Performance of Routine *Helicobacter pylori* Tests in Patients with Atrophic Gastritis

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Abstract

**Background.** Decreased density of *H. pylori* in atrophic gastritis may lead to low sensitivity of the routine tests. **Aims.** To evaluate the accuracy of routinely used *H. pylori* tests in atrophic gastritis. **Methods.** We compared 5 *H. pylori* diagnostic tests in 119 dyspeptic patients (28 males/91 females) with a mean age of 67 years (range 55-84). Patients with gastric cancer, peptic ulcer, previous gastric surgery, or those who have received eradication therapy were excluded. The following tests were performed: histology, rapid urease test (RUT), culture, 13C- urease breath tests (UBT), and *H. pylori* IgG/IgA antibody test (serology). **Results.** Atrophic gastritis was diagnosed in 26.1% of the patients; *H. pylori* was present in 87.1%. In the group with atrophy, the sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy were as follows: histology (100% for all parameters); UBT (96; 100; 100; 80; 97%); serology (96; 50; 93; 67; 90%); culture (96; 100; 100; 80; 97%); and RUT (78; 100; 100; 40; 81%), respectively. **Conclusions.** Histology, UBT and culture were the three best tests for diagnosing *H. pylori* infection. We cannot recommend using serology as a single test in a case of atrophy, but it would be reasonable to combine serology with one of the above tests.

Key words


Introduction

Multiple diagnostic methods, both invasive (rapid urease test - RUT, histology, and culture) and non-invasive (urea breath test - UBT, serology, and stool antigen test - HpSA) are used to identify *H. pylori* infection. Considering the broad spectrum of diagnostic methods, only highly accurate tests should be used in clinical practice under specific circumstances; nowadays, the sensitivity and specificity of an adequate test should exceed 90%.

Atrophy of the stomach mucosa develops in ~50% of *H. pylori* infected individuals by the age of 65 [1], and is considered a pre-malignant lesion for gastric cancer [2]. Although atrophy may be the result of autoimmune gastritis, *H. pylori* may also play a role in this condition [3]. *H. pylori* eradication is recommended in the presence of atrophy [4], since atrophy may reverse after successful eradication therapy [5]. It is critically important and challenging, therefore, to determine the presence or absence of *H. pylori* in patients with atrophic gastritis.

In the course of atrophy progression, however, the density of *H. pylori* in the stomach mucosa decreases, and during the late stages of atrophy the infection may completely disappear [6]. The density of *H. pylori* colonization may significantly influence test results, in particular those that are biopsy-based [7-8]. This may explain the markedly lower sensitivity of biopsy-based tests (RUT, histology, culture) in the presence of atrophy [9]. Similarly, UBT and HpSA can also give false-negative results in these circumstances [10]. In contrast, serology is not influenced to such an extent by a lower density of the microorganism, and is reliable even in advanced gastric body atrophy [11].

The Maastricht II guidelines have not listed serology among the first-choice methods for *H. pylori* detection (although it considered serology as an alternative), while subsequent Maastricht updates have reserved serology for special situations, including extensive atrophy of the stomach mucosa on the basis that other tests might be misleading at a low bacterial density [4]. The debate continues regarding the most appropriate *H. pylori* diagnostic method in atrophic gastritis. We have therefore decided to re-evaluate the
performance of routinely used \textit{H. pylori} tests in patients with
gastric mucosa atrophy.

**Methods**

**Patients**

A total of 127 patients aged 55 and above, referred
following upper endoscopy to the Digestive Centre GASTRO
due to dyspeptic complaints, were enrolled. Patients with
peptic ulcer disease, previous upper gastrointestinal surgery,
a personal or first-degree family history of gastric cancer,
a known or a suspected past history of \textit{H. pylori} eradication
therapy, and those having received antibiotic or proton pump
inhibitor treatment one month beforehand were excluded.

**Methods**

Altogether five \textit{H. pylori} diagnostic tests were performed
in each of the individuals: histology, RUT, culture, UBT, and
combined \textit{H. pylori} IgG/IgA antibody test (serology). All the
patients underwent upper endoscopy with nine standardized
gastric biopsies being taken: five for histology, two for RUT,
and two for microbiological examination.

