained from all patients.

A routine endoscopy was carried out on all patients using an Olympus GIF-D3 or GIF-Q endoscope. The distance between the oesophagogastric junction and the incisor teeth was carefully noted. When the endoscope was removed a Quinton suction biopsy instrument with a capsule containing four biopsy ports was inserted into the oesophagus.

Table 1 Symptoms of gastro-oesophageal reflux

Severity of symptoms	Grade	Degree of incapacity
<i>Heartburn: flow of gastric contents into oesophagus</i>		
None	0	No heartburn
Minimal	1	Occasional episodes
Moderate	2	Reason for medical visit
Severe	3	Interference with daily activities
<i>Regurgitation: flow of gastric contents into mouth</i>		
None	0	No regurgitation
Minimal	1	Occasional episodes
Moderate	2	Predictable on position or straining
Severe	3	Episodes of pulmonary aspiration
<i>Dysphagia</i>		
None	0	No dysphagia
Minimal	1	Occasional episodes
Moderate	2	Requires liquids to clear
Severe	3	Episodes of meat impaction requiring medical treatment

Value for heartburn plus regurgitation plus dysphagia = total symptomatic score.

This was positioned to obtain the biopsies about 5 cm above the oesophagogastric junction. Suction was applied with a 20 ml syringe and the biopsy knife fired manually. If only one or no biopsy was obtained the instrument was introduced a second time. Informed written consent was obtained from each patient before endoscopy, and all biopsies were taken in the routine assessment of oesophageal disease.

Biopsies were orientated carefully on gauze, fixed in 10% buffered formalin solution, sectioned at 5  $\mu$ m, and stained with haematoxylin and eosin. If sections were poorly orientated additional levels were examined. When all sections had been collected they were submitted in a randomised fashion and without endoscopic or clinical information for histological assessment, carried out independently by two histopathologists, to assess interobserver variation in diagnosis. Intraobserver variation was assessed by coding the biopsies and resubmitting them to the consultant pathologist.

#### HISTOLOGICAL ASSESSMENT

The following variables were assessed in each section:

- 1 Basal zone height, expressed as percentage of epithelial thickness.
- 2 Papillary length, expressed as percentage of epithelial thickness.
- 3 Dilatation of intraepithelial blood vessels.
- 4 Congestion of intraepithelial blood vessels.
- 5 The presence of neutrophils.
- 6 The presence of eosinophils.
- 7 The presence of lymphocyte aggregates.

The thickness of the basal cell zone was estimated using an eyepiece graticule in areas of the biopsy that showed perpendicular orientation of at least two consecutive papillae to the mucosal surface. If the basal zone thickness varied in any section the

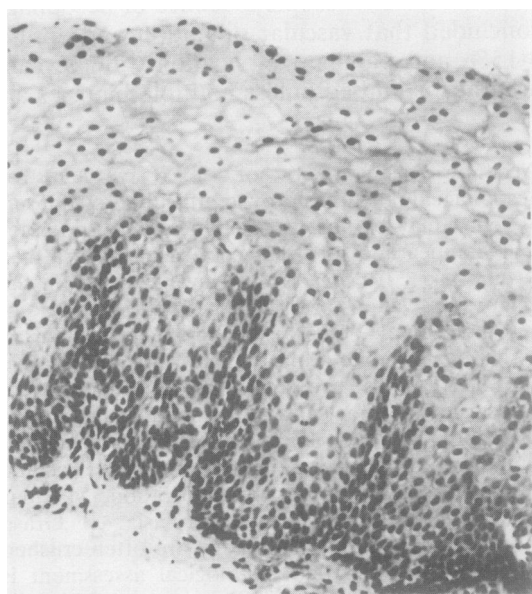


Fig. 1 Oesophageal suction biopsy from asymptomatic subject, showing thin basal zone layer and papillae less than 50% of epithelial thickness. Haematoxylin and eosin.  $\times 100$  (original magnification).

maximum value was recorded. Papillary length was also estimated in areas that showed perpendicular orientation of papillae to the mucosal surface, and maximum values were recorded (Figs. 1–3). Ismail-Beigi's criteria for epithelial hyperplasia were met if one or more biopsies showed a basal zone height  $\geq 15\%$  and in the same region of the biopsy a papillary length  $\geq 66\%$ . Behar and Sheahan described epithelial hyperplasia as the occurrence of basal zone height  $\geq 15\%$  and papillary length  $\geq 50\%$  in at least two biopsies. In several patients, however,

