Gastric mucosal disease
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Citation for published version (APA):
Liu, Y. (2003). Gastric mucosal disease

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Chapter 6

Gastrin (G) cells and Somatostatin (D) cells in patients with dyspeptic symptoms

--- *Helicobacter pylori* associated and non-associated gastritis

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This manuscript was revised by *The Journal of Clinical Pathology*
Abstract

**Background:** Gastrin G cells and Somatostatin D cells are important regulators of gastric acid secretion. It has been suggested that an alteration in the relationship between gastrin and somatostatin plays a key role in gastroduodenal disease. Several factors, including *H. pylori* infection, can interfere with this physiological balance, resulting in disturbances in gastric acid secretion.

**Objective:** The aim of this study was to investigate the expression of gastrin G cells and somatostatin D cells in gastric biopsies of a cohort of patients with dyspeptic complaints, especially in relation with *H. pylori* status.

**Methods:** For this study 122 individuals with dyspeptic complaints were enrolled. Two antrum and two corpus biopsies were taken during upper endoscopy and evaluated semi-quantitatively according to the updated Sydney system. To evaluate the amount and distribution of neuro-endocrine cells, especially gastrin G cells and Somatostatin D cells, the sections were additional immunohistochemically studied. All subjects were divided into three groups: patients with *Hp* positive gastritis, with *Hp* negative gastritis and histologically normal gastric mucosa. The statistical evaluation was done using Chi-square test, Mann-Whitney U test and the Spearman rank correlation test.

**Results:** The number of G cells was significantly higher in patients with *Hp* positive gastritis when compared with the *Hp* negative gastritis and histological normal gastric mucosa. In contrast, the number of D cells was significantly lower in the *Hp* infected...
patients than in non-infected patients with and without gastritis. In Hp positive subjects, the percentage of G cells was elevated, but D cells were decreased.

Conclusions: Our results strongly suggest that Hp infection induces elevation of the number of antral G cells and reduction of the number of antral D cells. The resulting hypofunction of the inhibitory action of D cell against G cells and hyperfunction of the stimulating action of G cells itself may contribute to increased gastric acid output.

Key words: Gastritis; Helicobacter pylori; Gastrin; Somatostatin; Chromogranin
Introduction

The secretion of acid is an important function of the human stomach. In man, acid is continuously secreted by the gastric mucosa, though the rate of secretion varies. During periods of fasting, acid secretory rate is low but sufficient to maintain an intragastric pH below 2. Feeding stimulates acid secretion. The regulation of gastric acid secretion is achieved by the interplay between two major gastric endocrine cells: the gastrin G cell and the somatostatin D cell. Regulation of these cells occurs via stimulatory or inhibitory paracrine, endocrine and neural pathways. When food enters the stomach, the protein component stimulates G cells situated in the antral region of the stomach to release the hormone gastrin which stimulates the ECL cells and the parietal cells in the body region to secrete acid. As the acidity of the stomach and duodenum increases, protective feedback pathways are activated to inhibit further acid secretion. One important acid-mediated inhibitory control concerns the release of somatostatin by D cells. This hormone exerts paracrine inhibitory control on gastrin release by the antral G cells. So gastrin G cells and somatostatin D cells are important regulators of gastric acid secretion. It has been suggested that disturbance of the balance between gastrin and somatostatin might play a key role in gastroduodenal disease. *Hp* infection can interfere with these physiological control processes, resulting in alterations of gastric acid secretion. Several clinical and animal studies have been performed to clarify the influence of *Hp* on regulation of gastric acid secretion. Most of them are based on hormonal concentration, only a few have focused on the endocrine cells themselves. It
was reported that after infection, gastrin levels were found to be consistently elevated and that normal physiological negative feedback control of secretion was lost. Furthermore, after *Hp* eradication, gastrin levels decline and normal feedback control of gastrin secretion is restored \(^{15-17}\). So the purpose of our study was to investigate the relation between *Hp* status and the expression of gastrin G cells and somatostatin D cells in the antrum of gastric mucosa, to further clarify the mechanism of altered acid secretion due to *Hp* infection.
Material and Methods

**Trial design** 173 Patients (F/M=1.25) between the ages of 17 and 78 yrs (mean ± SD, 42.02 ± 15.63 yrs) with dyspepsia were recruited from the Dutch family doctors in 1993. Biopsies were taken from all patients to rule out severe gastric pathology. If the patients had reflux esophagitis, peptic ulcers and malignancy, they were excluded from this study. Ethics committee approval and informed consent were obtained. Besides the routine pathological examination of these patients, we also did additional immunostaining on endocrine cells in these biopsies.