**Histology.** Two biopsies from the antral part (one from
the greater and the other from the lesser curvature), one from
the incisura (the lesser curvature), and two from the corpus
of the stomach (one from the greater and the other from the
lesser curvature) were used. The slides were stained with
haematoxylin and eosin and Giemsa; the latter was used to
confirm presence or absence of \textit{H. pylori}. Cases identified
with \textit{H. pylori} in any of the biopsy specimens were considered
positive. Presence of atrophy was assessed according to
the updated Sydney System classification. Biopsies were
analyzed independently by two expert gastrointestinal
pathologists. Cases of disagreement for any grade of atrophy
were simultaneously re-evaluated, and the consensus
achieved [12].

**RUT.** Two biopsy specimens (one from the antrum and
the other from the corpus) were analyzed from each patient.
The \textit{H. pylori} Quick Test (Biohit, Plc., Finland) was used
according to the instructions of the manufacturer. The color
change within 30 min of the biopsy being placed in the gel
was used to check positivity.

**Culture.** Two biopsy specimens (one from the antrum and
the other from the corpus) were used for culturing from each
patient. Biopsy specimens were stored in freezing media at
\(-70^\circ\text{C}\) until analyzed; transportation was arranged on dry ice.
They were analyzed at the laboratory of the Swedish Institute
for Infectious Disease Control, Solna, Stockholm.

The biopsies were homogenized and cultured on
both selective and non-selective agar plates in a moist
microaerophilic atmosphere (10% CO\textsubscript{2}, 5% O\textsubscript{2}, 85% N\textsubscript{2})
at 37°C. Colonies of \textit{H. pylori} were tested by Gram’s stain and
biochemically (oxidase, catalase and urease positive) [13].

**UBT** was performed with 75 mg $^{13}$C-urea in 200 ml
orange juice. Basal and the second breath samples 30 min
after the intake of the substrate were analyzed. A positive
threshold was determined when the difference between
the baseline ratio of $^{13}$CO\textsubscript{2}/$^{12}$CO\textsubscript{2} and the 30 min value
exceeded 4.0 delta over baseline (DOB) values, a negative
being below 2.5 DOB. Measurements were performed by
isotope-selective non-dispersive infrared spectrometry
(IRIS, Wagner Analyzen Technik, GmbH, Germany).

**Serology.** Combined \textit{H. pylori} IgG/IgA antibody ELISA
test-system method (Biohit, Plc., Finland) was used on
plasma samples. Blood samples were collected in EDTA
vials; the samples were centrifuged within 30 min, the
plasma separated, and the samples immediately frozen.
Plasma samples remained frozen (up to 1 week at -20°C,
but for a longer period at -70°C) until tested. Transportation
was arranged on dry ice. The analysis was performed at the
Biohit, Plc. service laboratory according to the instructions
of the manufacturer. IgG/IgA $\geq$30 IU was defined as positive
\textit{H. pylori} (Hp) infection and IgG/IgA $\leq$30 IU excluded an
infection.

**Interpretation of results**

The positivity and negativity criteria for \textit{H. pylori}
infection were set prior to the results being obtained. The
rationale was based upon the consideration that the culture
was expected to be less sensitive, but with a high specificity
[14]. The following criteria were used:

\textit{Definition of \textit{H. pylori} positivity}

Patients were considered positive for \textit{H. pylori} infection
if any of the two criteria was positive:

1) the culture and any additional (at least one) \textit{H. pylori}
test was positive;

2) four of the five \textit{H. pylori} diagnostic tests were positive

\textit{Definition of \textit{H. pylori} negativity}

Patients were considered uninfected with \textit{H. pylori} if 4 of
the five \textit{H. pylori} diagnostic tests were negative.

\textit{Definition of atrophy group}

Presence of atrophy was considered, if any grade of
atrophy was identified at least in one of the investigated
sites.

**Statistical analysis**

Methods of descriptive statistics were used for group
characteristics. Sensitivity and specificity of each \textit{H. pylori}
diagnostic test, as well as the positive (PPV) and negative
(NPV) predictive values and overall accuracy (OA)
were used to characterize each \textit{H. pylori} diagnostic test
versus consensus. The chi-square test was used for group
comparisons and the 95% CI's were calculated using Statistica
(Version 6, StatSoft, Inc., USA) for Windows.