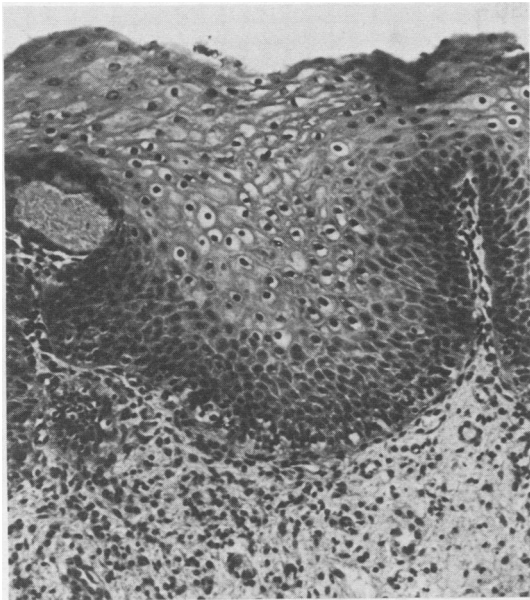


Fig. 2 Oesophageal suction biopsy from patient with erosive oesophagitis, showing considerable papillary elongation, mild basal zone hyperplasia, and dilated blood vessel. Haematoxylin and eosin.  $\times 80$  (original magnification).

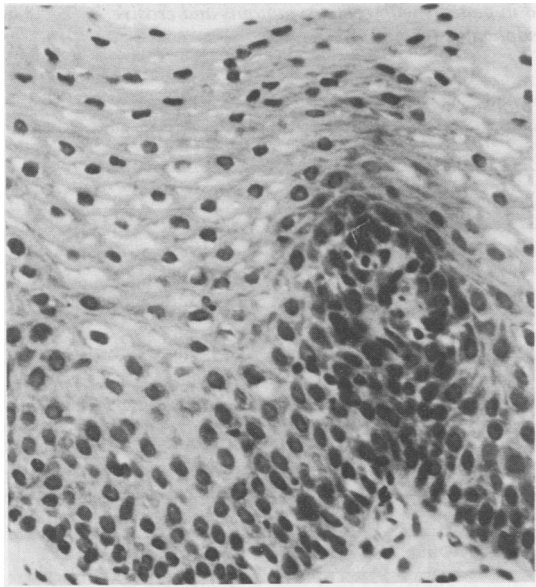


Fig. 3 Oesophageal suction biopsy from patient with erosive oesophagitis, showing mild papillary elongation and moderate basal zone hyperplasia. Haematoxylin and eosin.  $\times 200$  (original magnification).

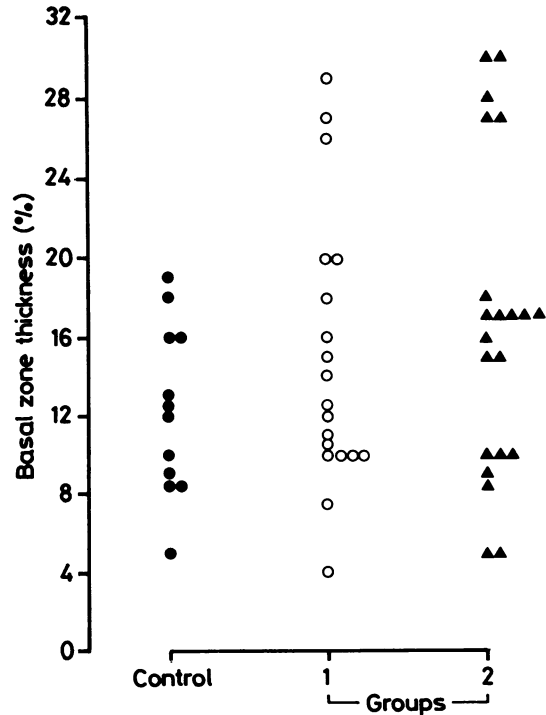


Fig. 4 Basal zone thickness (%) reported by consultant histopathologist in control subjects, in patients with reflux symptoms and normal endoscopic appearance (group 1), and in patients with reflux symptoms and erosive oesophagitis (group 2).

only one biopsy was adequately orientated for assessment, and we accepted this criterion if it was found in one or more biopsies.

