ORIGINAL ARTICLE

Inflammation and cytokeratin 7/20 staining of cardiac mucosa in young patients with and without *Helicobacter pylori* infection

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Background: Both *Helicobacter pylori* and gastro–oesophageal reflux disease (GORD) may cause inflammation in cardiac mucosa. Intestinal metaplasia (IM) is found more often in GORD associated inflammation than in inflammation caused by *H pylori*, especially in young individuals.
Aim: To examine morphological differences in chronic inflammation in these two conditions by immunohistochemistry.
Patients/Methods: Tissue blocks from cardiac mucosa of patients <45 years were available as follows: 10 patients with chronic inflammation of cardiac mucosa (carditis) and *H pylori* gastritis (group 1); 10 patients with (possibly GORD related) carditis, but normal antrum and corpus (group 2); and 10 patients with non-inflamed cardiac mucosa and normal antrum and corpus (group 3). Haematoxylin and eosin staining and immunohistochemical staining for various inflammatory cells were performed for patients in groups 1 and 2 as follows: CD20 (B cells), CD3 (T cells), CD4 (T helper cells), CD8 (T suppressor cells), CD163 (macrophages), CD138 (plasma cells), and CD117 (mast cells). For all patients, cytokeratin 7/20 (CK7/20) staining was performed.
Results: No clear differences were seen in the morphology of chronic inflammation between groups 1 and 2. In both, plasma cells were most abundant. CK7/20 staining showed no differences between these groups.
Conclusion: *Helicobacter pylori* negative (possibly GORD associated) and *H pylori* related carditis cannot be distinguished on a morphological basis. The stronger tendency towards IM in the first entity cannot be explained by differences in the type of inflammation. Barrett-type CK7/20 staining seems typical for cardiac mucosa, irrespective of the type of inflammation or presence of IM.

There has been a rapid rise in cardiac and oesophageal adenocarcinoma in Western countries,[1] and much attention has focused on its classic risk factor—Barrett's oesophagus—which is a consequence of gastro–oesophageal reflux disease (GORD). Along with the classic or long segment Barrett's oesophagus, new entities such as short segment and ultrashort segment Barrett's oesophagus have been introduced. Of these, the first is defined as segments or tongues of columnar epithelium less than 3 cm in length in the tubular oesophagus showing incomplete intestinal metaplasia (IM) on histological examination, and the second as IM in the columnar epithelium immediately adjacent to a normal looking Z-line.[2] However, endoscopic recognition of these findings may be difficult, and even if biopsy samples are available, it is not easy to distinguish Barrett's mucosa from IM associated with *H pylori* gastritis. For this reason, cytokeratin 7/20 (CK7/20) staining has been used to differentiate these two types of IM.[3 4] Barrett-type IM seems to be a stronger risk factor for malignancy than non-Barrett IM in cardiac mucosa[5 6]; factors behind this difference remain unknown.

> "It is not easy to distinguish Barrett's mucosa from intestinal metaplasia associated with *Helicobacter pylori* gastritis"

IM in the gastric mucosa is associated with chronic inflammation.[7] Inflammation in the cardiac mucosa may be associated with *H pylori* gastritis or with GORD.[8] IM may be present in both types of inflammation, but is more often found in cardiac inflammation associated with GORD.[9 10] IM is a risk factor for cancer, and thus these findings may have prognostic implications, especially in young patients. Whether there is a morphological difference in the inflammatory infiltrate of cardiac mucosa of young patients with *H pylori* gastritis, or in those with possible GORD associated, sole carditis, has not been previously studied.

