Abstract. Background: Screening gastroscopic examinations were performed in a cohort of individuals at high risk for developing gastric carcinoma (GC). Patients and Methods: Five gastric biopsies were obtained following the Houston schema. Five histological parameters of gastritis were investigated: acute gastritis, chronic gastritis, and its sequelae; mucosal atrophy, intestinal metaplasia and pseudopyloric metaplasia. Results: Out of 134 patients, 50% (n=67) had Helicobacter pylori (HP) infection. The sum of scores for the first four parameters was significantly higher in HP-positive cases than in HP-negative ones (p<0.0001). The frequency of these histological parameters was similar to other series from Northern and Central Italy. Hence, none of the histological parameters of gastritis explain the high GC risk in this borough of Florence, considering that the incidence rate of GC is higher in Central than in Northern Italy. Conclusion: Similarities in the frequency of chronic gastritis and sequelae in Northern and Central Italy substantiate the conviction that the difference in GC risk in these regions might be the result of local environmental or lifestyle factors, rather than HP infection. This knowledge is crucial, considering that environmentally related diseases are theoretically preventable.

In 1862, Crouvelhier (1) observed that mucosal inflammation was invariably present in stomachs with gastric ulcers. Since then, many have investigated the pathogenesis of the mucosal inflammation in the stomach (i.e. gastritis). It has long been known that ethanol, aspirin, radiation, viruses and fungi can cause gastric mucosal inflammation (2). In the absence of those agents, it was observed that the most common form of gastric mucosal inflammation occurred in the lower social classes and in people with blue eyes (3). In 1973, Strickland and McKay (4) described atrophic gastritis of types A and B. The A-type was a corpus-limited gastritis of autoimmune origin, while the B-type gastritis represented gastritis of the antrum. In 1983, it was found that the most common etiological agent of B-type gastritis was a bacterium called Helicobacter pylori (HP) (5).

The simple classification of Strickland and McKay into A and B gastritis (4) and the presence of HP infection (5) were adopted at a workshop in Sydney in 1990 (6). In order to assess the topographic distribution of gastritis, the workshop recommended that four sets of gastric biopsies, two from the antrum and two from the corpus (known as the Sydney System) should be obtained.

Twenty years after the Sydney Workshop two of the participating co-authors of the Sydney workshop wrote (7): “The Sydney system was not a classification in strict terms but more of a practical guideline that provided what we hoped might become a flexible universal gastritis reporting grid”. According to Sipponen and Price (7), a report summary based on the Sydney System would read: “H. pylori pangastritis, severely active with moderate atrophy and intestinal metaplasia”, while another read: “Autoimmune corpus gastritis with severe atrophy; no intestinal metaplasia”. In further attempts to resolve some of the problems associated with the Sydney System, another working group met in Houston in 1994 (8). This working group recommended a fifth biopsy from the incisura angularis. The histological changes were described in more detail and the degree of inflammation, used in the Sydney system, namely mild, moderate and severe, were...
ever since to be called mild, moderate and marked. In addition, in the Houston Update, visual analogue drawings schematically illustrated the changes. It was clarified however, that "readers should keep in mind that the drawings were not intended to represent realistically the histopathological appearance of the gastric mucosa but rather provided a schematic representation of the magnitude of each feature and, as such, they had certain limitations, since changes may considerably vary in intensity within the same biopsy sample" (8). In such cases, "the observer should attempt to average the different areas and score the specimen accordingly" (8).

The protracted mucosal infection by the ubiquitous bacterium HP often induces chronic active mucosal inflammation, leading in time, to glandular atrophy and to intestinal metaplasia (8). The latter two mucosal changes are claimed to have a particular proclivity to undergo cellular mutations leading to epithelial dysplasia, a precursor of gastric carcinoma. Since HP might set aflame this series of histological events in the gastric mucosa, the International Agency for Research on Cancer (IARC) regarded this bacterium as a first-degree carcinogen (9).