Intraepithelial vessels of  $>50 \mu\text{m}$  diameter were arbitrarily classified as dilated (Fig. 2). The diameter of the largest vessel was recorded for each section. Measurements were made only on vessels seen in the well orientated sections of the biopsy and when they appeared to be in transverse section. Congestion of the vessels was diagnosed if large numbers of red cells were seen in the vessel lumen. This was a subjective assessment by each histopathologist. A careful search for intraepithelial and subepithelial neutrophils, eosinophils, and aggregates of lymphocytes was made on each section.

STATISTICAL ANALYSIS

A comparison of maximum basal zone height and papillary length between patients with and without reflux symptoms was made using the Mann-Whitney U test from data provided by the consultant histopathologist. The incidence of the different histological criteria for diagnosing reflux oesophagitis was compared between groups by using the  $\chi^2$  test.

## Results

Patients were divided into three groups on the basis of their symptoms and endoscopic examination, and the histological findings in each group were compared. The 12 patients with no symptoms of reflux and a normal endoscopic appearance were assigned to the control group. Of the 44 patients with symptoms of reflux, 20 had no endoscopic abnormality of the oesophageal mucosa. These were designated group 1. The remaining 24 patients with symptoms of reflux had erosions and friability of the oesophageal mucosa and were considered to have definite reflux oesophagitis. They were designated group 2.

One specimen only was obtained from eight of the 56 patients biopsied. Although two or more biopsies were obtained from the remaining 48 patients, only one biopsy was adequately orientated for full assessment in 14 patients (five from group 1, six from group 2, and three from the control group). All the biopsies were poorly orientated in four patients, so that full histological assessment was impossible, and these were excluded from further analysis. Thus one or more biopsies were assessed from 52 patients, of whom 12 were control patients, 19 from group 1, and 21 from group 2. Two or more biopsies were assessed from 30 patients, of whom seven were control patients, 12 from group 1, and 11 from group 2.

A wide range of basal zone heights and papillary

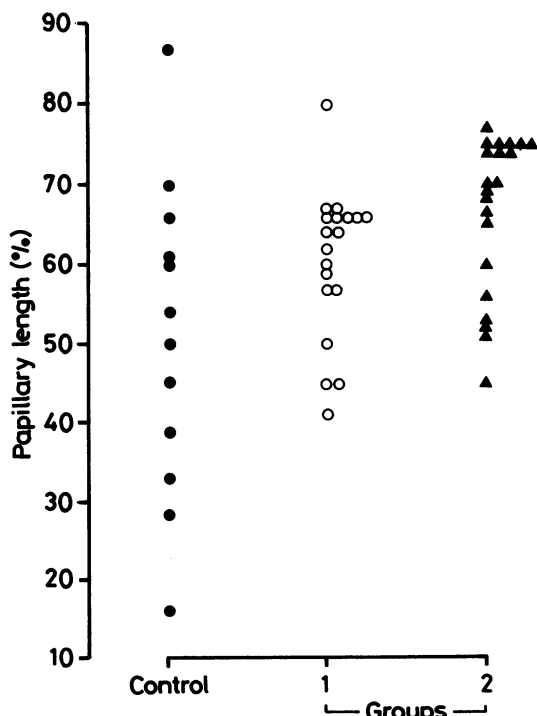


Fig. 5 Papillary length (%) reported by consultant histopathologist in control subjects, in patients with reflux symptoms and normal endoscopic appearance (group 1), and in patients with reflux symptoms and erosive oesophagitis (group 2).

Table 2 Histological findings in patients for whom one or more biopsies was examined

Diagnostic criteria	No. (%) of patients		
	Controls (n = 12)	Group 1 (n = 19)	Group 2 (n = 21)
Basal zone height $\geq$ 15%	4 (33)	8 (42)	14 (67)
Papillary length $\geq$ 50%	7 (58)	16 (84)	20 (95)
Papillary length $\geq$ 66%	3 (25)	8 (42)	14 (67)
Behar and Sheahan criteria	3 (25)	7 (37)	14 (67)
Ismail-Beigi criteria	2 (17)	6 (32)	11 (52)
Eosinophils	3 (25)	5 (29)	11 (52)
Neutrophils	1 (8)	4 (21)	8 (38)
Lymphocyte aggregates	11 (92)	14 (74)	16 (76)
Dilated vascular channels	4 (33)	9 (47)	15 (71)
Congested vascular channels	5 (42)	11 (58)	16 (76)

