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Original Article

A Morphometric Study of Antral G-Cell Density in a Sample of Adult General Population: Comparison of Three Different Methods and Correlation with Patient Demography, *Helicobacter pylori* Infection, Histomorphology and Circulating Gastrin Levels

Fredrik Petersson[1], Kurt Borch[2], Jens F Rehfeld[3] and Lennart E Franzén[4]

[1]Pathology Research Department, Ryhov Hospital, Jönköping; Departments of Surgery[2] and [1]Pathology II, University Hospital of Linköping, Sweden; [3]Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Denmark and [4]Department of Pathology, University Hospital, Örebro, Sweden

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Abstract: *Helicobacter pylori* infection has been linked to hypergastrinemia and either decreased or normal G-cell content in the antral mucosa. To clarify this controversial issue, we quantitatively determined antral G-cell content on the same biopsy specimens with three different methods and examined whether these methods are intercorrelated and the relation of these methods to plasma gastrin concentrations, demography, the occurrence of *H. pylori* infection and chronic gastritis. Gastric antral mucosal biopsy sections from 273 adults (188 with and 85 without *H pylori* infection) from a general population sample were examined immunohistochemically for G-cells using cell counting, stereology (point counting) and computerized image analysis. Gastritis was scored according to the updated Sydney system. Basal plasma gastrin concentrations were measured by radioimmunoassay. The three methods for G-cell quantification were poorly correlated and the results showed no correlation with basal plasma gastrin concentrations. The antral G-cell density and scores for *H. pylori* colonization were positively related to age. Neither the scores for chronic inflammation, nor the scores for inflammatory activity, atrophy or intestinal metaplasia were consistently related to the antral G-cell content. In conclusion, the results of three techniques for G-cell quantification in the gastric antral mucosa were poorly intercorrelated and none of the methods correlated with plasma gastrin concentrations. Age and scores for *H pylori* colonization seem to be determinants of the G-cell density. That common morphometric techniques correlate poorly is of utmost importance to bear in mind when quantitative morphological studies are planned, compared or interpreted.
Key Words: Gastrin, cell counting, computerized image analysis, gastritis, inflammation, morphometry, point counting, stereology, stomach

Introduction

Helicobacter pylori (*H. pylori*) is a widespread human pathogen infecting the gastric mucosa. It is the main cause of chronic gastritis [1-5] and peptic ulcer disease [6]. *H. pylori* infection is associated with increased gastrin concentration in the circulation [7, 8]. This association has also been shown in an adult general population where the titers of anti-*H. pylori* IgG antibodies were positively correlated with basal gastrin concentrations in plasma [9]. Both basal and postprandial gastrin concentrations are reported to be increased [10, 11] and to return to normal, when *H. pylori* is eradicated [12]. The mucosal content of bioactive amidated gastrin and pro-gastrin was increased in patients with dyspeptic symptoms and *H. pylori* infection, but no correlation was found between the antral mucosal content of gastrin measured biochemically and the plasma concentrations [13]. Morphological studies investigating the antral G-cell content have also been performed. In these, G-cells were quantified in various ways after immunohistochemical staining. Some focused on a certain area or a certain length of the mucosa, with or without

Table 1 Subject distribution according to age, sex and *H. pylori* infection in the study population

	Subjects (N)			
	H Pylori-negative		*H Pylori*-positive	
Age (years)	Male	Female	Male	Female
35-44	10	10	6	5
45-54	9	9	22	14
55-64	10	8	29	20
65-74	12	8	32	35
≥75	3	6	13	12

inclusion of the mucosal surface component [14-18]. Thus, the studies have yielded different results regarding the antral G-cell density.

The aims of the present study were (1) to quantify the antral mucosal content of G-cells in a sample of a general population using immunohistochemistry and applying three different morphometric methods on the same biopsy specimens; (2) to investigate whether these methods correlate with each other and (3) to relate the morphometric findings to circulating gastrin levels, demographic factors, *H. pylori* infection and gastric mucosal inflammatory changes, atrophy and intestinal metaplasia. To the best of our knowledge, no previous study comparing the three studied morphometric methods, i.e., cell counting, stereology and computerized image analysis on the same biopsy specimens, with regard to the content of endocrine cells in the gastrointestinal tract has been published in the English language.