Until the mid-1990s, gastric cancer (GC) was the first cause of cancer death worldwide (10). In recent decades, the incidence of GC has declined and, for obscure reasons, GC has become relatively rare in North America and in most countries in Northern and Western Europe, but not in Eastern Europe, Russia and in certain areas of Central and South America and East Asia (11-13). Puzzlingly, the incidence of GC may also vary between different regions within a single country. According to the Italian Network of Cancer Registries (14), the incidence rates of GC vary across Italy, and the ratio between the highest (usually in Central Italy) and lowest rates (in Southern Italy) is about 3 for both males and females.

In the borough of Mugello, an area with a high GC incidence in Northern Italy, surveillance gastroscopies were carried out in a cohort of individuals considered to be at high risk of developing GC: firstly for being first-degree relatives of patients with GC and secondly for dwelling in a district in Northern Italy with a high GC risk (14, 15). Since the cascade theory of HP infection, acute gastritis, chronic gastritis, mucosal atrophy and intestinal metaplasia have been regarded as the obligate pathway of gastric carcinogenesis of the intestinal type (16). The possibility was explored that one or more of these histological parameters might be more frequent in individuals in Mugello than in those reported in Central Italy (17-19).

**Patients and Methods**

**Pre-requisites for including participants.** i) At least one first-degree relative affected by GC; ii) residency in the district of Mugello, Florence County; and iii) age of 40 to 70 years.

**Participants.** Two hundred, first-degree relatives of 150 patients who had undergone surgery for GC in recent years were selected for the study (20). Out of the 200 first-degree relatives, 167 were enrolled between November 2004 and February 2008. Twenty-eight first-degree relatives residing outside the borough of Mugello, as well as two Mugello residents who had severe cardiovascular disease and three individuals who refused participation were excluded from this study. Filed gastric sections from 134 individuals were then investigated.

**Gastric biopsies.** Following the recommendations of the Houston Update of the Sydney classification (8) five gastric pinch biopsies were obtained in separately labeled tubes containing fixative agents, as follows: two from the antrum (anterior and posterior wall), one from the incisura angularis and two from the corpus (anterior and posterior wall). Sections were stained with diluted Giemsa. Two pathologists (CAR and GN) simultaneously assessed the histological parameters using a double-headed microscope.

**Histopathological evaluation: Sections from five separate sets of biopsies corresponding to the five aforementioned biopsy sites were registered according to the classification of gastritis proposed elsewhere (21) and summarized below.**

**Definitions. Acute inflammation:** The presence of polymorphonuclear granulocytes, usually neutrophils, in the epithelium and/or in the lamina propria mucosae.

**Chronic inflammation:** Increased numbers of lymphocytes/plasma cells above the normal limits. Lymphoid aggregates with germinal centers are characteristic of chronic HP-associated gastritis (8). The normal number of gastric mucosal mononuclear leukocytes (lymphocytes/plasma cells/macrophages) in the lamina propria is two to five per high power (×40 objective) field or, two to three lymphocytes or plasma cells between foveolae (the area in which chronic inflammatory cells are most often found). Occasionally, lymphocytes may also be observed in the surface epithelium.

**Glandular atrophy:** According to Dixon et al. (8) antral atrophy is demonstrated if the three to four gland cross sections that normally span to the lower antral mucosa are reduced to two or fewer cross sections. This loss may be followed by fibrous replacement or by a collapse of the existing supporting matrix.

**Intestinal metaplasia:** The state characterized by the replacement of the foveolar and/or the glandular epithelium of the stomach by intestinal-like epithelium (absorptive enterocyte-like and mucus-producing goblet-like cells, with or without Paneth cells. Intestinal metaplastic cells up-regulate the production of lysozyme (a natural antibacterial enzyme) (22) to halt the proliferation of bacteria resulting from the high pH generated by atrophy of the oxytic glands.

**Pseudopyloric metaplasia:** Pseudopyloric glands differ from true pyloric glands of the antrum in that endocrine cells associated with pseudopyloric glands do not include G cells (evidenced by gastrin immunostain).