PATIENTS

Tissue blocks comprising histologically defined cardiac mucosa from within 5 mm of the squamocolumnar junction (Z-line) were available from our earlier study on IM and inflammation in cardiac mucosa comprising outpatients ⩽ 45 years old.[10] These blocks were originally stained with Alcian blue (pH 2.5) and periodic acid Schiff. Based on the original histological examination, blocks came from patients with *H pylori* gastritis and chronic inflammation of the cardiac mucosa (group 1), from patients with normal antrum and corpus but chronic inflammation of the cardiac mucosa (group 2), and from patients with normal antrum and corpus and non-inflamed cardiac mucosa (group 3). Cardiac mucosa was defined as mucosa with mucin secreting glands, occasionally with some parietal cells, but without the typical fundic mucosa appearance. Each group was chosen with the view of obtaining sufficient material to perform immunohistochemistry. No patients had classic Barrett's oesophagus. In several of them, however, the squamocolumnar junction was

Abbreviations: CK, cytokeratin; GORD, gastro–oesophageal reflux disease; IM, intestinal metaplasia

Table 1 Clinical data for the three groups of patients

	Group 1	Group 2	Group 3
Male/Female	3/7	4/6	2/8
Median age (range)	38 (19–44)	37 (24–40)	32 (22–42)
Oesophagitis*	1	7	3
Hiatus hernia	3	6	3
Reflux symptoms†	4	6	5
Z-line irregular	5	8	7

Group 1: patients with *Helicobacter pylori* gastritis and carditis; group 2: patients with normal antrum and corpus but inflammation of cardiac mucosa; group 3: patients with normal antrum and corpus and non-inflamed cardiac mucosa; 10 patients in each group.
*Erosive and/or histological oesophagitis; †reflux-like symptoms as main indication for gastroscopy.
There was a significant difference in the presence of oesophagitis between groups 1 and 2 (p = 0.0102, Fischer's exact test), and a borderline significant difference between *H pylori* positive (group 1) and *H pylori* negative (groups 2 and 3) patients (p = 0.0485).

irregular at endoscopy. Table 1 provides details of the clinical data.

METHODS

For patients with inflammation in the cardiac mucosa (groups 1 and 2), haematoxylin and eosin staining and immunohistochemical staining for different types of inflammatory cells were performed as follows: CD3 (T cells), CD4 (T helper cells), CD8 (T suppressor cells), CD20 (B cells), CD117 (mast cells), CD138 (plasma cells), and CD163 (macrophages). In addition, all specimens were stained for CK7/20.

All tissues were fixed in 10% buffered formalin, processed, and embedded in paraffin wax. From each block, several 5 µm thick sections were cut on to coated slides and dried overnight at 37°C. Before staining, the sections were dewaxed in xylene and rehydrated through graded concentrations of ethanol to distilled water. One section from each case was stained with haematoxylin and eosin, and the rest of the sections were pretreated for immunohistochemical staining. Table 2 lists the different primary antibodies used and their target cells. Immunohistochemical staining was performed with an automated stainer (TechMate 500; Ventana Medical Systems, Tucson, Arizona, USA) using a peroxidase/diaminobenzidine detection kit (ChemMate, DakoCytomation, Glostrup, Denmark). After immunostaining, the sections were lightly counterstained with Mayer's haematoxylin and mounted in Mountex (HistoLab, Gothenburg, Sweden). A known positive control section for each primary antibody was included in every staining run.

One pathologist (AS) evaluated all specimens, blinded to patients' clinical background.

RESULTS

Histology for patients with chronic inflammation in cardiac mucosa

Specimens from nine of the 10 patients with *H pylori* infection (group 1) showed cardiac mucosa. In one patient, the biopsy was too superficial for proper evaluation of the glandular status. None of the patients with *H pylori* infection showed IM in the specimens.

In the 10 patients with histologically normal antrum and corpus, but with inflammation in the cardiac mucosa (group 2), all specimens showed cardiac mucosa. Four patients had IM in the specimens; of these, metaplasia was incomplete in three and complete in one. None of the patients showed dysplasia.

Table 3 shows the overall grade of inflammation in these patients and the density of neutrophils, as assessed by haematoxylin and eosin staining.