Materials and Methods

Study Population

The study was approved by the local ethics committee and informed written consent was obtained from all the volunteers. Results of screening with gastroscopy for gastritis and *H. pylori* infection, including classification of gastritis according to the updated Sydney system, in this population have previously been reported in detail [5]. From this group of 501 subjects, 273 were selected for the present study (**Table 1**). All subjects with *H. pylori* infection were included. Up to 20 uninfected controls were randomly chosen from each age group. In age groups with fewer than 20 individuals all subjects were included. Results of the histomorphological evaluation according to the Sydney system in the *H. pylori* infected subjects are shown in **Table 2**.

Gastroscopy and Routine Histology

During gastroscopy, three biopsy specimens for histological examination were collected from the gastric body and antrum, respectively. One additional biopsy specimen from each location was analyzed for *H. pylori* by urease test (CLO-test, Delta West Pty Ltd, Bentley, Australia), which was read after twenty minutes (score 4 if positive), one hour (score 3 if positive), three hours (score 2 if positive) and twelve hours (score 1 if positive).

Table 2 Histological features of the gastric biopsy among *H. pylori* infected subjects.

	Subjects (N)					
	Score (Antrum)			Score (Corpus)		
Histological variable	1	2	3	1	2	3
Chronic inflammation	62	103	15	132	33	2
Inflammatory activity	109	33	6	63	12	1
Intestinal metaplasia	71	7	3	11	3	1
Atrophy	68	19	1	17	8	2
H. pylori colonization	146	64	52	91	45	19

After orientation, the biopsy specimens collected for histological examinations were fixed in 4% neutral formaldehyde and embedded in paraffin. After routine processing and staining with haematoxylin and eosin, alcian blue, periodic acid-Schiff and Giemsa, the biopsy sections were semi-quantitatively assessed (according to the Sydney system) on a four graded scale (0: none, 1: mild, 2: moderate, 3: severe) regarding chronic inflammation, inflammatory activity (i.e. the density of neutrophilic granulocytes), intestinal metaplasia, glandular atrophy and colonization by *H. pylori*. According to the Sydney system, the scores for each variable given to each case are based on a weighted result of examination of three sections from all three biopsies from respective location.

Immunohistochemistry and Morphometry

For the G-cell studies, 4 micron thick sections were cut perpendicular to the surface onto silano slides for DAKO TechMate Horizon. After deparaffinization, the slides were treated in a microwave oven for antigen retrieval (2 times 5 minutes each at 780 W in citrate buffer at pH 6.0).

The negative controls (one for each case) were processed with Antibody Diluent from DAKO. The immunohistochemical processing was performed in DAKO Techmate Horizon (DAKOPATTS, Älvsjö, Sweden) using the DAKO ChemMate Reagents. One positive control section was put on each slide. The primary antibody for gastrin labeling was a rabbit polyclonal (A 0568, dilution 1:3000) and the secondary was a biotinylated goat anti-mouse and anti-rabbit antibody (AB2, streptavidin peroxidase, DAKO, Glostrup, Denmark). The gastrin antibody measures gastrin-17 and -34 in both sulphated and non-sulphated forms equally well.

Cells showing distinct cytoplasmic gastrin immunoreactivity were classified as G-cells. The procedure for double staining (for computerized image analysis) was as follows: The cover glasses from the gastrin-stained slides were removed and another immunohistochemical staining for cytokeratins was performed. The slides were treated in a microwave oven for antigen retrieval (20 min. at 650 W in Tris-EDTA buffer at pH 9.0). The antibody used was Cam5.2 (Becton and Dickinson, U.K, dilution 1:5) and anti-Mouse Rabbit IgG BA 1400, Vectastatin ABC-AP kit AK5000, Vector Red Substrate Kit SK- 5100.

Cell counting was performed on the best-oriented biopsy section chosen on low power (40x) magnification. The number of G-cells and epithelial cells (surface, neck and glandular areas included) were counted. In order to avoid mechanical artifacts, the counting started on high power field (400x) from the end of the biopsy section located at the nearest long side of the slide. Results are expressed as number of G-cells per 1000 epithelial cells. For stereology (point counting), the same sections (and biopsy specimens) as for cell counting were used. The procedure started (as with the cell counting) on high power field (400x) from the end of the biopsy located at the nearest long side of the slide. Microscopic images, ten high power fields for each subject, were digitized into a computer (Macintosh G3) and a point grid with 100 points (+) was superimposed. The whole thickness of the mucosa was analyzed using 40x objective, starting in the basal part and then sequentially moving upwards to the surface and then down in a zigzag fashion. The final magnitude on the screen was 1080x. The distance between the points was 35 microns. The results of the point counting are given as percentage of positive hits on the cytoplasm of the G-cells out of all hits on G-cell cytoplasm and epithelial cytoplasm.