**Statistical analysis.** Statistical analysis was carried out with the SPSS 8 for Windows (SPSS Inc., Chicago, Illinois, USA). A non-parametric test (Mann Whitney test or Kruskal Wallis test) was used to evaluate possible differences in specific parameters among different groups. A p-value <0.05 was considered significant.

**Results**

Out of 134 patients, 67 had evidence of HP infection, while the remaining 67 patients did not. The five histological parameters as assessed in the biopsies from the 134 patients
are summarized in Tables I and II. The missing values in some sites were mainly due to poor orientation of histological sections.

**Histological parameters at five biopsy sites.**

1. **Acute inflammation:** Table I shows that in individuals with HP infection, acute inflammation was present in 66% (43/65) of the biopsies from the anterior wall of the antrum, in 58% (38/66) from the posterior wall of the antrum, in 66% (42/64) of the biopsies from the *incisura*, in 45% (29/65) from the anterior wall of the corpus and in 39% (26/66) of these from the posterior wall of the corpus.

2. **Chronic inflammation:** Table I shows that in individuals with HP infection, chronic inflammation was found in 83% (52/63) of the biopsies from the anterior wall of the antrum, in 78% (51/65) of these from the posterior wall of the antrum, in 66% (42/64) of the biopsies from the *incisura*, in 45% (29/65) from the anterior wall of the corpus and in 39% (26/66) of these from the posterior wall of the corpus.

3. **Atrophy:** In individuals with HP infection (Table I), atrophic mucosa was present in 40% (22/55) of the biopsies from the anterior wall of the antrum, in 22% (13/59) from the posterior wall of the antrum, in 40% (22/55) of the biopsies from the *incisura*, in 23% (14/62) from biopsies of the anterior wall of the corpus and in 17% (11/60) of these from the posterior wall of the corpus.

4. **Intestinal metaplasia:** In cases with HP infection (Table I), intestinal metaplasia was recorded in 29% (19/66) of the biopsies from the anterior wall of the antrum, in 16% (11/67) from the posterior wall of the antrum, in 19% (12/64) from the *incisura*, in 6% (4/66) from the anterior wall of the corpus and in 7% (5/67) of these from the posterior wall of the corpus.

5. **Pseudopyloric metaplasia:** In individuals with HP infection (Table I), pseudopyloric metaplasia was present in 15% (9/62) of the biopsies from the anterior wall of the corpus and in 25% (15/61) of these from the posterior wall.

### Table I. Histological parameters of gastritis in 67 individuals with HP infection in Mugello.

<table>
<thead>
<tr>
<th></th>
<th>Antrum A</th>
<th>Antrum P</th>
<th><em>Incisura angularis</em></th>
<th>Corpus A</th>
<th>Corpus P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute inflammation</td>
<td>43</td>
<td>38</td>
<td>42</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>52</td>
<td>51</td>
<td>51</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>Atrophy</td>
<td>22</td>
<td>13</td>
<td>22</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>19</td>
<td>11</td>
<td>12</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>PPM</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>

*Anterior wall, P=Posterior wall.*

### Table II. Histological parameters of gastritis in 67 individuals without HP infection in Mugello.

<table>
<thead>
<tr>
<th></th>
<th>Antrum A</th>
<th>Antrum B</th>
<th><em>Incisura angularis</em></th>
<th>Corpus A</th>
<th>Corpus B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute inflammation</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Atrophy</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>PPM</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

*Anterior wall, P=Posterior wall.*
In individuals without HP infection, pseudopyloric metaplasia was present in 11% (7/64) of the biopsies from the anterior wall of the corpus and in 14% (9/64) of these from the posterior wall.

Discussion

In this survey, gastric biopsies from first-degree relatives of patients with GC residing in a district with high GC-risk were reviewed, with the aim of assessing the histological characteristics of the gastric mucosa at the five sites proposed by the Houston Update (8). The possibility that the prevalence of HP infection was higher in this borough than for those reported of other areas in Italy was explored. This working hypothesis was based on the claim by the IARC, that HP is a group I carcinogen (9).