Table 3 Histological findings in patients for whom two or more biopsies were examined

Diagnostic criteria	No. (%) of patients		
	Controls (n = 7)	Group 1 (n = 12)	Group 2 (n = 11)
Behar and Sheahan criteria	1 (14)	3 (25)	9 (82)
Ismail Beigi criteria	0	2 (17)	7 (64)
Eosinophils	2 (29)	2 (17)	7 (64)
Neutrophils	0	3 (25)	5 (45)
Lymphocyte aggregates	6 (86)	10 (83)	9 (82)
Dilated vascular channels	2 (29)	9 (75)	9 (82)

Table 4 Influence of cigarettes and alcohol on incidence of hyperplastic epithelial changes in oesophageal biopsies (figures are numbers (%) of patients)

Reflux criteria	Cigarette consumption		Alcohol consumption	
	Smokers (n = 9)	Non-smokers (n = 31)	Drinkers (n = 16)	Non-drinkers (n = 24)
Ismail-Beigi	4 (44)	13 (42)	9 (56)	8 (33)
Behar and Sheahan	5 (56)	16 (52)	11 (69)	10 (42)

Table 5 Percentage agreement in diagnosis between pathologists for each histological criterion

Diagnostic criteria	Controls	Group 1	Group 2	Total
Ismail-Beigi criteria	75	71	84	77
Behar and Sheahan criteria	58	53	84	67
Dilated vessels	83	71	90	81
Neutrophils	92	85	67	79
Eosinophils	83	75	57	70

Table 6 Mean percentage values for basal zone height and papillary length recorded by each pathologist

	Basal zone height		Papillary length	
	Pathologist 1	Pathologist 2	Pathologist 1	Pathologist 2
Controls	12.3	22.5	50.8	62.4
Group 1	14.7	26.3	59.1	64.5
Group 2	17.3	29.4	66.8	72.5

lengths were recorded for each group (Figs. 4 and 5). No significant difference was detected for basal zone height between patients in the control group and those in groups 1 or 2. Patients in group 2, however, had longer papillae than either those in group 1 ( $p < 0.05$ ) or those in the control group ( $p < 0.01$ ).

Table 2 details the incidence of the criteria of epithelial hyperplasia described by Ismail-Beigi and by Behar and Sheahan and the other criteria of excessive reflux detected by the consultant histopathologist. A higher diagnostic sensitivity was noted for most of the criteria when only data from patients who had multiple biopsies suitable for assessment were analysed (Table 3).

Of the patients with two or more biopsies available for histological assessment (Table 3), those with erosive oesophagitis had a higher incidence of the criteria of epithelial hyperplasia described by Behar and Sheahan ( $p < 0.05$ ) and by Ismail-Beigi ( $p < 0.05$ ) than the control group ( $\chi^2$  test). Dilated intraepithelial blood vessels were found more commonly in the patients with erosive oesophagitis than in the control group, but this difference did not reach significance ( $0.05 < p < 0.10$ );  $\chi^2$  test. No significant differences were detected for the other criteria. When patients in whom only one biopsy was available for histological assessment were included in the analysis differences between patients with erosive oesophagitis and control patients were less

pronounced (Table 2). Only the criteria of Behar and Sheahan showed a significantly higher incidence in the group with erosive oesophagitis than in the control group ( $p < 0.05$ ;  $\chi^2$  test).

Patients in groups 1 and 2 were combined to permit an assessment of the influence of smoking and alcohol on hyperplastic epithelial changes in suspected or definite reflux oesophagitis. No significant difference in the detection of these changes between smokers and non-smokers, or between drinkers and non-drinkers, was observed ( $\chi^2$  test). Epithelial hyperplasia was more common in patients who drank alcohol, but this did not reach significance ( $\chi^2$  test) (Table 4).

Table 5 details the agreement between the two histopathologists on the detection of histological criteria. The main area of disagreement was in the reporting of basal zone height: higher values were consistently recorded by one observer (Table 6).

