Histological changes in the oesophageal squamous mucosa: correlation with ambulatory 24 hour pH monitoring

D J Bowrey, G T Williams, G W B Clark

Aims: To determine the value of squamous mucosal histology in the assessment of patients with gastro-oesophageal reflux symptoms.

Methods: Sixty six patients with reflux symptoms underwent endoscopy with oesophageal biopsy, manometry, and 24 hour oesophageal pH testing. The following histological features were assessed in squamous mucosa: the degree of basal cell hyperplasia, the degree of papillary zone elongation, and the density of neutrophil and eosinophil infiltration. Comparisons were made between the histological findings and the oesophageal function tests.

Results: The correlation between the traditionally accepted histological markers of gastro-oesophageal reflux disease in squamous mucosa and 24 hour pH testing was predominantly negative, with the exception of neutrophil inflammation in the squamous mucosa of patients with complicated reflux disease.

Conclusions: This study was unable to confirm the value of the Ismail-Beigi criteria as histological markers of acid reflux. By inference, biopsy of the oesophageal squamous mucosa is of limited value in the assessment of patients with reflux symptoms.

Endoscopy
Endoscopy was performed in a standardised fashion, with visualisation of the oesophagus, stomach, and duodenum. A hiatal hernia was defined by a ≥ 2 cm disparity between the location of the crural impression and the location of the top of the gastric rugal folds. The presence of oesophagitis was identified and graded according to the Savary-Miller classification. A positive pH test but absence of erosive oesophagitis was termed uncomplicated reflux disease, whereas complicated reflux disease was defined as a positive pH test and the presence of either erosion, stricture, or Barrett’s oesophagus. Biopsies of the squamous oesophagus, including those patients with Barrett’s oesophagus, were obtained from a location 2 cm proximal to the squamo-columnar junction.

Histology
Biopsy specimens were fixed in 4% buffered formalin and 4 μm thick paraffin wax embedded sections were stained with haematoxylin and eosin and reviewed by a single pathologist who was blinded to the endoscopic findings. The following parameters were assessed:

1. The degree of basal cell hyperplasia, expressed as a percentage of epithelial thickness: none (0–15%), mild (16–33%), moderate (34–67%), and severe (>67%).
2. The presence or absence of papillary zone elongation, determined by calculating papillary length as a percentage of epithelial thickness: absent (0–67%) and present (> 67%).
3. The density of neutrophil and eosinophil infiltration: none (0/high power field), mild (1–2/high power field), moderate (3–10/high power field), and severe (> 10/high power field).

The area of one high power field was 0.229 mm².

When there was disparity between biopsies, the score for the most abnormal area was recorded.
Manometry and 24 hour pH studies

Oesophageal manometry was performed using a five channel, water perfused polyethylene catheter. For each of the five channels, measurements of the lower oesophageal sphincter total length, abdominal length, and the mean resting pressure at the respiratory inversion point were made. The averages of these values were used. Oesophageal peristaltic function was assessed by placing the catheter so that the pressure transducers were located 1, 6, 11, 16, and 21 cm below the distal border of the upper oesophageal sphincter. Eleven water swallows, each of 5 ml, were given to the patient, allowing a 30 second interval between each. Manometric oesophageal length was defined as the distance between the distal border of the upper oesophageal sphincter and the proximal border of the lower oesophageal sphincter.

Following calibration to pH 1 and pH 7, an antimony pH electrode was positioned 5 cm above the proximal border of the lower oesophageal sphincter. pH measurements were recorded on a microdigitrapper (Synectics, Middlesex, UK) and downloaded upon completion of the study to a desktop computer for automated analysis using the Gastrosoft software (Synectics).

During the study period, subjects were asked to consume two meals and to refrain from taking carbonated beverages, citrus fruits or their juices, alcohol, and coffee. Patients were instructed to remain in the upright position during the daytime and to assume the recumbent position only upon retiring to bed.