Table 2 Antibodies, pretreatments, and dilutions

Antibody	Clone	Source	Dilution	Target cells
CD3	PS1	N-C	1/200*	T cells
CD4	1F6	N-C	1/20*	T helper cells
CD8	4B11	N-C	1/25*	T suppressor cells
CD20	L26	Dako	1/1000*	B cells
CD117	Polyclonal	Dako	1/300*	Mast cells
CD138	B-B4	Serotec	1/75*	Plasma cells
CD163	10D6	N-C	1/100*	Macrophages
CK7	OV-TL 12/30	Dako	1/1000†	Epithelial cells
CK20	KS 20.8	Dako	1/125†	Epithelial cells

Suppliers: N-C, Novocastra Laboratories, Newcastle upon Tyne, UK; Dako, Glostrup, Denmark; Serotec, Oxford, UK.
*Pretreatment: microwave oven, boiled in citric acid pH 6.0 for 20 minutes; †pretreatment: enzyme digestion, 0.5% trypsin at 37°C for 30 minutes.
CK, cytokeratin.

Results of immunohistochemical staining

Table 3 shows the results of the semiquantitative assessment of the density of different inflammatory cells in groups 1 and 2, as assessed by immunohistochemical staining. In both groups, most of the inflammatory cells were CD138 positive plasma cells, located as a dense infiltrate in the superficial (upper) part of the lamina propria (fig 1). In addition, variable numbers of CD3 positive T cells and CD20 positive B cells were seen in both patient groups, but their number never exceeded that of the plasma cells. B cells were more abundant in the lamina propria of the patients with *H pylori* infection. The CD3 positive T cells were divided equally between CD4 positive helper T cells, scattered in the lamina propria, and CD8 positive T cells, mainly located in the superficial epithelium. CD117 positive cells were sparsely distributed in the lamina propria in all biopsy specimens in both patient groups. CD163 positive macrophages were slightly more abundant in the specimens of the patients with *H pylori* infection. However, as tested by the linear exact trend test, there were no significant differences between groups in the densities of the various lymphocyte subsets.

CK7/20 staining

Table 4 provides details of the CK7/20 staining patterns. Barrett-type CK7/20 staining was defined as moderate or strong CK7 staining of the surface epithelium and glands (fig 2) and strong CK20 staining of the surface epithelium. This pattern was found in six patients in group 1, eight in group 2, and seven in group 3 (table 4). Thus, the number of patients with Barrett-type staining did not differ between the groups. However, comparing CK7 staining with haematoxylin and eosin staining gave the clear impression that, in patients who had only true cardiac-type glands and no parietal cells, CK7 was positive in a Barrett-like manner, whereas in those with parietal cells and thus an oxyntho-cardiac-type mucosa, CK7 tended not to stain in a Barrett-like manner. In patient 28, from whom both cardiac-type and corpus-type mucosa from the vicinity of the squamocolumnar junction were available, the cardiac mucosa was clearly CK7 positive, but the corpus mucosa was negative (fig 3).

DISCUSSION

Using immunohistochemical staining of cardiac biopsy specimens from young individuals, inflammatory infiltrates in patients with *H pylori* gastritis and in those with no distal gastritis but (possibly GORD associated) carditis could not be distinguished on a morphological basis. This suggests that the tendency to develop IM at a younger age[10] in GORD related inflammation of the cardiac mucosa may not result from differences in the type of inflammation. Although our findings could not exclude the possibility of more subtle

Table 3 Densities of different inflammatory cells as detected by immunohistochemistry in group 1 and group 2 patients