Image analysis (same sections and biopsy specimen as with cell counting and point counting) by color threshholding was performed on a computerized image analysis system using a Leica DMRXE microscope (Leica Microsystems Wetzlar GmbH, Germany). The image processing and analysis was performed with the Leica Q-Win (Leica Microsystems Imaging Solutions Ltd, Cambridge, U.K.). Ten fields of image (40x) were used for each subject. The final magnitude on the screen was 525x. The red stain of the epithelial cells and the brown-black stain of the G-cells were thresholded in the HSI color space and the number of positive pixels recorded. The results are given as percentage area of gastrin positivity of the total cytokeratin positive area. The whole thickness of the mucosa was analyzed just as for cell- and point counting. All morphometric analyses were performed by the same pathologist (FP).

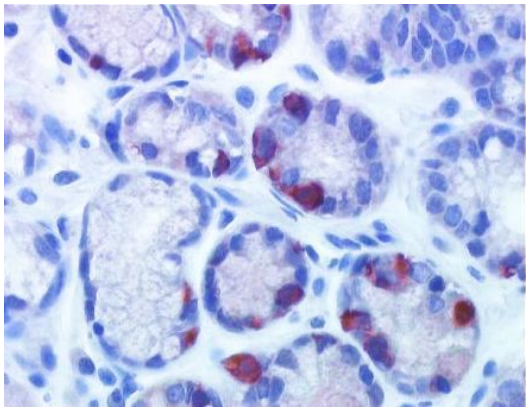

Figure 1 Photomicrograph of the glandular part of the antral mucosa of a subject in the general population sample. It has been immunohistochemically stained for gastrin and shows G-cells mainly located within the basement membrane of the glands.

Measurement of Gastrin Concentrations in Plasma

Plasma samples for gastrin measurement were collected in the fasting state immediately preceding gastroscopy and stored at –80°C until analysis. The gastrin concentration (sulphated and non-sulphated) was measured using antibody no. 2604 by radioimmunoassay as previously described by Stadil and Rehfeldt. The upper limit of gastrin concentration in plasma of healthy humans is 50 pmol/L.

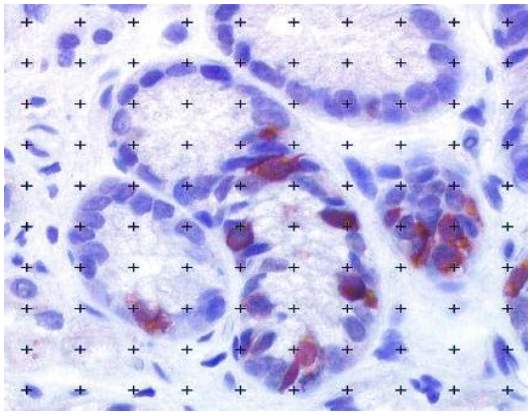

Figure 2 Photomicrograph of the glandular part of the antral mucosa of a subject in the general population sample. It has been immunohistochemically stained for gastrin and shows G-cells with a superimposed grid used for assessing G-cell density with point counting.

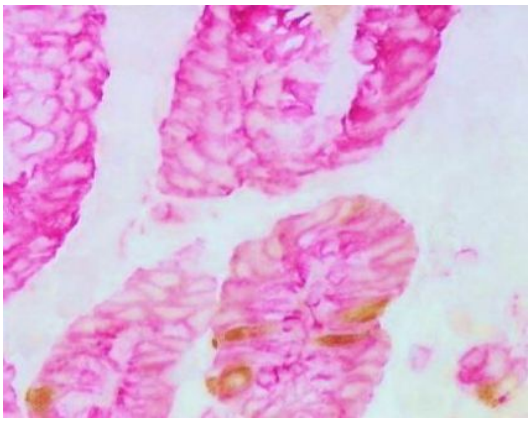

Figure 3 Photomicrograph of the glandular part of the antral mucosa of a subject in the general population sample. It has been immunohistochemically double stained for gastrin for visualizing G-cells (brown) and cytokeratin (Cam 5.2) for visualising epithelial cell cytoplasm (red).