**Endoscopy** All the patients underwent upper gastrointestinal endoscopy. Two antral and two corpus biopsies were obtained. The biopsies were used for the histological evaluation (Haematoxylin and eosin, Giemsa staining) and for the immunohistological staining for gastrin, somatostatin and chromogranin. Also an additional biopsy for a rapid urease test (CLO test) was obtained. Patients were classified as positive for *Hp* if one of the two tests (CLO and Giemsa staining) was positive and as negative if both tests were negative.

**Histological examination** Specimens were fixed in 10% formalin and routinely processed, and paraffin wax sections were cut into 3-4 um thick serial section and stained with Haematoxylin and eosin. The Giemsa staining was routine performed to detect the presence or absence of *H pylori*. The sections were interpreted by two experienced pathologists (TK and YL), who were unaware of the clinical and endoscopic findings.
The following four features were evaluated and graded according to the updated Sydney System: (1) chronic inflammation, which scored the chronic inflammatory infiltrate in the lamina propria; (2) activity through assessing the polymorphonuclear cell activity (neutrophil infiltration); (3) atrophy, which scored on the basis of proportion of the specialized gastric glandular loss with or without the replacement of intestinal metaplastic cells; and (4) intestinal metaplasia, scored on whether absent or occupying less than one third, more than one third, or more than two thirds of the mucosa present.

For both antral and corpus, biopsies were assessed semiquantitatively by a score (0, absent; 1-2, mild; 3-4, moderate; and 5-6, severe). The quantity of \( H. pylori \) in each Giemsa-stained specimen was graded according to Marshall et al\(^\text{18}\): no bacteria (grade 0), occasional bacteria found after searching (grade 1, +), scattered bacteria in most high power fields or occasional groups of numerous bacteria (grade 2, ++), and numerous bacteria in most high power fields (grade 3, +++).

All subjects were divided into three categories on the basis of histology and rapid urease test: \( Hp \)-positive gastritis, \( Hp \)-negative gastritis and histological normal gastric mucosa.

**Immunohistochemistry** All the specimens were immunostained with polyclonal antibodies to gastrin, somatostatin and chromogranine by the ABC Method (DakoCorp., Copenhagen). In brief, sections were dewaxed and rehydrated in graded alcohols. Endogenous peroxidase activity was quenched, antigen retrieval was performed by heating for 10 min at 100 °C in 0.01 M sodium citrate; and non-specific staining was reduced by a blocking step. The rabbit antibodies against gastrin (dilution, 1:1000),
somatostatin (dilution, 1:1600) and chromogranin (1:1000) were applied in PBS containing 1% bovine serum albumin and 0.1% Triton and incubated overnight at 4 °C (all three antibodies were from DakoCorp., Copenhagen). The following day, a three-step detection method was used as previously described, using a biotinylated goat anti-rabbit Ig antibody (DAKO, 1:500). Detection was performed with HRP conjugated ABcomplex (DAKO) for 60 min and peroxidase activity was detected with diaminobenzidine (fast DAB, sigma, St.Louis, MO) used according to the manufactures instructions, resulting in the formation of a brown reaction product. Finally the sections were briefly counterstained with haematoxyline, dehydrated in graded alcohols and mounted. Further controls consisted of omitting the primary and secondary antibodies and use of an appropriate Ig control.

Only sections demonstrating the entire axis from the superficial epithelium to the muscularis mucosa were examined. Cells that stained positive for gastrin G cells, somatostatin D cells and the endocrine markers were counted in a minimum of three high-power fields per specimens, without reference to the clinical histories. Each cell was identified as a G, D or endocrine type if a dark-brown granular reaction was produced by the ABC method. The numbers of endocrine cells were evaluated by two pathologists (TK and YL). 10 well-oriented vertical glands in random fields were counted for each patient. The results were expressed as the total number of cells counted per 10 adjacent glands. If the glandular structure was not well-oriented, then we counted two areas, with the most and the least intensive 10 adjacent glands, and calculated the mean. The average number of positively counted cells per 10 vertical glands was compared between groups.
Moreover the distribution of the cells was also reported (even or uneven).

**Statistical analyses** Data were analyzed using SPSS statistical Package. The Chi-square test was used to calculate the difference of G cells and D cells with respect to *Hp* status; the Mann-Whitney U test for testing the difference in the grading of gastritis features; and the Spearman rank correlation test for testing the correlation between different gastritis features and *Hp* status, and between G cells and D cells. A *P* value of less than 0.05 was considered to be statistically significant.
Results

From the original 173, a total of 122 subjects, who underwent the endoscopy for dyspeptic complaints, and for whom all the data were complete, were analyzed.