No	Grade	Neutrophils	IM	CD3	CD4	CD8	CD20	CD117	CD138	CD163
1	2	N+	–	1	1	1	1	1	2	1
2	3	N++	–	2	2	1	3	1	3	1
3	3	N++, E++	–	1	1	1	2	1	3	2
4	2	N+	–	0	0	0	0	1	1	2
5	1	E+	–	1	1	1	2	1	2	2
6	2	E++	–	1	1	2	2	1	2	1
7	2	N++, E+	–	0	0	0	0	1	2	1
8	3	N++, E++	–	2	1	1	2	1	3	2
9	1	–	–	1	1	1	0	1	1	1
10	2	E++	–	2	1	2	0	1	1	0
11	2	E+	–	0	0	1	1	1	2	2
12	2	E+	+	1	1	1	0	1	2	1
13	3	N++, E++	–	1	1	1	1	1	3	1
14	2	N++, E+	–	1	1	1	0	1	2	1
15	1	–	–	0	0	0	0	1	1	1
16	1	E+	–	1	1	1	1	1	1	1
17	1	N+, E+	+	1	1	1	0	1	1	1
18	3	E++	+	2	2	2	2	1	3	1
19	1	E+	+	1	1	1	0	1	1	1
20	1	E++	–	0	0	1	0	1	1	1

Group 1 (patients 1–10) were *Helicobacter pylori* positive and group 2 (patients11–20) were *H pylori* negative young patients with inflammation of the cardiac mucosa.
Grade (grade of general inflammation) scored as follows: 1, mild; 2, moderate; 3, strong.
Neutrophils (density of neutrophils (N) and eosinophils (E)) as detected by haematoxylin and eosin staining: +, sparse; ++, moderate.
Immunohistochemical staining: CD3 (T cells), CD4 (T helper cells), CD8 (T suppressor cells), CD20 (B cells), CD117 (mast cells), CD138 (plasma cells), and CD163 (macrophages). The density of cell infiltrates was scored as follows: 0, no cells; 1, low density; 2, moderate density; 3, high density.
IM, intestinal metaplasia in the cardiac mucosa.

differences in the nature of these two types of inflammation, they are supported by a recent study, in which cytokine profiles in these two types of inflammation were similar.[11]

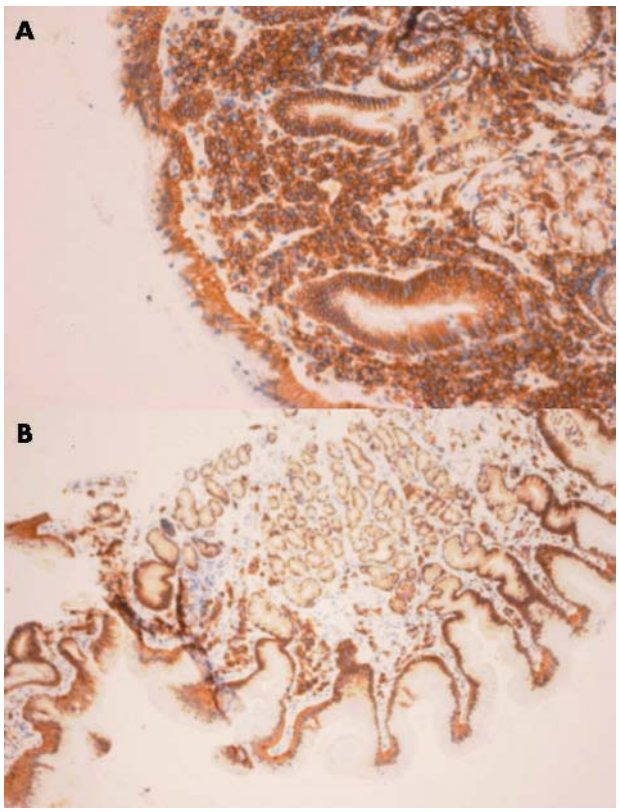

Figure 1 (A) CD138 staining for cardiac mucosa in a patient with *Helicobacter pylori* gastritis and inflammation of the cardiac mucosa (patient 8) and (B) in a patient with no distal gastritis but with possible gastro–oesophageal reflux disease associated carditis (patient 15).