Statistical Evaluation

Results regarding continuous data are summarized as median with range. In the statistical analyses, the null hypothesis was tested with the Mann-Whitney U-test for comparisons between groups. The Spearman rank correlation analysis was used to test correlations between two variables. A general linear model (GLM) was applied with analysis of variance regarding the categorical data and with regression analysis regarding the continuous data. Age was a co-factor and the other variables were co-variants. *H. pylori* colonization assessed microscopically according to the Sydney system, and the scores of the urease test were included in the GLM analysis separately. $P<0.05$ was considered significant.

Results

The immunohistochemical staining for gastrin was distinct and G-cells were easily detected and separated from other cells in the mucosa (**Figure 1**). This distinction remained when images were digitized onto the computer for point counting and image analysis (**Figures 2 and 3**). The staining for cytokeratin (Cam 5.2) was heterogeneous with weaker staining of the epithelial cells located in the basal third of the glandular compartment. However, this was uniform and did not differ between mucosa with or without inflammation.

Table 3 Correlation coefficients between the three different methods for quantification of gastrin cells in the antral mucosa and basal plasma gastrin levels

	Plasma gastrin	Cell counting	Image analysis
Cell counting	0.13		
Image analysis	0.06	0.44	
Stereology	0.07	0.49	0.11

Correlations between Morphometric Methods and Plasma Gastrin Concentrations

The correlations between the three methods for G-cell quantification as well as between each of these methods and the plasma gastrin concentrations were poor (**Table 3**). The plasma concentrations of gastrin are given in **Table 4**.

General Linear Model on Cell Counting

Age ($p<0.001$), *H. pylori* colonization assessed histologically ($p=0.044$) and atrophy in the corpus ($p=0.037$) were positively related to the density of G-cells. When *H. pylori* colonization assessed histologically was exchanged with the scores of the urease test, no significant association with G-cell density was found. However, age and atrophy in the corpus were still positively related to the G-cell density ($p<0.001$ and $p=0.016$, respectively).

General Linear Model on Point Counting

Age and the degree of chronic inflammation in the antrum were positively related to the percentage of G-cell hits ($p=0.017$ and $p=0.021$, respectively) when *H. pylori* infection was determined as positive urease test. When *H. pylori* colonization according to histological assessment was included, the degree of colonization in the corpus ($p=0.009$) and the degree of chronic inflammation in both the corpus ($p=0.028$) and antrum ($p=0.001$) were positively correlated with the percentage of G-cell hits.

General Linear Model on Image Analysis

Increasing age was the only variable that was associated with increasing percentage of G-cell positivity, when *H. pylori* colonization was included, as assessed histologically or when *H. pylori* infection was determined as positive urease test ($p<0.001$).

Group Comparisons

Age: When all subjects were stratified into five groups according to their age, some significant differences appeared regarding the G-cell content as measured with image analysis and cell counting (**Table 5**). A similar pattern appeared in G-cell content between different age groups in both the *H. pylori* positive and *H. pylori* negative groups, respectively (**Tables 6 and 7**).

H. pylori: When *H. pylori* infected subjects (assessed histologically) with *H. pylori* in both the corpus and antrum were compared with non-infected subjects, antral G-cell content as measured by point counting was higher in the group with infection (7.4 [0-36.5] vs 2.7 [0-15.2], $p<0.001$). However, neither cell counting nor image analysis showed any difference in this respect. When the group with *H. pylori* infection in the corpus only was compared with the non-infected group, antral G-cell content also was higher in the infected group (6.5 [0-11.8] vs 2.7 [0-15.2], $p=0.003$), as measured by point counting. No differences were found between subjects with infection strictly located in the antrum and the non-infected group. However, there were only four subjects with selective antral infection. No differences were found regarding cell counting

Table 4 Basal plasma gastrin level in subjects of different age group with or without *H. pylori* infection

