Campylobacter like organisms and reflux gastritis

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SUMMARY A total of 98 patients, who had undergone gastric surgery (23), or who had peptic ulcers (56), or who had normal endoscopic findings (19) underwent gastric biopsy, together with measurement of pH and total bile acid concentration, in their fasting gastric juice. The biopsy specimens were stained by the Warthin-Starry method for *Campylobacter* like organisms and were also graded "blind," as described in the preceding paper, for the five features that we believe may constitute the histological picture of reflux gastritis. The individual grades were added together to give a composite "reflux score" (0-15) for each patient. We found a notable association between the absence of *Campylobacter* like organisms and previous surgery for peptic ulceration, high reflux scores (> 10), hypochlorhydria (pH ≥ 4), and increased bile acid concentrations (≥ 1 mmol/l) in the stomach.

These findings further support our contention that reflux gastritis represents a distinct histopathological entity causally related to the effects of enterogastric reflux on the gastric mucosa and suggest that there may be two major categories of chronic gastritis: chronic superficial, or atrophic gastritis related to *Campylobacter* like organisms and reflux gastritis. Our data also imply that patients with peptic ulceration may, after gastric surgery, revert from being positive for these organisms to being negative and may undergo a possible transition from *Campylobacter* related chronic gastritis to reflux gastritis.

The consistent finding of *Campylobacter* like organisms in antral gastric biopsy specimens showing non-specific (type B) chronic gastritis¹⁻⁷ has led to a reconsideration of existing views on the aetiology of this condition. Based on an experiment that entailed the self administration of a culture of *Campylobacter pyloridis*, Marshall *et al* suggested that chronic gastritis results from a failure to clear *Campylobacter* organisms after an initial acute infective gastritis.⁸

In the preceding paper⁹ we described a constellation of histological features, which we believe to be associated with reflux of alkaline duodenal content into the stomach. If reflux gastritis is truly a separate clinicopathological entity with a distinctive histological picture a radically different pattern of association with *Campylobacter* like organisms to that found in "conventional" chronic gastritis might be expected.

To explore this hypothesis we compared the histological findings, especially those we consider to be indicative of reflux, with the presence or absence of *Campylobacter* like organisms in the same biopsy series. Additionally, we used the pH and bile acid

concentrations in fasting gastric juice as measures of alkaline reflux and related these to *Campylobacter* like organism status.

Methods

Ninety eight patients were included in this study (Table 1). As before⁹ they were drawn from five clinical groups, normal findings at endoscopy (n = 19), active benign gastric ulceration (n = 25), duodenal ulceration (n = 16), combined duodenal and gastric ulceration (n = 15), and a group of postoperative patients (n = 23).

Fasting gastric juice was aspirated at endoscopy, and four biopsy specimens were taken from within 5 cm of pylorus or the stoma in the operated group.

The routine sections stained with haematoxylin and eosin and with periodic acid Schiff and alcian Blue, pH 2.5, were examined by MFD, who was unaware of the endoscopic findings, gastric pH, or bile acid concentrations. The following histological features were assessed and given a score of 0-3 equivalent to normal or absent (0), mild (1), moderate (2), and severe abnormality or severe increase (3); foveolar hyperplasia; lamina propria oedema; vasodilatation and

Table 1 Details of patients and their *Campylobacter* status

Group	No of patients	Sex		Age (year)		No positive
		M	F	Mean	Range	
Normal endoscopy	19	10	9	41.7	22-75	12
Duodenal ulceration	16	9	7	44.4	21-76	14
Gastric ulceration	25	12	13	62.9	36-83	20
Combined duodenal and gastric ulceration	15	6	9	54.5	37-77	12
Postoperative	23	19	4	56.9	26-78	6

congestion; neutrophil polymorph infiltration; chronic inflammatory cell infiltration; intestinal metaplasia.

To calculate a composite reflux gastritis score the scores for acute and chronic inflammatory cells were modified to reflect their paucity—that is, a severe increase became 0, moderate increase 1, minor increase 2, and normal numbers of chronic inflammatory cells and absence of polymorphs 3. These modified scores were added to the scores given to the first three variables listed yielding a reflux gastritis score in the range 0–15.

A further section was taken from each set of biopsies and stained by the Warthin-Starry method for *Campylobacter* like organisms. These sections were examined by JIW, who was unaware of the findings in the routinely stained sections and the other investigations. The presence of *Campylobacter* like organisms in any of the biopsy specimens was recorded. Bile acid concentrations and pH were measured according to previous methods.⁹

Differences between the groups positive and negative for *Campylobacter* like organisms in reflux gastritis scores, gastric pH, and bile acid concentrations were tested for significance by χ^2 analysis and the Mann-Whitney U test.