Little is known about the inflammatory infiltrate in *H pylori* gastritis. In our present study, because of the small size of the biopsy specimens, cell counts were not applicable, and thus the density of the inflammatory cells could be assessed only semiquantitatively. This, together with the relatively small number of patients renders our study incapable of finding minor differences in lymphocyte populations. Our findings that CD4 positive cells were scattered in the lamina propria, and CD8 cells were mainly located in the superficial epithelium of the cardiac mucosa, fully agree with two earlier reports on distal *H pylori* gastritis, based on immunohistochemistry.[12 13] Thus, it seems that inflammation in both the distal gastric mucosa and in the cardiac mucosa share similar features.

> "The tendency to develop intestinal metaplasia at a younger age in gastro–oesophageal reflux disease related inflammation of the cardiac mucosa may not result from differences in the type of inflammation"

As far as we are aware, the presence of plasma cells in the cardiac mucosa has not previously been studied by immunohistochemical methods. Plasma cells—B cells committed to antibody secretion—were the most abundant cells in the inflammatory infiltrate of the cardiac mucosa in both *H pylori* negative and *H pylori* positive patients. In *H pylori* gastritis, antibody secreting cells specific to various *H pylori* antigens are detectable in the gastric mucosa,[12 14] and the density of these cells correlated with the amount of *H pylori* specific immunoglobulin produced and secreted by the mucosa.[15] All our patients with *H pylori* gastritis and inflammation in the cardiac mucosa had *H pylori* specific antibodies in their serum, as measured by an in house enzyme immunoassay, whereas none of the individuals with inflammation in the cardiac mucosa but with normal antrum and corpus were seropositive. The role and specificity of the plasma cells found in the cardiac mucosa of patients without *H pylori* gastritis remains to be studied.

Helicobacter pylori causes gastritis, and these bacteria can be found in the cardiac mucosa also[10]; thus, *H pylori* is most

