Helicobacter heilmannii gastritis: a histological and immunohistochemical trait

E Ierardi, R A Monno, A Gentile, R Francavilla, O Burattini, S Marangi, L Pollice, A Francavilla

Abstract

Aim—Biopsies of the gastric antrum were reviewed over a period of 10 years to determine the prevalence of Helicobacter heilmannii in symptomatic subjects from this geographical area and to relate its presence to distinctive histopathological and immunohistochemical features.

Methods—Biopsies from 7926 symptomatic patients were reviewed. Ten serial sections were stained with haematoxylin and eosin for conventional histology. Another 10 sections were stained with the Gram method for spiral bacteria. When H heilmannii was suspected, 10 additional serial sections were stained with methylene blue to obtain homogeneous colouring. An equal number of sections from patients affected by isolated H helimannii or H pylori gastritis were analysed by immunohistochemistry to evaluate lymphoid aggregate/mucosal lymphocyte clonality (CD20 and CD3) and tumour necrosis factor alpha (TNF-α) in stromal cells.

Results—The prevalence of H heilmannii was 0.1% (eight of 7926), whereas H pylori was present in 60.7% of patients (4813 of 7926). In two of the eight H heilmannii positive patients both helicobacters were found. In all subjects infected by H helimannii only, distinctive histology (lymphocyte exudation into gastric foveolae) was seen. Lymphoid aggregates, chronic mucosal inflammation with patchy activity, and the absence of epithelial mucus depletion were regular features of H heilmannii gastritis. Immunohistochemistry did not reveal different lymphocyte clonal patterns between H pylori and H helimannii gastritis: CD20 positive cells were predominant in the centre of aggregates and mucosal infiltrates, whereas CD3 positive cells were prevalent at the periphery of follicles. Only H pylori gastritis showed a significant increase in TNF-α positive stromal cells.

Conclusion—These data suggest that an unusual lymphocyte reaction, with the tendency to invade the foveolar lumen, is a distinctive histopathological aspect of H heilmannii chronic gastritis, although further studies in a larger series are necessary to confirm this fact. Nevertheless, lymphocyte clones do not differ qualitatively from those found in H pylori infection. Moreover, compared with H helimannii, H pylori provokes a more intense release of TNF-α, suggesting that different inflammatory responses exist to these two organisms.

Keywords: Helicobacter heilmannii; Helicobacter pylori; tumour necrosis factor α

In 1987, Dent et al reported the finding of a bacterium different from H pylori in the human gastric mucosa; this organism was named Gas-trospirillum hominis. Since then, other cases have been described in association with gastritis, confirming the worldwide distribution of this microorganism. Most infected patients had been in contact with dogs, cats, and even pigs, thus suggesting an animal transmission of this infection. Various attempts to grow the microorganism in vitro failed, until Andersen et al isolated it in an artificial medium in 1995. Moreover, it was propagated and maintained by taking gastric biopsies from patients and feeding them to mice. Finally, by cloning and sequencing the bacterial 16S rRNA gene, G hominis was shown to be a helicobacter, and the name of Helicobacter heilmannii was proposed in honour of the German histopathologist K Heilmann. The low prevalence of H heilmannii infection in histopathological series reflects the small number of cases published until now.

Following on from our previous reports, we have reviewed endoscopic biopsies of the gastric antrum over the past 10 years. Aims of the study were to detect the histological prevalence of H heilmannii in symptomatic subjects in our geographical area (Puglia, southern Italy) and to relate the presence of this bacterium to distinctive histopathological and immunohistochemical changes in the gastric mucosa.

Material and methods

STUDY DESIGN

A retrospective study was performed on biopsy specimens taken at the level of the gastric antrum at a distance of 3 cm from the pylorus. Our series comprised 7926 patients (4131 men and 3795 women; age range, 17–75 years; mean age, 44.8) undergoing oesophagogastro-toduodenoscopy for upper gastrointestinal symptoms over a total period of 10 years (1989–99) in the section of gastroenterology of the department of emergency and organ transplantation of the University of Bari, Italy. At least two biopsy specimens had been taken for each patient. Routinely, 10 serial sections had been stained with haematoxylin and eosin for

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histological examination of gastric mucosa and an additional 10 with the Gram stain for the detection of spiral bacteria.

**HELIcobacter HEILMANNII GASTRITIS**

The morphological criteria for _H heilmannii_ identification that we used for this study and our previous ones were in agreement with those described by Dent and colleagues and successively stated by Heilmann and Borchard. In particular, “tightly spiral shaped bacteria (corkscrew shape)” were suspected to be _H heilmannii_. They were characterised by their predominantly straight appearance and large size (about 10 μm). When _H pylori_ was also present, the distinction was based on the smaller dimensions and curved shape of this last bacterium when compared with _H heilmannii_.