Age (years)	Basal plasma gastrin concentration (pmol/L, median[range])	
	H. pylori-positive	H. pylori-negative
35-44	14.0 [8-23]	15.0 [8-34]
45-54	18.2 [4-61]	13.4 [7-26]
55-64	18.0 [7-83]	12.0 [9-19]
65-74	23.0 [7-198]	13.0 [6-171]
≥75	24.0 [9-285]	14.0 [12-255]

Table 5 Comparison of antral G-cell content between different age groups of the entire study population (N=273)

Age groups compared (years)	Image analysis	Point counting	Cell counting
35-44 vs 45-54	p<0.0001	NS	NS
35-44 vs 55-64	p<0.0001	p=0.02	p<0.0001
35-44 vs 65-74	p<0.0001	NS	p<0.0001
35-44 vs ≥75	p=0.0002	NS	p<0.0001
45-54 vs 55-64	p=0.002	NS	p<0.0001
45-54 vs 65-74	NS	NS	p<0.0001
45-54 vs ≥75	NS	NS	p=0.0009
55-64 vs 65-74	p=0.004	NS	p=0.005
55-64 vs ≥75	p=0.001	NS	p=0.03
65-74 vs ≥75	NS	NS	NS

NS, no statistical difference (p≥0.05) according to Mann-Whitney U-test.

Table 6 Comparison of antral G-cell content between different age groups among subjects with *H. pylori* infection (N=188)

Age groups compared (years)	Image analysis	Point counting	Cell counting
35-44 vs 45-54	p=0.02	NS	p=0.001
35-44 vs 55-64	p=0.002	NS	p=0.001
35-44 vs 65-74	p=0.003	p=0.03	p=0.0008
35-44 vs ≥75	p=0.02	NS	p=0.004
45-54 vs 55-64	p=0.03	NS	p<0.0001
45-54 vs 65-74	NS	NS	p<0.0001
45-54 vs ≥75	NS	NS	p=0.001
55-64 vs 65-74	p=0.02	p=0.02	NS
55-64 vs ≥75	p=0.007	NS	NS
65-74 vs ≥75	NS	NS	NS

NS, no statistical difference (p≥0.05) according to Mann Whitney U-test.

Table 7 Comparison of G-cell content between different age groups among controls without *H. pylori* infection (N=85)

Age groups compared (years)	Image analysis	Point counting	Cell counting
35-44 vs 45-54	p=0.0008	NS	NS
35-44 vs 55-64	p<0.0001	p=0.04	p=0.001
35-44 vs 65-74	p=0.0006	NS	p=0.006
35-44 vs ≥75	p=0.002	NS	p=0.02
45-54 vs 55-64	p=0.005	NS	p=0.04
45-54 vs 65-74	NS	NS	NS
45-54 vs ≥75	NS	NS	NS
55-64 vs 65-74	NS	p=0.0001	NS
55-64 vs ≥75	p=0.01	NS	p=0.002
65-74 vs ≥75	NS	NS	NS

NS, no statistical difference (p≥0.05) according to Mann Whitney U-test.

or image analysis.

In the group with *H. pylori* in both the antrum and corpus, comparisons of G-cell content were made separately between subjects with different histological scores of *H. pylori* colonization in the antrum and corpus, respectively. No differences appeared regarding different scores of *H. pylori* colonization in the corpus; but considering the

antrum, the group with scores 3 and 2 had higher density of G-cells (cell counting) than the group with score 1 (10.4 [1.8-18.1]% and 9.0 [0.1-22.3]%. vs 6.7 [0-14.1]%, p=0.0004 and p=0.04, respectively). We also found that the subjects with score 3 in the antrum had a higher G-cell content on image analysis than those with score 1 in the antrum (1.0 [0.2-2.0]% vs 0.3 [0.1-1.0]%, p=0.001). There were too few subjects with selective infection in either the corpus or the antrum for a meaningful subgroup analysis.

Considering the urease test, there were no differences with regard to the results of the three methods for G-cell quantification between infected subjects with different scores in the corpus. Regarding the antrum, however, subjects with score 4 on the urease test had significantly higher density of G-cells by cell counting compared to the group with score 1 (10.2 [0.8-22.4]% vs 6.6 [0.1-12.7]%, p=0.03). Similarly, the group with score 4 on the antral urease test showed increased G-cell density (cell counting) compared to those with score 2 (10.2 [0.8-22.4]% vs 7.2[0.1-17.8], p=0.008). None of the other group comparisons with regard to the urease test score revealed any differences in G-cell content.