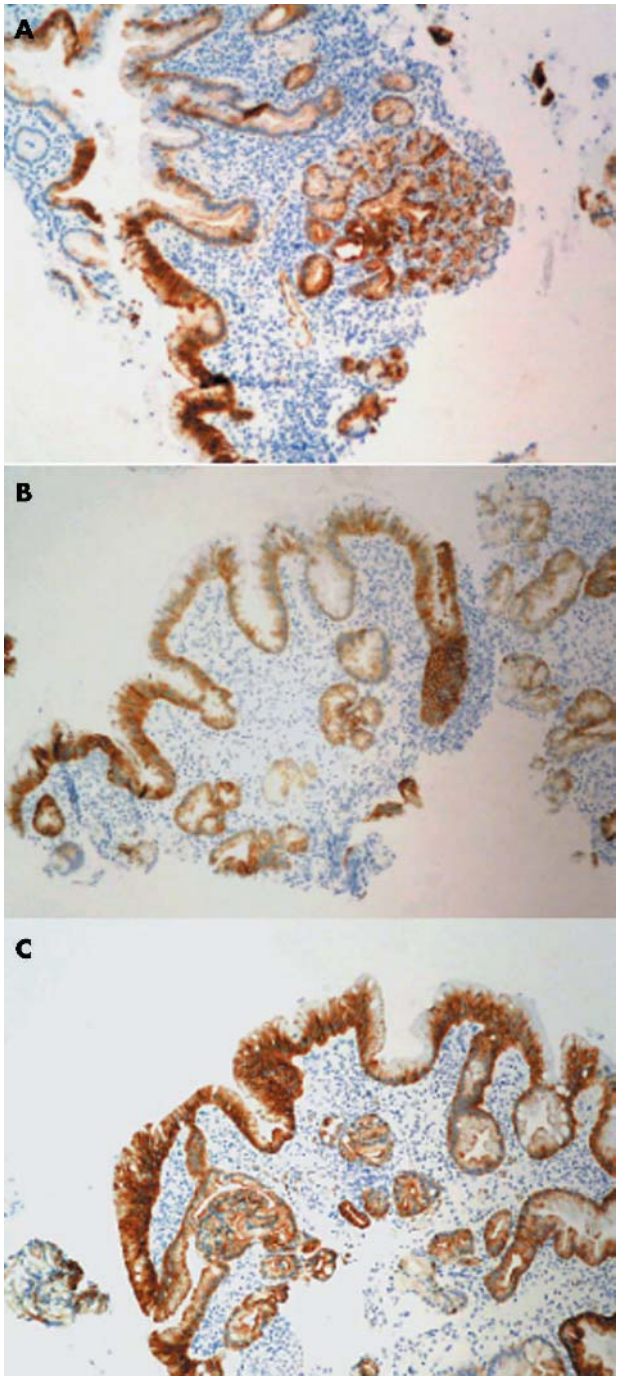

Figure 2 (A) Cytokeratin 7 staining for cardiac mucosa in a patient with *Helicobacter pylori* gastritis and carditis (patient 8); (B) in a patient with no distal gastritis but with possible gastro–oesophageal reflux disease (GORD) related carditis without intestinal metaplasia (IM) (patient 13); and (C) in a patient with GORD related carditis and IM (patient 12).

probably the cause of inflammation at this site also in *H pylori* positive patients. In *H pylori* negative patients, inflammation in the cardiac mucosa may be considered a consequence of GORD.[8] Indeed, in our present study, erosive or histological oesophagitis was significantly more common in *H pylori* negative (group 2) than in *H pylori* positive (group 1) patients with inflammation of the cardiac mucosa. However, 24 hour pH measurement—the gold standard test for GORD—was not performed, so that the presence of GORD cannot be proved in group 2 or excluded in group 1, which may make it more difficult to detect differences between the groups.

The cardiac mucosa is often inflamed, even in patients with no distal gastritis. Therefore, it has been proposed that the cardiac mucosa may actually be metaplastic oesophageal mucosa.[16] However, in our earlier study comprising young patients, the cardiac mucosa was normal in more than half of those with no distal gastritis.[10] Some degree of gastro–oesophageal reflux occurs in most individuals; the fact that inflammation is seen more often in studies comprising older patients might merely reflect the fact that these individuals have had reflux for a longer time. Furthermore, none of the patients in our study had classic Barrett's oesophagus; the Z-line was grossly at the normal site. However, the exact location of the gastro–oesophageal junction is difficult to define endoscopically. In several of our patients, the Z-line was somewhat irregular, which implies that the possibility of a short segment Barrett's oesophagus cannot be ruled out macroscopically. The fact that this was equally common in *H pylori* negative patients with and without inflammation in the cardiac mucosa is not in favour of the idea that inflammation is a sign of metaplasia. However, this small study does not justify conclusions about the origin of cardiac mucosa.

Helicobacter pylori infection is most often acquired in childhood.[17] Thus, young individuals with *H pylori* gastritis and inflammation in the cardiac mucosa, like those in our present study, would be expected to have had the inflammation for several decades. The duration of possible GORD associated inflammation is difficult to assess, but it probably does not exceed that of *H pylori* related carditis. GORD associated inflammation of the cardiac mucosa seems to be milder in nature than *H pylori* related inflammation.[8 10 18] Thus, neither the duration nor intensity of inflammation seems to explain the higher prevalence of IM found in GORD related inflammation of the cardiac mucosa.

> "In gastro–oesophageal reflux disease, intestinal metaplasia in the cardiac mucosa may develop as a result of the direct toxic effects of acid, nitrosoamines, and possibly bile"

IM in Barrett-type mucosa is more often incomplete,[19] and has a stronger tendency to develop dysplasia and cancer than does IM at the squamocolumnar junction in non-Barrett mucosa, which is often associated with *H pylori* gastritis.[5 6] An explanation for this may be that the mechanism by which IM develops differs. In *H pylori* gastritis, IM most probably develops as a consequence of decades of mucosal inflammation. This is supported by some studies in which even IM has been reported to vanish several years after *H pylori* eradication.[20] According to our present findings that the type of inflammation does not seem to differ between *H pylori* related and GORD associated carditis, factors other than inflammation may be crucial in the development of IM in the cardiac mucosa in patients with GORD. This assumption is supported by the finding that proinflammatory polymorphisms, which strongly increase the risk for distal gastric carcinoma, have little effect on the risk for cardiac carcinoma.[21]