Characteristics of the patients studied are shown in Table 1. The age and sex distribution did not differ significantly among groups.

Hp status and histopathological changes

H. pylori infection was found in 58 cases (58/122=47.54 %) with both procedures (CLO and Giemsa staining), the remaining 64 patients being H pylori negative (64/122=52.46 %). Among the non-infected individuals, 47 were without any histological changes, and only 17 had features of chronic gastritis.

The histological scores for chronic inflammation, activity, gland atrophy and intestinal metaplasia were higher in Hp positive than in Hp negative patients (P<0.05). Hp infection was significantly associated with an increased antral inflammation and activity (P<0.01). Mucosal atrophy was more prevalent in patients with Hp infection than Hp negative individuals. Antral atrophy was more marked than corpus atrophy (P<0.01).

Expression of gastrin, Somatostatin and Chromogranin

There was no immunoreactivity when the primary antibodies were omitted from the staining procedure. G cells were only present in the antrum, while D cells and chromogranin positive cells were expressed both in antrum and corpus.
The staining patterns of the gastrin, somatostatin and chromogranin were similar. Positive cells revealed cytoplasmic granules, located between the nucleus and basement membrane. Most of the positive cells were located in the middle third of the gastric glands, few in the upper or deeper part. However in \textit{Hp} positive patients, the distribution of chromogranin- and gastrin-positive cells moved slightly upwards to just beneath the foveolar gastric pit.

The \textit{Hp} infected individuals had significantly higher numbers of G cells and lower numbers of D cells than both non-infected individuals and the histological normal gastric mucosa. Significant differences existed between infected and non-infected individuals or normal groups both for G cells and D cells (Table 2). Significant correlation was found between antrum G cells and D cells with a correlation coefficient of 0.293 (P<0.01).

The density of G cells and D cells in each group were compared in Fig 1. The highest score for G cells was found in \textit{Hp} infected patients. Also the lowest score for D cells was observed in \textit{Hp} infected patients. The mean density of G cells and D cells in non-infected and normal groups revealed no differences.

Fig 2 summarizes G cells and D cells' density in relation to the amount of \textit{Hp}. The score of G cells was lowest in the \textit{Hp} negative group. It showed a phenomenon that the mean density elevated with the amount of \textit{Hp}, however, the highest value was observed in the group with moderate amount of \textit{Hp}, not in the group with the highest amount of \textit{Hp}. As for the D cells, the highest score was found in the \textit{Hp} negative group.
The cell density in relation to the severity of inflammation is shown in Fig 3. With the development of inflammation degree, G cells’ density was increasing and D cells’ was decreasing.

**Percentage of G cells and D cells in the stomach**

In addition to the gastrin and somatostatin, chromogranin was used to identify all endocrine cells. The total number of chromogranin positive cells was 49.12 ± 21.68 q 49.00 ± 19.45 q 50.47 ± 22.87 in Hp infected, non-infected and normal individuals respectively. There was no significant difference among the three groups (P>0.05).

The percentage of G cells and D cells of all endocrine cells in the stomach was 52.03 % and 17.5% in normal individuals, and 47.06% and 15% in the Hp negative group. No statistical difference was detected between these two groups.

However in the Hp infected subjects, G cells were elevated to 70.05% and D cells were decreased significantly to 4.91%. The difference between Hp infected and non-infected or normal individuals was significant (P< 0.01) (Table 3).
Discussion

Endocrine cells related to the acid secretion in the stomach

The major endocrine cells in the stomach, which are known to play an important role in acid secretion, are the gastrin (G) and the somatostatin (D) cells. Unlike other secretory products of the mucosa, these endocrine cells discharge their granules into the lamina propria rather than into the gastric lumen. From here the hormones enter the blood to exert an endocrine effect or influence neighboring cells (paracrine effect). The location of these endocrine cells is not random, since the somatostatin released by antral or corpus D cells should have equal access to the G cells of these regions of the gastric mucosa.

In the antrum about 50% of the whole endocrine cell population are G cells and 15% are D cells. In the corpus mucosa, however, a major portion of the endocrine cells are ECL cells. However, no thorough study has been done about the exact percentage of these endocrine cells. To our knowledge, this study provides the first clinical evidence about the precise proportion of G cells and D cells in the normal stomach and the range in Hp positive subjects. It was shown in our study that about 52% of all the endocrine cells in the antrum are G cells and 17.5% are D cells. While in corpus, 8.3% are D cells and no G cells are found. Our results are comparable to other studies. We also found that the percentage of G and D cells was variable when infected with Hp—elevated to 70% for G cells and decreased to 3% for D cells.

