We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

4,200 Open access books available
116,000 International authors and editors
125M Downloads

154 Countries delivered to
TOP 1% Our authors are among the most cited scientists
12.2% Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us? Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected. For more information visit www.intechopen.com
A Study of the Correlation between Bacterial Culture and Histological Examination in Children with Helicobacter pylori Gastritis

Felicia Galoș, Gabriela Năstase, Cătălin Boboc, Cristina Coldea, Mălina Anghel, Anca Orzan and Mihaela Bălgrădean

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80257

Abstract

Helicobacter pylori (H. pylori) is one of the most common chronic bacterial infections in the world, and it is currently estimated that approximately half of the world’s population is infected with the bacterium. The correct diagnosis and effective treatment of H. pylori gastric infection are essential in controlling this condition. The available diagnostic methods have advantages and limitations related to factors such as age of patients, technical difficulty level, costs and extensive accessibility in hospitals. The eradication therapy of H. pylori infection is still a challenge for gastroenterologists. One of the main causes of failure in H. pylori eradication is antibiotic resistance. Biopsy cultures are the most widely used methods among the antimicrobial susceptibility tests. In case of a negative culture, H. pylori can be clearly recognised in histological sections. The sensitivity and specificity of histology for the diagnosis depend on clinical settings, density of colonisation and the experience of the histopathologist. A prospective study was performed in order to analyse patients with H. pylori gastric infection with positive histology and positive culture versus positive histology and negative culture.

Keywords: Helicobacter pylori culture, histology, children

1. Introduction

Helicobacter pylori (H. pylori) is a Gram-negative spiral-shaped bacterium which colonises the gastrointestinal tract of nearly half of the world’s population, causing local inflammation in
the stomach and duodenum and inducing a systemic humoral immune response [1]. *H. pylori* survival in the acidic gastric environment is mediated by mechanisms such as activity of the urease enzyme, which catalyses and hydrolyses urea to form carbon dioxide and ammonia, producing a neutral environment that is essential for its survival. The primary routes of transmission are considered to be faecal-oral and oral-oral, but some indirect evidences report that the infection can also be acquired by drinking water and by other environmental sources [2, 3].

*H. pylori* represents a key factor in the aetiology of various gastrointestinal diseases, ranging from asymptomatic chronic active gastritis to peptic ulceration, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Other diseases caused by the pathogen are iron deficiency anaemia, chronic idiopathic thrombocytopenic purpura and growth retardation. *H. pylori* was classified as a class I human carcinogen by the World Health Organization in 1994.

Numerous diagnostic tests are available for detecting *H. pylori* infection: invasive techniques, which means endoscopy with biopsies for a rapid urease test (RUT), histology and culture and noninvasive techniques, such as serology, 13C-Urea breath test (13C-UBT) and stool antigen test. There is no single method to detect *H. pylori* infection reliably and accurately. The choice of the diagnostic method depends on patients’ age and complaints, technical difficulty level, costs and extensive accessibility in hospitals.

Two tests are recommended to define *H. pylori* status in children: bacterial culture of gastric biopsy and histology [4]. Bacterial culture of gastric biopsy has 100% specificity, but its sensitivity is low. *H. pylori* can be cultured from gastric biopsies, although this method often presents some difficulties. *H. pylori* soon loses viability when exposed to the environment, and biopsies should be cultured quickly. If it is not possible, a transport media may be used. Histology provides an excellent diagnostic accuracy, allowing for the detection of the bacteria as well as for the grading of gastritis. The sensitivity and specificity of histology for the diagnosis depend on clinical settings, density of colonisation and experience of the histopathologist [5].

The aims of our study were to assess the histological findings and to compare them with the results of bacterial cultures, obtained through gastric biopsy, in children with *H. pylori* gastritis. We also wanted to find out the possible factors that may influence bacterial culture outcomes.