Chronic Inflammation: When comparing groups with different topographical distribution of gastritis, we found that those with chronic inflammation in both the antrum and corpus had increased G-cell content compared to the group with normal gastric mucosa as measured by point counting (7.5[0-36.5] vs 3.4[0-15.2], p<0.001). Also, the group with strictly antral inflammation (N=19) had increased G-cell count compared to those with normal gastric mucosa as measured with point counting (9.1[2.5-21.4] vs 3.4[0-15.2], p<0.001). The group with chronic inflammation in the corpus only showed no significant change in G-cell content compared to subjects without gastritis. However, this group comprised of only 9 subjects. When comparisons were made in the group with inflammation in both the corpus and the antrum regarding different degrees of chronic inflammation either in the corpus or the antrum, we found no differences in G-cell content as measured with any of the methods.

Inflammatory Activity, Atrophy, Intestinal Metaplasia and Gender: When subjects with different degrees of inflammatory activity in the antrum or corpus were compared, no differences in G-cell content appeared with any method. The same was true regarding atrophy and intestinal metaplasia.

The results of the three methods for G-cell quantification did not differ between men and women, neither in the gastritis group nor in the control group.

Discussion

This study was performed in order to compare three different techniques for quantifying the G-cell content of the gastric antral mucosa in an adult general population and to relate the findings to age, sex, *H. pylori* infection, mucosal inflammatory changes and basal plasma gastrin levels. Earlier morphometric studies [14-18] have shown conflicting results regarding the gastric mucosal content of G-cells. Therefore, we used different morphometric techniques on the same biopsy specimens in order to elucidate whether previously reported discrepant results may be related to the choice of method. We are not aware of any previous study published in the English language, which has addressed this issue in the way that we have done.

Increasing age was the only variable that almost consistently correlated with increased G-cell content in the general linear model. This was substantiated by the findings in the group comparisons. However, point counting did not show these differences. That age may influence the distribution of endocrine cells in the gastrointestinal tract has been shown in a previous study [19], where the duodenal mucosal contents of cholecystokinin, somatostatin and serotonin-secreting cells were negatively related to age. The authors used immunohistochemically stained tissue sections in which endocrine cells were quantified with an image analysis system (number of positive cells/mm^2), a method which bears some resemblance to our image analysis technique. On the other hand, Yacoub *et al* [20] found no effect of age (using multivariate ANOVA statistics) on endocrine cell densities, including antral G-cells, using a computerized image analysis system (number of cells/mm^2 mucosal area) in patients with reflux esophagitis, gastric or duodenal ulcer and non-ulcer dyspepsia. The *H. pylori* status or occurrence of chronic gastritis was not

included. As in the present study, G-cell density did not differ between women and men.

H. pylori colonization and chronic inflammation showed a complex association with G-cell density. In monovariate analysis and as compared with subjects with normal mucosa, the *H. pylori* infected population and the population with chronic gastritis had an increased G-cell density as measured with the point counting method. Furthermore, the subgroup with gastritis limited to the corpus mucosa showed increased G-cell content as measured with the point counting method, when compared to subjects with normal mucosa. There was also a positive correlation between the G-cell density according to the cell counting method and the scores of *H. pylori* colonization assessed histologically in the antrum. However, subgroup analysis regarding different degrees of chronic inflammation yielded no differences. In multivariate analysis, increasing score of *H. pylori* colonization as assessed histologically was associated with increased G-cell density as determined with cell counting and point counting. The degree of inflammatory activity showed no associations with G-cell density. Taken together this may indicate that *H. pylori per se*, rather than the inflammatory process, has an impact on the G-cell density. However, due to the large number of statistical calculations, the occurrence of statistical type 2 errors probably blurs the picture and makes the interpretation difficult regarding the true relations between the various demographic and morphological parameters and the antral G-cell content, even more so when taking into account the poor correlations between the three morphometric methods for G-cell quantification.

The finding that the three morphometric methods showed poor correlation with the plasma levels of gastrin can be explained by the fact that the morphological strategies focused on the number or volume of G-cells or their intracellular gastrin content rather than on their secretion activity. This is in accordance with previous published results showing a lack of association between serum gastrin levels and the number of G-cells per mm mucosa [22].