Results

Campylobacter like organisms were detected in 64 of the 98 patients (65%): Table 1 shows the distribution of the positive cases. Considerably fewer postoperative patients (six of 23) were positive compared with patients in the other clinical groups studied. Of

the negative cases, three (one normal endoscopy, one duodenal ulceration, and one postoperative) were considered to have normal histological findings, and these patients were eliminated from subsequent analysis.

REFLUX GASTRITIS SCORES AND *CAMPYLOBACTER* LIKE ORGANISM STATUS

When the distribution of *Campylobacter* positive and negative cases was tabulated with regard to reflux gastritis scores (Table 2), there was a highly significant difference in distribution between patients with higher (> 10) and lower (≤ 10) reflux scores. Of the 31 patients who were negative, 24 had a score > 10 compared with only 12 of 64 in the positive group. Reflux gastritis scores (mean (SD)) were also significantly higher in the negative group (11.7 (2.3)) compared with those of the positive group (8.2 (2.5)) ($p = 0.0004$).

Contingency tables were constructed for individual histological variables thought to be related to *Campylobacter* like organism status (Table 2), and these showed highly significant associations between increased neutrophil polymorphs and increased chronic inflammatory cells, and *Campylobacter* like organism positivity. To exclude the possibility that observed differences in reflux gastritis scores between the negative and positive groups might be accounted for solely on the basis of differences in the intensity of the inflammatory cell infiltrate a modified reflux score was calculated for each patient by adding the scores allotted for foveolar hyperplasia, lamina propria oedema, and vasodilatation and congestion. This

Table 2 Histological grades and *Campylobacter* like organism status

	No of patients	Reflux gastritis score		Increased polymorphs		Increased chronic inflammatory cells		Intestinal metaplasia	
		≤ 10	> 10	Normal	Mild to severe	Normal	Mild to severe	Normal	Mild to severe
<i>Campylobacter</i> like organisms									
Negative	31	7	24	18	13	16	15	20	11
Positive	64	52	12	6	58	2	62	41	23
Total	95								

$\chi^2 = 30.545$;
 $p < 0.0001$

$\chi^2 = 26.222$;
 $p < 0.0001$

$\chi^2 = 31.972$;
 $p < 0.0001$

$\chi^2 = 0.002$;
 $p > 0.0001$

Campylobacter like organisms and reflux gastritisTable 3 Gastric pH, bile acid concentrations, and *Campylobacter* like organism status

Gastric pH		Bile acid concentrations (mmol/l)	
≥ 4	< 4	≥ 1	< 1
Negative 16	15	11	11
Positive 7	57	4	46
$\chi^2 = 18.831$; $p < 0.001$		$\chi^2 = 16.340$; $p < 0.001$	

modified score, reflecting the epithelial and vascular components of the inflammatory reaction, was also significantly higher in the *Campylobacter* negative group (data not shown).

No significant difference was found in the distribution of intestinal metaplasia with regard to overall *Campylobacter* like organism status, although bacteria were never present on focal areas of intestinal metaplasia in colonised biopsy specimens.

GASTRIC pH, BILE ACID CONCENTRATIONS, AND *CAMPYLOBACTER* LIKE ORGANISM STATUS

When the relation between *Campylobacter* like organism status and both fasting intragastric pH and bile acid concentrations was assessed (Table 3), there was a highly significant association between the absence of *Campylobacter* like organisms and both hypochlorhydria and high bile acid concentrations in the stomach. Of the 31 patients who were negative for *Campylobacter* like organisms, 16 had a gastric pH ≥ 4 , whereas only seven of the 64 positive patients were hypochlorhydric ($p < 0.001$). Likewise, of the 72 patients who had bile acid concentrations measured, 22 were negative, and 11 of these patients had bile acid concentrations ≥ 1 mmol/l whereas of the 50 who were *Campylobacter* positive, only four had abnormally high bile acid concentrations ($p < 0.001$). Furthermore, intragastric pH (mean (SD)) was significantly higher in the negative group 4.3 (2.3) than in the positive group 2.5 (1.5) ($p = 0.0015$), as were bile acid concentrations 3.76 (5.86) v 0.73 (2.90) ($p = 0.0039$).

Discussion

This is the first study in which an attempt has been made to correlate *Campylobacter* like organism status with evidence of alkaline reflux into the stomach. In using histological evidence based on our contention that reflux gastritis can give rise to a distinctive histopathological picture, we should further emphasise that lesser degrees of foveolar hyperplasia, lamina propria oedema, and capillary congestion are common to all forms of gastritis. Thus we typically found "reflux" scores of 5–9 in biopsy specimens, which would be diagnosed as chronic superficial, or chronic

atrophic gastritis. Only where the key features were severe and linked to a paucity of acute and chronic inflammatory cells, giving a score of 11–15 in our scheme, was the diagnosis of reflux gastritis indicated.