## 2. Materials and methods

### 2.1. Patients

This was a prospective, single-centre study (in Maria Sklodowska Curie Children’s Emergency Hospital Bucharest, Romania) that evaluated consecutive children referred by their physicians for an upper endoscopy because of dyspepsia. They were all screened for *H. pylori* and presented a positive stool antigen test.

Excluding criteria were the use of proton pump inhibitors or H2-receptor antagonists and antibiotics as well as non-steroidal anti-inflammatory drugs or steroidal treatment 2 weeks
before the beginning of the study, previous intestinal surgery (except for polypectomy and appendectomy), concomitant severe disease (heart, lungs, kidney and endocrine diseases) and smoking or alcohol consumption among adolescents.

The study was approved by the ethics committee.

2.2. Endoscopy

All patients underwent endoscopy with biopsy specimens for histology (one for the antrum, one for the corpus). One sample from the antrum was used for rapid urease test. Two additional biopsies were taken from the antrum for bacterial culture. The samples were placed into separate vials, previously identified, containing the appropriate medium for each test. The first sample was used for bacterial culture.

This procedure was performed in patients with a minimum of 10 hours of fasting, under general anaesthesia or conscious sedation. Vital signs were continuously monitored for the entire procedure.

Written informed consent was obtained from the parent or guardian of each child included in the study.

2.3. Bacterial culture

The biopsy specimens collected for bacterial culture were transported in commercial selective transport *H. pylori* medium, Portagerm pylori (BioMérieux SA, Marcy l’Etoile, France), and were inoculated after a few hours onto selective medium pylori agar (BioMérieux Italia). The plates were incubated under microaerobic condition at 37° for 72 hours. Once incubated, the colonies resembling *H. pylori* were identified by Gram stain and by oxidase, catalase and urease tests. Suspensions from the primary plates were prepared in sterile solution to perform an E-test on pylori agar. An agar plate was streaked in three directions with a swab dipped into each bacterial suspension to produce a lawn of growth; an E-test strip (E-test; AB Biodisk, Solna, Sweden) was placed onto separate plates, which was immediately incubated in a microaerobic atmosphere at 37° for 72 hours. Isolated strains were tested for amoxicillin, clarithromycin, metronidazole and levofloxacin resistance following the recommendations of the European Committee on Antimicrobial Susceptibility Testing.

2.4. Histology

A biopsy of the gastric body and antrum was fixed in a solution of formaldehyde 10%. Subsequently, the gastric mucosa samples were processed, following the usual steps of dehydration and paraffin embedding.

Two stains were used for histological study: haematoxylin-eosin and Giemsa. Haematoxylin-eosin stain was used to evaluate inflammatory cells and *H. pylori* (Figure 1). Giemsa stain was needed when haematoxylin-eosin stain failed to identify the bacterium (Figure 2). Giemsa stain is the preferred stain for detecting *H. pylori* because of its technical simplicity, high sensitivity and low cost.
Figure 2. *Helicobacter pylori* in histological section of the gastric mucosa stained with Giemsa. This is a 400× histological section of Giemsa-stained gastric mucosa biopsy. There can be seen colonisation with *Helicobacter pylori* (white arrows) of the gastric glands, represented by small curved structures.

Gastritis was graded according to the Sydney system [6] that assesses the severity of inflammation, the level of activity (the degree of polymorph neutrophil inflammation) and the presence of atrophy and of intestinal metaplasia on a scale from 0 to 3.

In accordance with the Sydney system, the density of *H. pylori* infection was also graded semiquantitatively on a scale from 0 to 3 (mild, moderate and marked).

*H. pylori* was recognised in the histological section appearing as a short-curved or spiral bacillus resting on the epithelial surface or in the mucus layer.

2.5. Statistical analysis

The data was collected and analysed with Microsoft Excel 2013 and PSPP version 1.0.1. Continuous variables with a normal distribution were expressed as a mean with standard
deviation (SD). Differences and relationships between variables were analysed using Fisher’s exact for low expected frequencies. A p < 0.05 was considered statistically significant for all the analysed parameters.