**Ethical considerations**

The project complied with the World Medical Association
Helsinki Declaration. The protocol was approved by the
Committee of Ethics of the Institute of Experimental and
Clinical Medicine, University of Latvia, Riga, Latvia. All
the patients signed consent forms prior to enrolment.

**Results**

A total of 127 patients were enrolled. From them, 119
patients fitted the diagnostic criteria for \textit{H. pylori} positivity
and negativity and were included in the final sample: 28/119
(24%) males and 91/119 (76%) females with the mean age of 67 years (range 55-84). Eight individuals did not fully comply with the criteria for definition of \textit{H. pylori} positivity and negativity and were not included in the main study analyses (Table I).

No differences were observed between the atrophy and non-atrophy groups analyzed by gender and age.

A significantly higher prevalence of \textit{H. pylori} infection occurred in the group with atrophy than in the non-atrophy group: 27/31 (87.1%, 95\%CI: 74.3\% - 99.6\%) vs. 53/88 (60.2\%; 95\%CI: 50.4\% - 61.1\%); \( p = 0.007 \).

Furthermore, each \textit{H. pylori} diagnostic test was evaluated in both patient groups with and without atrophy (Tables III and IV).

In the group with atrophy, 26 cases were \textit{H. pylori} positive according to both diagnostic approaches. Compatibility between \textit{H. pylori} diagnostic approach No.1 and No.2 was 96\% (25 cases). In the group with atrophy, histology identified all the \textit{H. pylori} positive cases, while UBT, serology and culture were equally sensitive (96\%).

The specificity was equally good for RUT, histology, UBT and culture (Table III), but only 50\% for serology. The overall accuracy (OA) was the best in histology (100\%), followed by UBT and culture (97\% each), and serology (90\%), with the poorest OA being for RUT (81\%).

The sensitivity of the tests was comparable in both groups, while the specificity of serology was 50\% in the atrophy group compared to 74\% in patients without atrophy.

The OA of the tests did not differ between the atrophic and non-atrophic groups (Tables III and IV).

Eight patients left out from the final sample due to non-compliance of the diagnostic criteria were separately treated (Table I). In all cases in the gray-zone, both culture and histology gave negative results. In four of the cases with a positive serology (two in the atrophy, the other two in the non-atrophy group) UBT and RUT were also positive.

### Discussion

Our cases are linked to a relatively high gastric cancer risk population, since the incidence of gastric cancer in Latvia remains high [15]. Individuals over 55 and without eradication therapy in the past were enrolled to ensure a relatively higher proportion of atrophic gastritis in this cohort. Besides atrophy, elderly individuals also tend to have a lower density of \textit{H. pylori}, thereby creating a challenge for proper diagnosis [16].

In general, good concordance between the different test results (both invasive and non-invasive) was observed in patients with atrophic gastritis. The only test that was not included in the protocol was the \textit{H. pylori} stool antigen test.

### Table I. List of excluded patients due to lack of compliance with the criteria for definition of \textit{H. pylori} positivity and negativity

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>Histology</th>
<th>Serology</th>
<th>RUT</th>
<th>UBT</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No.2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No.3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nonatrophy group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No.6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No.7</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>No.8</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

RUT – rapid urease test; UBT – 13C-urea breath test; (+) – positive test result; (-) – negative test result

Atrophy of gastric mucosa was detected in 31/119 (26.1\%) patients. In the group with atrophic gastritis, atrophy in the corpus was present in 27/31 (87.1\%), in the antrum in 10/31 (32.3\%), but panatrophy (atrophy of both corpus and antrum) was present in 6/31 (19.4\%) patients. The distribution of patients based on atrophy grading is given in Table II.

### Table II. Distribution of patients with atrophic gastritis per grades of atrophy according to the presence or absence of \textit{H. pylori}

<table>
<thead>
<tr>
<th>Gastric glandular atrophy</th>
<th>Corpus atrophy (n=27)</th>
<th>Antrum atrophy (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hp positive</td>
<td>Hp negative</td>
</tr>
<tr>
<td>Grade 3</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Grade 2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Grade 1</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Total (%)</td>
<td>23 (85%)</td>
<td>4 (15%)</td>
</tr>
</tbody>
</table>

In the group with atrophic gastritis, 6/31 (19.4\%) were males and 25/31 (80.6\%) females, with a collective mean age of 68.9 (range from 55 to 81 years); in patients without atrophy, 22/119 (25.0\%) were males and 66/119 (75.0\%) females with the collective mean age of 66.5 (range from 55 to 84 years).