Twenty four hour pH data were recorded as the percentage time spent at pH < 4, including all time periods within the analysis. Increased oesophageal acid exposure was defined by a total time oesophageal pH < 4 of 4.4%.

Statistics

Non-parametric statistical tests were used, the Mann-Whitney U-test and Kruskal-Wallis test for continuous data, with comparison of proportions by the $\chi^2$ test. Significance was assumed at the 5% level.

RESULTS

The endoscopic findings were grade 0 oesophagitis in 39 patients, grades I–III oesophagitis in nine patients, grade IV oesophagitis in four patients, and grade V oesophagitis (Barrett's oesophagus) in 14 patients. Table 1 compares the characteristics of these 66 patients who underwent manometry and pH testing with those of the 84 patients from the original study cohort of 150 who did not undergo these investigations (see above). In all respects, the two groups were similar, indicating that a representative sample had been obtained.

Figure 1 shows that patients with complicated reflux disease had a higher frequency of severe neutrophil inflammation (nine of 21) compared with those with uncomplicated GORD (UncomplGORD) and patients without reflux (Negative pH).

Table 1: Characteristics of 66 study patients compared with the 84 patients not undergoing oesophageal manometry and 24 hour pH testing

<table>
<thead>
<tr>
<th></th>
<th>Study patients (n=66)</th>
<th>Non-studied patients (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (range)</td>
<td>57 (21–80)</td>
<td>56 (22–85)</td>
</tr>
<tr>
<td>Sex ratio (male:female)</td>
<td>42:24</td>
<td>48:36</td>
</tr>
<tr>
<td>Grade of oesophagitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>39 (59%)</td>
<td>50 (60%)</td>
</tr>
<tr>
<td>1–3</td>
<td>9 (14%)</td>
<td>21 (25%)</td>
</tr>
<tr>
<td>4</td>
<td>4 (6%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>5</td>
<td>14 (21%)</td>
<td>10 (12%)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of squamous mucosal histological abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Negative pH score</th>
<th>Uncomplicated reflux disease</th>
<th>Complicated reflux disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cell hyperplasia (n=61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10 (43%)</td>
<td>10 (56%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Mild</td>
<td>10 (43%)</td>
<td>6 (33%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (4%)</td>
<td>1 (6%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Severe</td>
<td>2 (5%)</td>
<td>1 (6%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Papillary zone lengthening (n=51)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>13 (72%)</td>
<td>14 (87%)</td>
<td>12 (71%)</td>
</tr>
<tr>
<td>Present</td>
<td>5 (28%)</td>
<td>2 (13%)</td>
<td>5 (29%)</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>16</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 3: Relation between severity of neutrophil inflammation in squamous mucosa and lower oesophageal sphincter/oesophageal body characteristics

<table>
<thead>
<tr>
<th></th>
<th>None (n=28)</th>
<th>Mild (n=12)</th>
<th>Moderate (n=11)</th>
<th>Severe (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS pressure (mm Hg)</td>
<td>11 (7–18)</td>
<td>10 (5–18)</td>
<td>10 (4–25)</td>
<td>5 (1–10)*</td>
</tr>
<tr>
<td>Contraction amplitude 6 cm</td>
<td>34 (22–55)</td>
<td>21 (20–31)†</td>
<td>49 (24–71)</td>
<td>18 (12–28)‡</td>
</tr>
<tr>
<td>Contraction amplitude 11 cm</td>
<td>64 (37–82)</td>
<td>52 (39–75)</td>
<td>60 (36–86)</td>
<td>31 (21–53)§</td>
</tr>
<tr>
<td>Contraction amplitude 16 cm</td>
<td>65 (43–91)</td>
<td>80 (43–120)</td>
<td>90 (43–118)</td>
<td>32 (18–43)¶</td>
</tr>
</tbody>
</table>

The contraction amplitude refers to the oesophageal contraction amplitudes 6, 11, and 16 cm below the upper oesophageal sphincter. *p<0.01 severe v none; †p<0.05 mild v none and v moderate; ‡p<0.01 severe v none and v moderate; §p<0.01 severe v none and p<0.05 severe v moderate; ¶p<0.01 severe v all other groups.