The poor correlations between the three morphometric methods may appear puzzling, but less so when one contemplates what they actually measure. The cell counting method includes the number of clearly defined G-cells, their volume disregarded, whereas the point counting and image analysis techniques assess the areas/volumes of these cells. One can then raise the question why the two last mentioned methods were also poorly correlated, since both methods focus on the same characteristics. The methods, however, assess different cellular structures. With the point counting method, all epithelial cytoplasm was included when appropriate according to the method. In contrast, in the computerized image analysis, we only included the cytoplasmic area that was positively labeled with a cytokeratin antibody. However, the epithelial cells showed a heterogenous cytokeratin staining mainly because numerous cells had their cytoplasm tightly packed with mucus, which means that less epithelial cell surface area/volume was included than in the stereological analysis. The fact that different methodological strategies for G-cell quantification produce different results with regard to antral G-cell content has not previously been emphasized by authors who have dealt with relations of G-cell content to gastrin in circulation or to gastrin concentrations in the antral mucosa. For example, when counting the number of G-cells per mm of muscularis mucosae, Tzaneva found a normal or decreased number of G-cells in patients with *H. pylori* associated chronic gastritis compared to healthy volunteers [17, 18]. However, the number of subjects included in these studies was limited (32 patients and 12 controls, and 12 patients and 5 controls, respectively). Sankey *et al* [22] reported an increase in antral G-cell content in patients with *H. pylori* associated gastritis compared to uninfected subjects, when cell counting ("number of G-cells per gland complex") and a computer-assisted image analysis system were used. The authors stated that "The results of the subjective analysis and computer assisted analysis corresponded well", but no statistical data supporting this statement were presented. Using cell counting (mid zones of the mucosa) Queiroz *et al* [14] found a decrease in G-cell content when children infected with *H. pylori* received eradication treatment. The study included non-infected controls, but it was not stated whether there were any differences between these groups, either in the infected or eradicated state. Kamada *et al* [15] reported a

decreased density of G-cells in patients with *H. pylori* associated chronic antral gastritis both with and without duodenal ulcer in comparison with subjects with normal antral mucosa. A cell counting method was used and only the glandular compartment was analyzed. Similarly, using cell counting and focusing on the glandular compartment, Graham *et al* [16] found no differences between subjects with normal antral mucosa and subjects with chronic antral *H. pylori* associated gastritis. However, patients with duodenal ulcer had decreased G-cell counts as compared to subjects with normal antral mucosa and subjects with antral *H. pylori* associated gastritis. Furthermore, eradication of the bacterium was not associated with increased density of G-cells among patients with duodenal ulcer. Absence of a change in G-cell density after *H. pylori* eradication among patients with duodenal ulcer was also found by Queiroz *et al* [14] using a cell counting method over the mid-zone of the mucosa. Chamouard *et al* [23] reported decreased G-cell density using cell counting (whole mucosal thickness) in duodenal ulcer patients compared to both *H. pylori* infected subjects without peptic ulcer disease and uninfected controls. Furthermore, they also concluded that the inflammatory status of the corpus mucosa did not affect the antral density of G- cells, which is in accordance with our findings. These varying results are difficult to interpret because many different morphometric techniques were employed. In the present study, we used three different morphometric techniques and still could not find any firm indications that the degree of chronic inflammation or the degree of inflammatory activity are involved in G-cell biology, at least not on a morphological level.

In conclusion, the main finding in this study is that there is a substantial lack of correlation between three common morphometric methods for endocrine cell quantification in the gastrointestinal tract. A lack of correlation between these methods and the plasma gastrin levels has previously been shown and our data clearly substantiates these findings. Age may be related to increased G-cell content in the antral mucosa. *H. pylori* colonization of the gastric mucosa may be associated with increased G-cell density as assessed with the cell- and point counting methods. It should be strongly emphasized that the choice of morphometric technique is of utmost importance to obtain results that are comparable to results from other studies and in repeat studies in the same individuals.

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Please address all correspondences to Fredrik Petersson, MD, PhD, Department of Pathology, Karolinska University Hospital, S-171 76, Stockholm, Sweden. Tel: +46733509002; Fax: +46851774524; Email: fredrikpetersson@live.se

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