Reassessment of coded biopsies from 43 patients according to the criteria of Ismail-Beigi by one of the pathologists, who did not know the results of his previous diagnosis, showed that the same diagnosis was made in 39 cases, representing an agreement of 91%.

## Discussion

This study examined the value of the Quinton suction biopsy instrument in obtaining adequate oesophageal mucosal biopsies. At least one biopsy

was well orientated in 52 out of 56 patients, so that the criteria for epithelial hyperplasia could be evaluated. Difficulty was experienced in obtaining biopsies from some patients with erosive oesophagitis, and reinsertion of the instrument was required. Mucus and blood, or air rising from the stomach, were probably factors that interfered with the biopsy technique. Histological assessment is not, however, essential to establish a diagnosis in such patients, and grasp biopsies under direct vision may be more appropriate to assess complicating lesions such as dysplasia and Barrett's metaplasia.

Routine processing of suction biopsies resulted in more inadequately orientated specimens than we had expected. We recommend, therefore, that preferably four or five suction biopsies should be taken from each patient to ensure that two or more specimens will be adequately orientated for histological assessment. We would probably have shown a greater sensitivity of criteria of histological reflux if we had obtained more samples from each patient. Our results, however, serve to illustrate the likely diagnostic yield that would result if the current recommendation of at least two biopsies from each patient was followed in routine clinical practice.

New criteria for the diagnosis of reflux oesophagitis cannot be derived from the data in this investigation as the presence or absence of abnormal gastro-oesophageal reflux was not formally tested. Possibly, a few of our control patients were "refluxers," presenting with atypical abdominal symptoms. The definition of a perfect control subject remains difficult, however, even when prolonged monitoring of oesophageal pH is used, as most asymptomatic volunteers show occasional episodes of gastro-oesophageal reflux.<sup>13</sup> None the less, further assessment of the specificity of histological criteria for reflux will require studies of asymptomatic subjects who have been shown to have normal reflux patterns during pH monitoring.

Demeester *et al* carried out prolonged pH monitoring in over 100 patients with symptoms of reflux and erosive oesophagitis and found abnormal gastro-oesophageal reflux in 90%.<sup>14</sup> Thus it is worth assessing the sensitivity of different histological criteria in the diagnosis of reflux oesophagitis from biopsy findings in patients of group 2. Patients with symptoms of reflux but a normal endoscopic appearance are more difficult to categorise, as Demeester *et al* found abnormal reflux in only 55% of similar patients.<sup>14</sup>

Before 1970 the histological diagnosis of oesophagitis rested on the presence of lymphocytes and neutrophils.<sup>6</sup> In this study subepithelial accumulation of lymphocytes was observed in most biopsy specimens, including those from all but one of the

control patients. This finding supports the view of other investigators that these cells do not signal oesophageal inflammation.<sup>9</sup> Subepithelial neutrophils were detected in one control patient and in eight patients with erosive oesophagitis (38%). This observation is in keeping with the consensus that neutrophil infiltration in blind oesophageal biopsies is a specific but relatively insensitive marker for oesophagitis.<sup>15</sup> A much higher yield would probably be obtained in endoscopic biopsies taken under direct vision from the margin of oesophageal erosions.

Winter *et al* recently recommended that intraepithelial eosinophils should be a specific diagnostic criterion for reflux oesophagitis.<sup>7</sup> Most of their patients were aged under 5 years, and only three asymptomatic control subjects were biopsied. Eosinophils were detected in only 52% of our patients with erosive oesophagitis, and this low sensitivity limits the diagnostic value. As these cells were also found in three of 12 control biopsies further evaluation of the specificity of this criterion is required.

Hyperplastic epithelial changes have been most widely accepted as histological criteria for the diagnosis of excessive reflux. Formal evaluation of these criteria, however, has been undertaken in only a few centres, and one major report failed to confirm their diagnostic value.<sup>15</sup> Different methods of assessing basal zone height and papillary length were used in different studies, and some investigators applied detailed but time consuming morphometric measurements.<sup>15</sup> In our study considerable variation in basal zone height and papillary length was frequently observed in the same biopsy specimen. As macroscopic oesophageal mucosal damage is often focal in its distribution we considered it appropriate to report basal zone and papillary dimensions in the most abnormal region of each biopsy. This approach, which was also used by Ismail-Beigi and colleagues,<sup>9</sup> permitted a rapid assessment of each specimen.