LOS, lower oesophageal sphincter.
reflux disease (none of 18; p < 0.01) and those without reflux (two of 23; p < 0.05). No differences were seen between the severity of neutrophil inflammation in patients with negative pH studies and those with uncomplicated reflux disease.

There was no significant association between the indices of basal cell thickening or papillary zone elongation and reflux disease (table 2), although there was a trend for moderate or severe basal cell hyperplasia to be more common in complicated reflux disease than in subjects with a negative pH study or those with uncomplicated reflux (30% vs 9% and 12%, respectively).

Eosinophils were not identified in the sections. Severe neutrophil inflammation was associated with reductions in lower oesophageal sphincter pressure and contraction amplitudes throughout the smooth muscle portion of the oesophagus (table 3). As the intensity of neutrophil inflammation in squamous mucosa increased, the abdominal length of the lower oesophageal sphincter (fig 2) and the manometric oesophageal length fell progressively (fig 3). Figure 4A–C shows histological examples.

DISCUSSION
The correlation between traditionally accepted markers of inflammation in squamous mucosa and acid reflux, as measured by pH monitoring, was predominantly negative in our study. Only neutrophil infiltration in squamous mucosa in patients with endoscopically visible oesophagitis correlated with the severity of reflux. By definition, this implies that in non-erosive reflux disease biopsy is unreliable, and underscores the importance of 24 hour oesophageal pH testing.

In 1970, Ismail-Beigi and colleagues published their much cited study evaluating the squamous mucosa of patients with symptoms of GORD. Based upon their observations in 33
subjects with GORD symptoms and 21 healthy volunteers, the investigators concluded that the normal thickness of the basal zone should be less than 15% of the entire thickness of the squamous epithelium, that the papillae should extend for less than two thirds of the distance to the surface epithelium, and that the squamous epithelium should not contain neutrophils. These criteria were soon afterwards revalidated, and have since been widely used as histological markers for GORD.

The findings of more recent studies have generally been less impressive than the findings of earlier work, possibly because the earlier reports included a high proportion of patients with erosive GORD. Based upon all published reports, basal zone thickening has been noted in 15–95% of patients with GORD and 6–40% of controls (median values, 56% v 14%); papillary zone thickening in 21–98% of patients with GORD and 6–47% of controls (median values, 50% v 14%); and neutrophil infiltration in 18–30% of patients with GORD and 0–8% of controls (median values, 23% v 0%). In our study, we did not look for intraepithelial balloon cells, although they have been reported to be another putative histological marker of oesophagitis.

In summary, we were unable to confirm the value of histological changes in the oesophageal squamous mucosa as markers of gastro-oesophageal reflux in patients with non-erosive reflux disease. Therefore, biopsy of the oesophageal squamous mucosa is of limited value in the assessment of patients with reflux symptoms.

**Take home messages**

- Except for neutrophil infiltration in the squamous mucosa of patients with complicated reflux disease, the traditionally accepted histological markers of gastro-oesophageal reflux disease in squamous mucosa did not correlate with 24 hour pH testing.
- Thus, we were unable to confirm the value of the Ismail-Beigi criteria as histological markers of acid reflux.
- It therefore seems that the biopsy of the oesophageal squamous mucosa is of limited value in the assessment of patients with reflux symptoms.

**REFERENCES**


**ACKNOWLEDGEMENTS**

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In summary, we were unable to confirm the value of histological changes in the oesophageal squamous mucosa as markers of gastro-oesophageal reflux in patients with non-erosive reflux disease. Therefore, biopsy of the oesophageal squamous mucosa is of limited value in the assessment of patients with GORD symptoms. It is possible that histological features in the oesophageal squamous mucosa might predict long term complications, although there is, to date, no good evidence for this.