We calculated the sensitivity and specificity for *H. pylori* culture and histology. Sensitivity and specificity may be defined, respectively, as the probability of having a positive test in a person with the disease (sensitivity) and the probability of having a negative test in a person without the disease (specificity).

3. Results

In the study, the culture findings and histological examination findings were accepted as “gold standard”. The detection of *H. pylori* in at least one of the two tests was accepted as *H. pylori* positivity. Negative results in both culture and histology were accepted as *H. pylori* negativity.

Of the 38 patients who underwent upper endoscopy with biopsies by protocol (Figure 3), nine were excluded because of negative results in both culture and histology.

Twenty-nine cases (76.31%) were included in the final analysis, nineteen females (65.51%) and ten males (34.49%). The ages were between 3 years and 7 months and 17 years and 8 months (mean age 13.5 ± 4.53 years).

The results for the diagnosis of *H. pylori* infection for each of the tests revealed that the haematoxylin-eosin and Giemsa stains of the antrum and body were the test that identified a higher number of *H. pylori* infection than the *H. pylori* culture. Indeed, the histological examination of samples was able to identify the presence of *H. pylori* in 28 patients (96.55%), while the culture resulted to be positive in only six cases (21.42%).

We did not analyse separately the presence of the *H. pylori* in the antral mucosa compared with the gastric body mucosa; the result was recorded positive, if the bacterium was isolated in any of the histological examinations.

The histology also showed that 14/28 (50%) patients had mild *H. pylori* density, 11 (38.29%) had moderate density and 3 (10.71%) had marked density.

In one case, the culture was positive, but the bacterium was not identified through the histological exam. Among the other five cases with positive culture, two were associated with a mild score of *H. pylori* density, the other two with a moderate score and one with a marked score (Table 1).

We analysed the correlation between densities of *H. pylori* in histological exam and positive *H. pylori* culture: 14.28% (2/14) of patients with mild *H. pylori* density, 18.18% (2/11) of those with moderate density and 33.33% (1/3) of those with marked density had positive results in bacterial culture. There was not a statistically significant correlation between the degree of *H. pylori* density observed at histology and the positive result of bacterial culture (p = 0.7). The limited number of patients with positive bacterial culture may have influenced these results.
Table 1. Correlations of *Helicobacter pylori* density and *Helicobacter pylori* culture.

<table>
<thead>
<tr>
<th>Helicobacter pylori density</th>
<th>Patients (n)</th>
<th>Helicobacter pylori culture</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>14</td>
<td>Positive</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>12</td>
</tr>
<tr>
<td>Moderate</td>
<td>11</td>
<td>Positive</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>9</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>Positive</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>1</td>
</tr>
</tbody>
</table>
We used haematoxylin-eosin as the main staining method, while Giemsa stain was reserved for a few cases, not identified through haematoxylin-eosin staining, in which the suspect of infection was high.

We also tried to find out if there existed a relationship between the activity of gastritis and the density of \textit{H. pylori} in the histological exam. Most of the patients with mild \textit{H. pylori} density presented a mild gastritis activity (50%), while the other half showed a similar frequency of moderate gastritis activity (28.57%) and of no activity (21.43%); among patients with moderate \textit{H. pylori} density, 54.54% had moderate activity, 36.36% a mild one and only 9.0% of them presented a marked gastritis activity. Those with marked bacterial density at histology displayed an identical frequency of absent, mild and moderate gastritis activity (33.33%). According to our results, there were not statistically significant differences between the groups that allow concluding that the severity of gastritis activity is related to \textit{H. pylori} density at histology \((p = 0.3)\) (Table 2).