After meals, highly acidic gastric juice is present at the squamocolumnar junction, which most probably contributes to the high prevalence of disease at this site.[22] The potential for acid nitrosation and formation of nitrosamines seems to be maximal in circumstances that exist in the cardiac region of the stomach.[23] Even ascorbic acid, a powerful inhibitor of the nitrosation reaction in general, might be expected to promote carcinogenesis at the squamocolumnar junction by enhancing the local production of nitric oxide in gastric

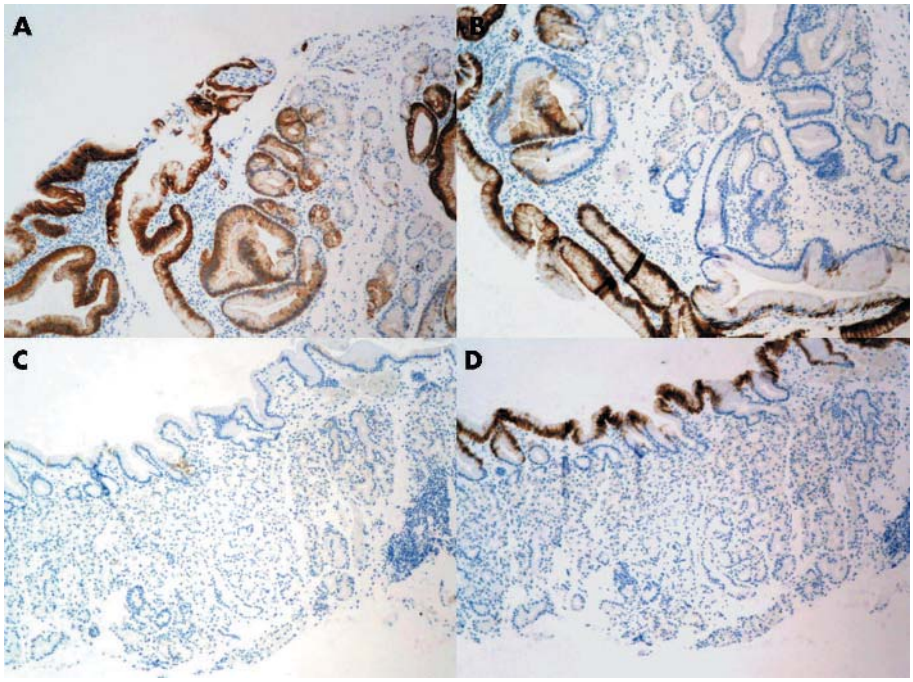

Figure 3 Cytokeratin staining in cardiac and corpus-type mucosa only millimetres from the squamocolumnar junction in a patient with non-inflamed cardiac mucosa (patient 28): (A) cytokeratin 7 (CK7) in the cardiac mucosa; (B) CK20 in cardiac mucosa; (C) CK7 in corpus-type mucosa; and (D) CK20 in corpus-type mucosa.

juice.[24] Thus, in GORD, IM in the cardiac mucosa may develop as a result of the direct toxic effects of acid, nitrosoamines, and possibly bile. This process may occur without a primary role for inflammation. Inflammation in the cardiac mucosa in these patients may be only an insignificant and secondary, non-specific reaction to damaged tissues. Differing mechanisms behind the development of IM may make GORD related IM more vulnerable to the development of dysplasia.