Our finding of increased papillary length in patients with erosive oesophagitis agrees with other reports. Considerable overlap with normal values, however, was observed, and the diagnostic value of this feature alone was limited. Only 52% of patients with erosive oesophagitis satisfied the criteria of Ismail-Beigi, 67% fulfilling the less rigorous features described by Behar and Sheahan. The relatively low sensitivity of these criteria in this study is disappointing, especially considering that only the most abnormal appearances were reported for each biopsy. Some improvement in the sensitivity of these criteria of reflux was noted when we examined only data from patients in whom at least two well orien-

tated biopsies were obtained. This observation again highlights the importance of taking sufficient biopsies so that multiple specimens are available for histological assessment.

As Ismail-Beigi and Behar and Sheahan derived their criteria from studies of predominantly male patients in Veterans Administration hospitals<sup>9, 11</sup> excessive cigarette or alcohol consumption might possibly have influenced the histological appearances. Our finding of more abnormal biopsies in patients who drank alcohol is interesting, especially as no patient was a heavy drinker (>60 g alcohol/day), and further assessment of this relation is required.

The site of biopsy may also be important. Our biopsies were taken 5 cm proximal to the oesophagogastric junction, and although Ismail-Beigi and Pope reported random distribution of "reflux" lesions over the distal 8 cm of the oesophagus,<sup>10</sup> possibly biopsies taken closer to this junction would show more histological abnormality in patients with reflux. Weinstein reported epithelial hyperplasia in biopsies from asymptomatic subjects taken within 2 cm of the oesophagogastric junction.<sup>16</sup> Thus the specificity of these criteria may be impaired if more distal biopsies are taken.

Dilated and congested vessels have been described in oesophageal biopsies from patients with reflux oesophagitis and those with oesophageal varices.<sup>8, 17</sup> A trend towards vessel dilatation being more common in patients with erosive oesophagitis was observed in this study, but four of 12 control patients had similar abnormalities. Possibly, slight dilatation of intraepithelial vessels occurs when blood is squeezed into the oesophageal epithelium during the biopsy procedure, or as a reaction to the preceding endoscopic examination. It would be interesting to evaluate further this criterion in biopsies from control subjects.

If any histological criterion is to find wide acceptance for routinely diagnosing excessive reflux it is important for it to be recognised accurately by the histopathologist. Our observations of the independent reporting of the same biopsies by two pathologists showed fairly good agreement in the interpretation of biopsies. Vascular dilatation was an easily recognised phenomenon, and over 80% of biopsies were classified in the same way by the two pathologists.

The principal area of disagreement was the measurement of basal zone height, and, as a result, Behar and Sheahan's criteria of epithelial hyperplasia provided the most difficulty, with only 67% of patients being classified in the same way. As papillary length >50% was observed in most patients, irrespective of symptoms, measurements of basal zone height were

the deciding factor for this histological marker. Periodic acid Schiff staining has been used to aid definition of the basal zone layer in oesophageal biopsies,<sup>18</sup> but in some preliminary studies we found no advantage with this stain, and other investigators have been similarly disappointed.<sup>19</sup>

Agreement between the pathologists using the criteria of Ismail-Beigi was better: 77% of patients were classified in the same way. Here, the more objectively defined measurements of papillary length were the major determinant of the presence or absence of this histological marker. Furthermore, when one pathologist re-examined biopsies from 43 of these patients he made the same diagnosis, using Ismail-Beigi's criteria, in 90% of them.

We conclude that the suction biopsy instrument provides satisfactory well orientated tissue samples for histological assessment, although in some patients it is difficult to obtain multiple biopsies. The accuracy of histological diagnosis of reflux oesophagitis seems to be limited unless multiple biopsies are examined. No totally reliable diagnostic criteria have emerged, and the established criteria are not detected in all patients with oesophagitis, even when multiple biopsies are examined. Vascular dilatation and the criteria of Ismail-Beigi can be recognised fairly easily in biopsy specimens, but further assessment of the relevance of vascular dilatation is required. As Ismail-Beigi's criteria are more easily detected by different pathologists than those of Behar and Sheahan we suggest that they are most suitable for the routine diagnostic assessment of oesophageal biopsies by a general histopathologist.

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