The relationship between the degree of inflammation and \textit{H. pylori} density was examined as well. Mild density was mostly associated with moderate (50.0%) and mild (42.80%) inflammation, while severe inflammation was demonstrated in only one case (7.20%). In patients with moderate \textit{H. pylori} density, a moderate inflammatory process was identified in most of the cases (63.64%), together with a mild inflammation (36.36%), whereas nobody presented with a severe one. In children with marked density, mild inflammation was predominant (66.67%), followed by a moderate one in 33.33% of patients, while none of them was severely affected. Our findings do not show the evidence of a significant correlation between inflammation and \textit{H. pylori} density at histology \((p = 0.7)\) (Table 3).

The specificity for \textit{H. pylori} culture in our study was 90.90%, but the sensitivity was low, 20.68%. The specificity for histology was 90.90%, and the sensitivity was 96.55%.

<table>
<thead>
<tr>
<th>Without activity</th>
<th>Mild activity</th>
<th>Moderate activity</th>
<th>Marked activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{H. pylori} density</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Mild</td>
<td>3 (21.43%)</td>
<td>7 (50%)</td>
<td>4 (28.57%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>4 (36.36%)</td>
<td>6 (54.54%)</td>
</tr>
<tr>
<td>Marked</td>
<td>1 (33.33%)</td>
<td>1 (33.33%)</td>
<td>1 (33.33%)</td>
</tr>
</tbody>
</table>

Table 2. Correlations of activity of \textit{Helicobacter pylori} gastritis and \textit{Helicobacter pylori} density.

<table>
<thead>
<tr>
<th>Mild inflammation</th>
<th>Moderate inflammation</th>
<th>Marked inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{H. pylori} density</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Mild</td>
<td>6 (42.80%)</td>
<td>7 (50.0%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (36.36%)</td>
<td>7 (63.64%)</td>
</tr>
<tr>
<td>Marked</td>
<td>2 (66.67%)</td>
<td>1 (33.33%)</td>
</tr>
</tbody>
</table>

Table 3. Correlations of inflammation of \textit{Helicobacter pylori} gastritis and \textit{Helicobacter pylori} density.
4. Discussions

The enthusiasm beginning with the isolation of *H. pylori* from gastric biopsies by Warren and Marshall in 1982 has increasingly continued after the important role of this agent in the aetiology of gastric cancer has been established; consequently, the interest for *H. pylori* has increased. The association of gastric cancer, one of the most frequent causes of death worldwide, with a treatable aetiological factor has led to a profound impact on researchers [7, 8]. *H. pylori* infection is generally acquired in childhood, and it persists throughout life. Spontaneous resolution is rare, and a targeted therapy is needed [9].

The correct diagnosis and effective treatment of *H. pylori* gastric infection are essential. The recent guidelines for the management of *H. pylori* in children and adolescents recommend the initial diagnosis of *H. pylori* infection to be performed using invasive gastric biopsy methods including the following: obtaining a positive bacterial culture or demonstrating *H. pylori* gastritis on histopathology; using the updated Sydney classification, with at least one other positive test such as RUT or molecular-based assays where available; and including polymerase chain reaction or fluorescent in situ hybridisation. The initial diagnosis of *H. pylori* infection should not be based on noninvasive tests (i.e. 13C-UBT and *H. pylori* stool antigen test) or other noninvasive methods. A positive noninvasive test, however, supports the diagnosis in cases in which positive histology is the only available invasive test [4].

76.31% of patients enrolled in the study were positive for *H. pylori* infection. The diagnosis was made in 96.55% by histology. In 17.25% of cases, both histology and bacterial culture were positive.

The *H. pylori* culture was positive in only six cases (21.42%). Our results are less significant if compared to other published data in which higher percentages of positive cultures were obtained, except one. Kaya et al. reported the sensitivity for *H. pylori* culture as 22.5%, and the specificity as 97.1% [10].

The specificity for *H. pylori* culture, in our study, was 90.90%, but the sensitivity was low, 20.68%.