CKs are structural proteins of epithelial cells. The differing patterns of expression of CK peptides can be useful to indicate the origin of malignant tumours. A specific Barrett-type CK7/20 staining pattern has been reported to differentiate Barrett's mucosa from *H pylori* associated and other types of IM at the gastro–oesophageal junction.[3 4] The Barrett-type CK7/20 staining pattern, consisting of band-like CK20 staining of the surface epithelium and superficial glands and moderate to strong CK7 staining of both superficial and deep glands, has been shown to correlate with reflux symptoms,[25] macroscopical findings of the Z-line,[26] and the absence of *H pylori*.[27] However, other studies, in which the cardiac mucosa was defined histologically rather than by biopsy site, have found no differences in the CK7/20 staining pattern between Barrett's mucosa and non-Barrett's IM.[19 28–30]

In earlier studies of non-intestinalised cardiac mucosa, cardiac glands have been CK7 negative[3 31] with superficial foveolar epithelium showing CK20 positivity.[3] In more recent studies, in which the cardiac mucosa was defined histologically, 55–85% of patients have shown Barrett-type staining in non-intestinalised cardiac mucosa.[19 30 32] In our present study, in which few patients had IM in the cardiac mucosa, the results of CK7/20 staining were similar for all patient groups; that is, for patients with *H pylori* related or GORD associated inflammation in the cardiac mucosa and those with healthy, non-inflamed cardiac mucosa. Because our present study defined cardiac mucosa as mucosa with mucous secreting glands, although some parietal cells could be present, patients with both cardiac or oxynthocardiac mucosa were included. Barrett-type staining seemed to be associated with pure cardiac, not oxynthocardiac, mucosa, which may indicate that the Barrett-type CK7/20 staining pattern is a

Table 4 CK7/20 staining of cardiac mucosa in the three patients groups

No	CK7	CK20	No	CK7	CK20	No	CK 7	CK20
1	S+, G+	S++	11	S++, G++	S++	21	S++, G++	S++
2	S+	S++	12	S++, G++	S++	22	S++, G++	S++
3	S++, G++	S++	13	S++, G++	S++	23	S+	S++
4	S+, G+	S++	14	S++, G++	S+	24	S+	S++
5	S+	S++	15	S++, G++	S++	25	S+,G+	S++
6	S++, G++	S++	16	S++, G++	S++	26	G+	S++
7	S+	S++	17	S+, G+	S++	27	S++, G++	S++
8	S++, G++	S++	18	S++, G++	S++	28	S++, G++	S++
9	S+	S++	19	S+, G+	S++	29	S++, G++	S++
10	S++, G++	S++	20	S+	S+	30	S++, G++	S++

Group 1 (1–10): patients with *Helicobacter pylori* gastritis and inflammation of the cardiac mucosa; group 2, (11–20): patients with normal antrum and corpus but inflammation of the cardiac mucosa; group 3, (21–30): patients with normal antrum and corpus and non-inflamed cardiac mucosa.
S, surface; G, glands; ++, strong; +, moderate.

Take home messages

- *Helicobacter pylori* negative (possibly gastro–oesophageal reflux disease (GORD) associated) and *H pylori* related carditis cannot be distinguished on a morphological basis
- The stronger tendency towards intestinal metaplasia (IM) in GORD related carditis cannot be explained by differences in the type of inflammation, so that other factors may contribute to the tissue damage and development of IM in these patients
- Plasma cells—B cells committed to antibody secretion—were the most abundant cells in the inflammatory infiltrate of the cardiac mucosa in both *H pylori* negative and *H pylori* positive patients
- Barrett-type CK7/20 staining appears to be typical for cardiac mucosa, irrespective of the type of inflammation or presence of IM

marker for pure cardiac mucosa with only mucous secreting glands and no parietal cells.

In conclusion, inflammation of the cardiac mucosa in young patients with *H pylori* gastritis and in those with no distal gastritis but with possible GORD associated carditis could not be distinguished on a morphological basis. This may indicate that factors other than inflammation contribute to the tissue damage and development of IM in patients with GORD related carditis. In most of the patients in each group, the cardiac mucosa showed Barrett-type CK7/20 staining. This suggests that the Barrett-type CK7/20 staining pattern may be typical of cardiac mucosa as such, irrespective of inflammation or of the presence of IM.

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