When the presence of _H heilmannii_ was suspected, 10 additional serial sections were cut and stained with methylene blue which, in our experience, yields a more homogeneous colouring of bacteria. Finally, the histological picture was accurately reviewed in these sections to detect any distinctive histopathological features.

**IMMUNOHISTOCHEMISTRY**

Sections from all six patients with isolated _H heilmannii_ infection and an equal number of _H pylori_ positive subjects, with chronic gastritis with moderate–severe activity, were studied by means of immunohistochemistry. The two groups were matched for sex, age, and endoscopic picture.

Clonal lymphocyte populations CD20 and CD3 were detected using monoclonal antibodies (Dako, Copenhagen, Denmark) and the labelled streptavidin–biotin technique, according to Saxena et al. Tumour necrosis factor alpha (TNF-α) was stained using a polyclonal rabbit antibody (PromoCell, Heidelberg, Germany). The reaction was visualised using a peroxidase/anti-peroxidase (PAP) technique with goat anti-rabbit immunoglobulins (Dako) and a complex of rabbit antibodies and horseradish peroxidase (Dako).

**STATISTICS**

The TNF-α labelling index (LI) was expressed as per cent of stromal positive cells (at least 1000 cells were counted for each specimen) as described by Baert et al. The Student’s _t_ test for unpaired data was used to compare the values of LI in _H pylori_ and _H heilmannii_ gastritis.

**Results**

The prevalence of _H heilmannii_ in our series of gastric biopsies was 0.1% (eight of 7926), whereas the prevalence of _H pylori_ was 60.7% (4813 of 7926). In two of the eight _H heilmannii_ positive patients both helicobacters (_H pylori_ and _H heilmannii_) were present. _Helicobacter heilmannii_ were seen as single elements or clumps of spiral bacteria. They had the typical feature of “corrugated cigars/corkscrew shape” and were found both in the gastric foveolar lumen and within the mucus. In some instances bacteria were seen in close association with the gastric epithelium (fig 1A and B).

The details of the _H heilmannii_ positive patients are as follows: five men and three women with a median age of 40.3 years and a range of 20–64. Contacts with domestic animals (cats and/or dogs) were reported by six of the eight patients. Table 1 details the endoscopic features of the patients. All patients were treated with conventional triple therapies. Eradication was achieved in all but one patient who dropped out for unknown reasons.

**HISTOPATHOLOGY**

The presence of _H heilmannii_ was always associated with chronic gastritis (an increase of lymphocytes and plasma cells in the lamina

<table>
<thead>
<tr>
<th>Endoscopic feature</th>
<th>Prevalence (positive patients/total patients)</th>
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</thead>
<tbody>
<tr>
<td>Hypaeraemia/oedema</td>
<td>5/8</td>
</tr>
<tr>
<td>Antral chronic erosions</td>
<td>5/8</td>
</tr>
<tr>
<td>Duodenal erosions</td>
<td>1/8</td>
</tr>
<tr>
<td>Desophagitis</td>
<td>3/8</td>
</tr>
<tr>
<td>Normal</td>
<td>2/8</td>
</tr>
</tbody>
</table>
Active inflammation (polymorphonuclear cells) showed a diffuse distribution in the lamina propria and epithelial cells only in the two patients affected by combined (H pylori/heilmannii) infection. In all six subjects with isolated H heilmannii infection, a small number of polymorphonuclear cells with a patchy distribution was always seen. Moreover, in these patients distinctive histology (lymphocyte exudation into gastric foveolae) was seen (fig 2A and B). This feature was never found in H pylori gastritis, in which the inflammatory cell exudate in the gastric foveolae, when present, was made up of polymorphonuclear cells (crypt abscesses).

Lymphoid aggregates were seen regularly in H heilmannii chronic gastritis and were predominantly located in the basal portion of the lamina propria.

A depletion of epithelial cell mucus was often seen in H pylori gastritis and its extent was related to the degree of inflammation. This feature was never seen in H heilmannii infection.

Table 2 summarises the differences in histopathology seen in H pylori and H heilmannii gastritis.

### IMMUNOHISTOCHEMISTRY

Immunohistochemistry did not reveal a different lymphocyte clonal pattern in the two types of gastritis. We found that CD20 positive cells were predominant in the centre of aggregates and mucosal infiltrate, whereas CD3 positive cells were prevalent in the peripheral area of the follicles.

There was a significant increase in TNF-α positive stromal cells in H pylori gastritis compared with H heilmannii gastritis (mean (SD) LI, 42.3 (8.7) and 9.4 (4.2), respectively; p < 0.001; Student’s t test; fig 3A and B).

### Discussion

It is well known that H heilmannii is an uncommon cause of chronic gastritis. The first cases of infection by this bacterium in Italian patients were described by Figura and colleagues and our laboratory. We have since reported other cases of isolated and H pylori associated H heilmannii gastritis. Following on from these previous reports, we have reviewed endoscopic biopsies of the gastric antrum over a period of 10 years with the aim of detecting the histological prevalence of H heilmannii in chronic gastritis.