A recent Israeli study, conducted in the paediatric population, reported 57.8% of positive *H. pylori* culture in 154 children with positive RUT [3]. In another study conducted on children and adolescents, the sensitivity for *H. pylori* culture was 79.3%, and the specificity was 100% [11].

The sensitivity of culture method to detect *H. pylori*, in adult population, ranges from 62.7% to 96.3% in the studies performed [8, 10, 12].

Although the culture method is accepted as a “gold standard” for the diagnosis, it is difficult to use alone as a routine diagnostic method. As the sensitivity of culture method is low, *H. pylori* positivity can be detected in case of growth. The absence of growth does not indicate *H. pylori* negativity.

Despite its long use, culture remains a challenge because of the fastidious nature of the bacterium, with particular growth requirements regarding environment and atmosphere [13]. Altering pH, the proton pump inhibitors (PPIs) indirectly interfere with *H. pylori* distribution.
in the stomach. The antrum has been found to be the most affected part of the stomach by PPIs as *H. pylori* almost disappears from this niche. To avoid false-negative results, it is recommended not to consume these drugs 2 weeks prior to endoscopy [14, 15].

In our study, exclusion criteria were of inhibitory proton pump or H2-receptor antagonists and antibiotics 2 weeks before the beginning of the study, similarly with another study and recommendations [15].

The recent Maastricht V/Florence Consensus Report recommends discontinuation of antibiotics 4 weeks before the study to allow an increase of detectable bacterial load [2].

The number of biopsies necessary to diagnose *H. pylori* infection is a subject of controversy. A single biopsy specimen taken from the antrum (2 cm from the pylorus) gives good sensitivity, but it is not sufficient for a reliable diagnosis. Indeed, *H. pylori* may have a patch distribution, and the more biopsy specimens analysed, the higher the chance of detecting the organism. There are some rare cases where the infection lies only in the corpus, but usually, *H. pylori* is present in all sites. After consumption of antisecretory drugs, as pointed out before, the corpus may be the only site that remains positive [15].

We took two biopsies from the antrum for *H. pylori* culture, and maybe this could be adjusted by obtaining another one/two samples from the gastric body as well in order to try to improve the culture success rate, but this option is difficult to apply in children.

The usual recommendation derived from the Sydney system is to obtain two biopsy specimens from the antrum and two specimens from the corpus. Bacteria are usually present at both sites even if the lesions occur essentially in the antrum. When topographic studies of *H. pylori* distribution and gastritis were performed, the best site suitable for diagnosis was the lesser curvature of the midantrum, while for the corpus, there was a discrepancy between greater and lesser curvatures [15, 16]. Others showed that two antral biopsies only were sufficient to detect *H. pylori* [17].

We took the biopsy specimens for culture before specimens for histology, and we used an appropriate commercially transport medium, according to the recommendations.

We analysed the correlation between densities of *H. pylori* in histological exam and positive *H. pylori* culture: in 14.28% the *H. pylori* culture was positive in mild *H. pylori* density in histology, 18.18% in moderate *H. pylori* density and 33.33% in marked *H. pylori* score. We have not had a significant statistics correlation between densities of *H. pylori* in histological exam and positive *H. pylori* culture (p = 0.7). Similar results were reported by other authors [8].

We analysed the correlation between gastritis activity and density of *H. pylori* in histological exam, and we have not had a significant statistics correlation (p = 0.30).

The results of this study are in agreement with published work, suggesting that a strain of the organism may be a more important factor than the density of infection in determining the gastric inflammatory response to *H. pylori* [18].

In our study, we had four children with bleeding, and all of them had negative *H. pylori* culture.
Peptic ulcer bleeding and atrophic gastritis decreased the accuracy of *H. pylori* diagnostic test. The histology was found to be a reliable test in the presence of bleeding [19].