Using such histological criteria, it is of considerable interest that we found a highly significant difference in *Campylobacter* status between patients with reflux scores above and below 10. Conversely, the features that we believe constitute the histological picture of reflux gastritis were more severe in the negative than positive patients. The much lower incidence of *Campylobacter* like organisms in those patients, who by histological and other criteria are considered to have alkaline reflux into the stomach, supports the proposition that reflux represents a distinct aetiological factor and that the resultant gastritis is unrelated to *Campylobacter* infection. Our data, however, do not suggest that these aetiological agents are mutually exclusive; 12 of the 36 patients with high reflux scores (> 10) had *Campylobacter* like organisms in at least one of their biopsy specimens. Nevertheless, corroborative evidence of reflux (pH ≥ 4 , bile acid concentrations ≥ 1) was present in only four of the 12 patients, so the finding of high scores and *Campylobacter* like organisms might not represent coexistence of reflux and infection in all these cases, but might reflect the inability of an arbitrary scoring system to distinguish between those histological features which are most discriminatory in making a diagnosis of reflux. For instance, six of the 12 cases showed a mild or moderate increase in both acute and chronic inflammatory cells, findings that militate against a diagnosis of reflux gastritis.

We found highly significant correlations between the presence of *Campylobacter* like organisms and an increase in both acute and chronic inflammatory cells in the gastric mucosa. The mononuclear cell infiltrate in the lamina propria is the major diagnostic criterion in chronic superficial and atrophic gastritis. Therefore our findings are in agreement with previous studies that have shown a strong association between the presence of *Campylobacter* like organisms and the finding of "conventional" chronic gastritis in antral biopsies.^{3–6} These studies, however, also claim that most biopsy specimens negative for *Campylobacter* like organisms are normal. We suspect that the reluctance to diagnose gastritis in the absence of an inflammatory cell infiltrate means that features of reflux gastritis have been previously overlooked, or regarded as part of the "normal" spectrum, and that a proportion of these so called normal biopsy specimens will show the changes of reflux gastritis.

Using fasting intragastric pH and bile acid concentrations as indices of alkaline reflux, we found a highly significant association between the absence of *Campylobacter* like organisms and both hypochlorhydria

and increased bile acid concentrations in the stomach. These data provide further evidence, albeit indirect, that the aetiology of chronic gastritis developing under conditions of increased enterogastric reflux is likely to be largely related to the noxious effects of reflux on the gastric mucosa rather than to *Campylobacter* infection.

Our observations lead us to conclude that there are two principal categories of non-autoimmune chronic gastritis. The first is the currently accepted entity of "chronic gastritis" elucidated by Whitehead,¹⁰ in which the chronic inflammatory cell response predominates and activity is manifest by neutrophil polymorph infiltration. There are now good grounds for believing that this form is initiated or perpetuated by *Campylobacter* infection, or both.

The second major category, we suggest, is gastritis causally related to reflux of alkaline duodenal content into the stomach. This gives rise to an essentially "chemical" injury to the mucosa, which is chronic by virtue of constant repetition but which evokes a predominantly epithelial response where activity is reflected in the degree of compensatory foveolar hyperplasia. The mucosal response embraces other components of the vascular inflammatory reaction—namely vasodilatation, congestion, and interstitial oedema—but the inflammatory cell response seems to be unimportant.

With regard to other forms of gastritis, we have shown previously¹¹ that there is a low incidence of gastric *Campylobacter* like organisms in cases of pernicious anaemia, and this finding is in keeping with the presumed autoimmune aetiology of the type A chronic gastritis found in such patients.

Another potentially important implication from our results is the suggestion that patients with peptic ulceration may revert from being positive for *Campylobacter* like organisms to being negative after gastric surgery. Of the 56 patients with peptic ulcer that we studied, 46 (82%) were positive compared with only six of 23 (26%) in the postoperative group. It is tempting to suggest that this radical change in *Campylobacter* like organism status may in some way be related to the effects of increased enterogastric reflux after surgery. Under normal conditions the presence of a pH gradient in the gastric mucus barrier creates near neutral conditions at the epithelial cell surface. The reflux of bile acids may disrupt the mucus barrier, thereby lowering pH at the cell surface and rendering the microenvironment unfavourable to colonisation

by acid intolerant *Campylobacter* like organisms. Patients with peptic ulceration may thus undergo a transition from one predominant type of chronic gastritis to another—that is, from chronic superficial, or atrophic gastritis related to *Campylobacter* like organisms, to reflux gastritis after operation. Whether effective surgery to divert bile (closure of gastro-enterostomy or construction of a Roux-en-Y loop) might cause the regression of the histological features we consider to be indicative of reflux gastritis or recolonisation of the gastric mucosa by *Campylobacter* like organisms, or both, remains to be assessed.

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