![Figure 2](image1.png)  
(A, B) Lymphocyte exudation into the lumen of gastric crypts in patient with Helicobacter heilmannii gastritis (original magnification, ×1000).

<table>
<thead>
<tr>
<th>Feature</th>
<th>H pylori</th>
<th>H heilmannii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic inflammation</td>
<td>+ ±</td>
<td>+ ±</td>
</tr>
<tr>
<td>Active inflammation</td>
<td>+ + +</td>
<td>± (patchy)</td>
</tr>
<tr>
<td>Epithelial mucus depletion</td>
<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Lymphoid aggregates</td>
<td>+ ±</td>
<td>+ ±</td>
</tr>
<tr>
<td>Lymphocyte exudation into gastric foveolae</td>
<td>−</td>
<td>+ +</td>
</tr>
<tr>
<td>Foveolar abscesses</td>
<td>+ +</td>
<td>−</td>
</tr>
</tbody>
</table>

![Figure 3](image2.png)  
(A, B) Immunohistochemical staining of tumour necrosis factor α (TNF-α) in stromal cells of gastric antrum from a Helicobacter pylori and H heilmannii positive patient. The cytoplasm of positive cells is stained red by aminoethylcarbazole and strong positivity is seen (original magnification, ×400).
symptomatic subjects in our geographical area (Puglia, southern Italy) and relating the presence of this bacterium to distinctive histopathological and immunohistochemical mucosal changes. In the period 1989 to 1999 we have seen eight cases of *H. heilmannii* infection with a prevalence of 0.1% in symptomatic subjects undergoing upper endoscopy, whereas *H. pylori* was found in 60.7%. These data are in agreement with the estimated prevalences of other studies from geographical areas with a similar socioeconomic development. 1

In particular, the prevalence in a recent series reported from Emilia, a region of Northern Italy, is very similar to ours. 11

Recently, Goteri et al. have described the association of *H. heilmannii* with gastric lymphoma. Moreover, Stolte and colleagues 7 and Holck and colleagues 12 found that *H. heilmannii* may trigger the onset of germinal centres in lymphoid aggregates more frequently than *H. pylori*, and this phenomenon may account for the increased risk of developing mucosal associated lymphoid tissue lymphoma. 13 We found a distinctive histological feature: lymphocyte exudation into the lumen of gastric foveolae. We have never seen this feature in histological sections from patients with *H. pylori* gastritis. In addition, in biopsy specimens from patients with isolated *H. heilmannii* infection, a few polymorphonuclear cells showing a patchy distribution were seen. These findings suggest that *H. heilmannii* may induce a peculiar lymphocytic mucosal reaction, whereby lymphocytes tend to diffuse into the foveolar lumen. However, immunohistochemistry did not reveal different lymphocyte clonal patterns in *H. pylori* and *H. heilmannii* gastritis. Definite conclusions cannot be drawn because of the small number of patients with *H. heilmannii* gastritis in our series, and our data need to be confirmed in a larger sample.

Despite the variability of its pattern, *H. pylori* induced inflammation is characterised by polymorphonuclear infiltration of the lamina propria, with the tendency to invade epithelial cells and for foveolar abscesses to develop. For this reason, mucus depletion often reflects epithelial cell damage in the course of *H. pylori* gastritis. We have never seen changes in the amount of cellular mucus in *H. heilmannii* gastritis, suggesting that this bacterium induces less epithelial damage than *H. pylori*. Nevertheless, it is well known that in some cases even *H. pylori* gastritis may be characterised by a paucity of active inflammation. This picture is currently related to the presence of non-cytotoxic strains 14 and is similar to the one described for *H. heilmannii* gastritis. For this reason, only *H. pylori* positive subjects showing chronic gastritis with moderate–severe activity were included in our immunohistochemical study.

Furthermore, in the active phase, *H. pylori* is known to induce a predominantly Th1 helper 1 type immune response, which is important for the development of related gastric disease and is predominantly mediated by TNF-α release. 21 Moreover, TNF-α is involved in the modulation, by polymorphonuclear cells, of epithelial cell apoptosis, which is increased in the course of *H. pylori* infection. 22 This increased cellular death may be reflected by epithelial damage, which is present in *H. pylori* and absent in *H. heilmannii* gastritis. Therefore, in *H. heilmannii* gastritis, low TNF-α expression might account for the absence of histological signs of epithelial damage. Our finding of low mucosal TNF-α release in *H. heilmannii* infection suggests that the mechanisms of induction of mucosal inflammation by *H. pylori* and *H. heilmannii* could be different.

In conclusion, our results suggest that *H. heilmannii* gastritis is associated with some unusual histopathological and immunohistochemical features, which may render it a distinctive histopathological entity.