When atrophic changes occur in the gastric mucosa, a high percentage of endoscopic biopsy samples become negative at bacterial histology [20, 21]. During atrophy progression the density of *H. pylori* in the stomach mucosa decreases and may disappear completely during the late stages of atrophy [22]. This may explain the lower sensitivity of biopsy-based tests in the presence of atrophy: RUT, histology and culture. UBT and antigen stool detection can also give false-negative results in this situation. Serology is the only diagnostic method not influenced by a lower density of microorganism, being reliable even in advanced gastric body atrophy. Maastricht guidelines updates have reserved serology for special situations, including extensive atrophy of the stomach mucosa, in conditions in which the other tests may be negative because of low bacterial density.

In childhood, advanced gastric atrophy is rare. We found only one case with atrophy, but the *H. pylori* culture was positive. It was an adolescent, 17 years and 8 months old, with a long history of illness (2 years) and with previous treatment failure. We could not find, in this case, antibiotic resistance to amoxicillin, clarithromycin, metronidazole and levofloxacin, and we suppose that the noncompliance to therapy is the cause of failure for bacterial eradication. Successful eradication is important to prevent the development of antibiotic resistance, as well as to reduce the number of treatments and procedures. Among children receiving the triple standard therapy regimen, eradication rate is declining [23]. In part, this decrease can be attributed to increased antibiotic resistance. Other reasons for treatment failure are, among others, host genetic factors, *H. pylori* virulent factors, inadequate compliance to therapy or insufficient duration of therapy and, not in the last row, smoking and household crowding [23].

The sensitivity of histology in our study was 96.55%, while the specificity was 90.90%.

The sensitivity and specificity of haematoxylin and eosin stain have been reported as 69–93% and 87–90%, respectively. The specificity can be improved to 90–100% by using special stains such as Giemsa stain, Warthin-Starry silver stain, Genta stain and immunohistochemical stain [15, 20, 21]. Immunohistochemical stain has a particular advantage in patients partially treated for *H. pylori* gastritis, a setting can result in atypical (including coccoid) forms, which may mimic bacteria or cell debris on haematoxylin and eosin preparation. The major advantages of immunohistochemical stain include shorter screening time and high specificity because it can exclude other similar-shaped organisms [15, 21].

In our study, we analysed the sensibility and specificity of histology in *H. pylori* infection, not focusing on the ones specific for the different types of stain. We used in all cases haematoxylin-eosin stain and in a few cases Giemsa stain.

**5. Conclusions**

Histology is an excellent method for detecting *H. pylori* infection. Haematoxylin-eosin, with or without Giemsa stains, is usually adequate. It is difficult to use culture method alone as routine diagnostic method. In our study, the specificity of histology in identifying *H. pylori* infection was 90.90%, and its sensitivity was 96.55%. Bacterial culture had the same specificity
as histology (90.90%), while its sensitivity was 20.68%. We did not find out a statistically significant positive correlation between *H. pylori* density observed at the histological exam and positive bacterial culture. This result may have been influenced by the limited number of patients and by the few cases with positive bacterial culture. Larger studies are needed in order to obtain relevant conclusions.

**Acknowledgements**

The authors thank Dr. Violeta Cristea and Dr. Augustina Enculescu for their laboratorial and histological support, respectively.

**Conflict of interest**

None declared.

**Author details**

Felicia Galoș¹²*, Gabriela Năstase²³, Cătălin Boboc², Cristina Coldea¹², Mălina Anghel², Anca Orzan¹² and Mihaela Bălgrădean¹²

*Address all correspondence to: felicia_galos@yahoo.com

1 University of Medicine and Pharmacy Carol Davila, Bucharest, Romania

2 Maria Sklodowska Curie Children’s Emergency Hospital, Bucharest, Romania

† These two authors contributed equally to this work.

**References**

[1] Sanders CJ, Yu Y, Moore DA, Williams IR, Gewirtz AT. Humoral immune response to flagellin requires T cells and activation of innate immunity. Journal of Immunology. 2006;177:2810-2818. DOI: 10.4049/jimmunol.177.5.2810