Gastrin vs acid secretion
Gastrin is released from G cells in the gastric antrum and acts via the circulation to stimulate acid secretion. The first report on the relationship between *Hp* infection and plasma gastrin concentrations was by Levi et al. in 1989. These authors reported that both basal and stimulated acid secretion plasma gastrin levels were significantly higher in *Hp* positive patients compared with *Hp* negative ones. Their data clearly demonstrated that *Hp* infection induced hypergastrinemia that was followed by an increase in acid secretion. Several investigators have demonstrated that elevated acid secretion decreases after *Hp* eradication, with simultaneous reduction of serum gastrin. There is now increasing evidence that *Hp* infection alters gastrin and acid secretory function through a variety of mechanisms. A number of investigators have shown that *Hp* infected patients with duodenal ulcer and *Hp* positive healthy volunteers have higher basal serum gastrin levels compared with uninfected controls. Examination of antral biopsies from *Hp* infected individuals has shown that gastrin synthesis is increased. Our results are consistent with the finding that the total number of G cells is significantly increased, although others suggest that the number of G cells is unchanged.

More recently, available evidence suggests that gastrin does not stimulate parietal cells directly, but that it acts by stimulating histamine from the ECL cells in the oxyntic mucosa. Histamine then stimulates the parietal cells to secrete HCl.

**Gastrin-somatostatin link**

Gastrin release is suppressed when the luminal antral pH falls below 3. In addition, there is an inhibitory control exerted on gastrin release by cholecystokinin (CCK). The
inhibition of gastrin release exerted by both gastric acid and CCK is mediated mainly via the release of somatostatin by D cells within the antral mucosa. These D cells lie in close proximity to the G cells and the somatostatin they release exerts a paracrine inhibitory control on both gastrin synthesis and release. Several studies have now demonstrated lowered concentrations of somatostatin within the antral mucosa of subjects with *Hp* antral gastritis \(^{30,33,36,40-43}\). In addition, somatostatin mRNA concentrations are lowered, indicating a reduced synthesis of this inhibitory hormone \(^{33,42}\). These findings are consistent with our results that the amount of D cells was significantly lower in *Hp*-infected persons compared with uninfected and normal individuals, while the amount of G cells was significantly higher in *Hp*-infected group. Therefore, the major defect leading to hyperfunction of gastrin appears disruption of the inhibitory effect of somatostatin on the G cell. Apparently *Hp* antral gastritis increases gastrin by producing deficiency of antral somatostatin and thus the normal inhibitory influence of this hormone exerts on gastrin release.

**Possible mechanisms in Helicobacter pylori infection**

The mechanism responsible for elevated gastrin secretion and diminished amount of somatostatin remains unclear, but several hypotheses exist. The first proposed by John Calam et al.\(^{20}\) suggest that ammonia generated by *Hp* urease may produce an alkaline environment in the vicinity of G cells, thus stimulating gastrin release. Acid inhibits gastrin release, probably by stimulating D cells to release somatostatin, so that local alkalization might have the opposite effect \(^{40}\).
Inflammatory mediators associated with antral gastritis might also affect the G and D cells. *Hp* infection increases the mucosal expression for many cytokines including: interleukins (IL)-1β, tumor necrosis factor (TNF-α), interferon – γ (IFN γ) and platelet-activating factor (PAF)\(^45\). Weigert N et al\(^46\) found that IL-1β and TNF-α had the same effect on releasing gastrin from the rabbit G cells. Calam et al\(^47\) found that the reduced somatostatin mRNA in *Hp* infection is attributable to the release of TNF-α that inhibits antral D cell function, and incubation of D cells with TNF- α for 24 hours resulted in a 40% decrease in somatostatin release in response to CCK.

It is possible that biogenic amines produced by *Hp* might also affect the regulation of D cells, but again this remains largely hypothetical\(^48\).