It was found that the frequency of HP in Mugello (50%) was similar to or somewhat lower than that of populations in Northern Italy (24-27). In one study on first-degree relatives of patients with GC carried-out in Aviano (Northern Italy) De Re et al. (24) recorded a similarly high frequency of HP infection (50%) as in Mugello. In Milan, Northern Italy, Testoni et al. (25) found HP infection in 52% of patients with atrophic gastritis; and in Parma, Rindi et al. (26) found HP infection in 73% of the biopsies from the corpus and in 87% in those from the antrum; in Turin, Palestro et al. (27) found HP positivity in 57% of the individuals consecutively admitted to the Emergency Care Unit. In Rome, Central Italy, Giuliani et al. (17) recorded HP colonization in 52% of patients with GC residing in a district with high GC-risk in the Mugello district (28).

Despite declining GC incidence in many countries, up to 92% of the inhabitants worldwide are infected with HP (29). In some African countries, the prevalence of HP infection is high (up to 90%) despite the frequency of GC being low, a phenomenon often referred to as The African Enigma (30). In contrast, the GC incidence continues to be high in Japan, despite the age-adjusted prevalence of HP infection dramatically decreasing from 70.5% in 1988 to 52.7% in 2005 (31). Today, HP infection has declined in most developed and developing countries; in England (32) and in Argentina (33), only 15% of individuals respectively are HP-positive.

It may be argued that geographical and ethnic prevalence of the HP virulence-associated genotypes can explain some of the conflicting biopathological peculiarities of this microorganism and the incidence of GC in Central, Northern and Southern Italy (14). However, Chiarini et al. (34) by studying biopsies with chronic gastritis from Sicilian patients (Southern Italy, where the incidence of GC is the lowest (14)), he identified virulent HP genotypes cagA+, vacA1, vacAm1, babA2+, and hopQ I, I/II in 51.8%, 80.4%, 35.2%, 47.3%, and 67.7%, respectively. They concluded that the prevalence of these virulent HP genotypes in Southern Italy was similar to those found in Northern Italy (34). Hence, if HP infection and the HP virulent genotype are not the reason for an increased cancer risk for individuals in the Mugello district, could an abnormally high frequency of chronic inflammation, atrophic gastritis, intestinal metaplasia or pseudopyloric metaplasia be the cause for the increased GC risk? The review of the literature regarding Northern and Central Italy (17-19, 24-27) showed, however, similar frequencies of histological parameters of gastritis as those recorded in Mugello.

Acute inflammation, chronic inflammation, mucosal atrophy, intestinal metaplasia and pseudopyloric metaplasia occurred both in HP infected and non HP-infected individuals. The possibility that the presence of histological changes in non HP-infected individuals might be more permanent than those generated by the HP infection (an infection which is amenable to therapy), remains to be explored. In this context it appears pertinent to mention that in a recent study, Marrelli et al. (35) found that negative HP status was an indicator of poor prognosis in patients with GC and that Meimarakis et al. (36) provided evidence of a better prognosis in GC patients with HP infection compared to patients without.

In conclusion, none of the histological parameters of gastritis analysed in this survey are capable of explaining the high GC risk in the Mugello district.

Previous studies on gastrectomies carrying a GC showed that intestinal metaplasia was more extensive in patients dwelling in the Pacific basin than in those residing in the Atlantic basin (37), despite their similarities in the frequency of the HP infection. It was speculated that the difference could be due to different environmental and/or lifestyle factors acting in the two basins (37). In the present study, similarities in the frequency of HP infection, chronic gastritis and its sequelae between Northern and Central Italy substantiate the conviction that the difference in GC risk in these regions might be the result of undisclosed factors related to the location of the patients, apparently unrelated to HP infection. This knowledge is crucial, considering that environmentally related diseases are theoretically preventable.

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References

Saieva et al: Gastritis in a High Cancer-risk Area in Northern Italy


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