**Clinical outcome related to acid secretion in *Hp* infected subjects.**

It is clear that *Hp* plays a major causative role in gastric and duodenal ulcers (DUs) as well as gastric cancer of the intestinal type. Studies of gastric physiology in patients are revealing complex interactions between the bacterium and gastric acid that seems to predict the clinical outcome. For example, among infected patients, acid secretion tends to be high in patients with DUs but low in those with gastric cancer and their close relatives. Why does the same infection produce different patterns of acid secretion in different individuals? Therefore, examining the mechanisms responsible for these various changes in physiology should illuminate the pathways that lead to the different clinical outcomes of this infection.
Recent work shows that more aggressive strains of *Hp* have greater effects on somatostatin/gastrin physiology. It was reported that the presence of antibodies to the Cag A protein has been linked with the DU disease and mucosal atrophy, gastric carcinoma, which are associated with high or low acid secretion. Another variable is the distribution of gastritis. *Hp* infection in some subjects results in an antral predominant gastritis with increased acid secretion and propensity to duodenal ulcer disease, in others a body gastritis with low acid secretion and a predisposition to atrophic gastritis and gastric cancer, and in the majority of subjects a mixed gastritis with no overall change in acid secretion. The net effect on acid presumably depends on which mechanism predominate. It is known that inhibition of acid secretion by proton pump inhibitors transforms antral predominant gastritis into body predominant gastritis and it has therefore been suggested that subjects with naturally low acid output prior to contracting *Hp* infection may develop a body predominant gastritis, whereas subjects with a naturally high acid output may develop an antral predominant gastritis.
Conclusion

Our results strongly suggest that *Hp* infection induces elevation of the number of antral G cells, but diminishes mucosal expression of D cells. The resulting relative hypofunction of the inhibitory action of D cell against G cells and hyperfunction of the stimulating action of G cells itself may be responsible for increased gastric acid output.

It is concluded that decreased D cells number in patients with *Hp* related chronic gastritis might be one of the reason for the existing hypergastrinemia. Increased circulating gastrin and diminished mucosal expression of somatostatin tend to increase acid secretion.
References


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Table 1. Characteristics of the patients

<table>
<thead>
<tr>
<th>Hp status</th>
<th>No of patients</th>
<th>Mean age (yr)</th>
<th>Sex (F/M)</th>
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<tr>
<td>positive</td>
<td>58</td>
<td>47.36±15.42</td>
<td>0.87 (27/31)</td>
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<tr>
<td>negative chronic gastritis</td>
<td>17</td>
<td>44.00±16.65</td>
<td>0.89 (8/9)</td>
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<tr>
<td>normal (no gastritis)</td>
<td>47</td>
<td>41.72±14.21</td>
<td>0.81 (21/26)</td>
</tr>
</tbody>
</table>

Table 2. The density of the endocrine cells, gastrin and somatostatin in Hp infected, non-infected and normal subjects

<table>
<thead>
<tr>
<th>Status</th>
<th>No of subjects</th>
<th>Chromogranin cells density (mean±SD)</th>
<th>G cells density (mean±SD)</th>
<th>D cells density (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Antrum</td>
<td>corpus</td>
<td>Antrum</td>
</tr>
<tr>
<td>Hp(+)</td>
<td>58</td>
<td>49.12±21.68</td>
<td>22.22±15.45</td>
<td>34.41±19.04*</td>
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<tr>
<td>Hp(-) chronic gastritis</td>
<td>17</td>
<td>49.00±19.45</td>
<td>28.18±16.88</td>
<td>23.06±10.66</td>
</tr>
<tr>
<td>normal (no gastritis)</td>
<td>47</td>
<td>50.47±22.87</td>
<td>19.13±9.49</td>
<td>26.26±13.81</td>
</tr>
</tbody>
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* Significant difference (P < 0.01)
Table 3. The percentage of gastrin and somatostatin positive cells in the whole amount of the endocrine cells in *Hp* infected and non-infected subjects

<table>
<thead>
<tr>
<th>Status</th>
<th>No of subjects</th>
<th>G cells %</th>
<th>D cells %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>antrum</td>
<td>corpus</td>
</tr>
<tr>
<td><em>Hp</em>(+)</td>
<td>58</td>
<td>70.05%</td>
<td>4.91%*</td>
</tr>
<tr>
<td><em>Hp</em>(−) chronic gastritis</td>
<td>17</td>
<td>47.06%</td>
<td>15%</td>
</tr>
<tr>
<td>normal (no gastritis)</td>
<td>47</td>
<td>52.03%</td>
<td>17.5%</td>
</tr>
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</table>

* Significant difference (P < 0.01)

Fig 1. The mean density of G cells and D cells in the antrum in the different groups.
Fig 2. The density of G cells and D cells in the antrum in relation to the amount of Hp

Fig 3. The density of G cells and D cells in the antrum in relation to the severity of inflammation