### Table III. Performances of the five invasive and noninvasive diagnostic tests for \textit{H. pylori} detection in atrophic group

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Overall accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid urease test</td>
<td>78% (21/27)</td>
<td>100% (4/4)</td>
<td>100% (21/21)</td>
<td>40% (4/10)</td>
<td>81% (25/31)</td>
</tr>
<tr>
<td>Histology</td>
<td>100% (27/27)</td>
<td>100% (4/4)</td>
<td>100% (27/27)</td>
<td>100% (4/4)</td>
<td>100% (31/31)</td>
</tr>
<tr>
<td>\textsuperscript{13}C urea breath test</td>
<td>96% (26/27)</td>
<td>100% (4/4)</td>
<td>100% (26/26)</td>
<td>80% (4/5)</td>
<td>97% (30/31)</td>
</tr>
<tr>
<td>Serology</td>
<td>96% (26/27)</td>
<td>50% (2/4)</td>
<td>93% (26/28)</td>
<td>67% (2/3)</td>
<td>90% (28/31)</td>
</tr>
<tr>
<td>Culture</td>
<td>96% (26/27)</td>
<td>100% (4/4)</td>
<td>100% (26/26)</td>
<td>80% (4/5)</td>
<td>97% (30/31)</td>
</tr>
</tbody>
</table>
Although all the biopsy-based tests may give false negatives in the case of a low density of H.pylori, including atrophy [8], both histology and culture were among the best-performing tests in our study group. The choice of the right biopsy sampling approach to detect pre-malignant lesions remains an issue [17-18], and the method is clearly expertise-dependent [19]. All our biopsies were collected according to the standard approach and assessed by two expert pathologists; therefore, any potential drawback of the method will have been eliminated. The sensitivity of H. pylori culture may be an important issue, and so the method is not routinely recommended as the primary tool for identifying the presence of the infection [4]. However, it is becoming increasingly important in populations with high antibacterial resistance [20]. We found 96% sensitivity and 100% specificity with this method; as expected, the OA was slightly higher in the group without atrophy compared to those with atrophy. It should be mentioned that the testing was performed in a specialist institution.

Yet, the performance of biopsy tests was not equal – RUT failed to meet the expectations by demonstrating low sensitivity. Some previous studies have shown false-negative results in up to 50% of cases over 60 years [21]. Similarly, false-positive results may occur due to contamination with urease-producing bacteria from the oral cavity [14]. Low RUT sensitivity in our study group was observed in both the groups with and without atrophy. This leads us to believe that atrophy itself may not be the critical factor responsible for the unsatisfactory (poor) performance of the tests. The test reading time recommended by the manufacturer might possibly be too short. However, other studies have also reported low RUT sensitivity [22].

UBT is among the recommended tests for primary identification of H. pylori [4], but inconsistency exists with respect to the test accuracy where there is a low density of bacteria. In addition, false-negative results of UBT in patients given H2-receptor antagonists remain unexplained [23]. Some studies have reported high false-negative UBT rates in corpus-predominant gastritis [24].

Although UBT has been shown to be a reliable method in the Japanese population with a high prevalence of atrophic gastritis, the values were affected by the degree of atrophy [25]. Similar data have been obtained among elderly patients in Europe [26]. Some European studies have suggested UBT as the test of choice for H. pylori in atrophy, admitting that combining UBT with serology under such circumstances might be beneficial [27]. Other studies have identified lower UBT performance in atrophic body gastritis [10] and even corpus-predominant gastritis [24]. In our group with atrophy, UBT gave good results (96% sensitivity and 100% specificity). However, the results were identical to the group without atrophy.

The draw-back of serology is its inability to differentiate current and recent H. pylori infection; therefore, the method can still give a positive result after successful eradication or spontaneous elimination of the microorganism. In a case of advanced atrophy, serology might be the method of choice, since other methods could give false-negative results depending on the density of H. pylori [28]. Nevertheless, our study did not confirm the hypothesis that serology could show better accuracy in patients with gastric atrophy: the accuracy of serology was marginal both in the group with or without atrophy. In addition, it performed worse than the histology, UBT and culture in patients with atrophic gastritis.

To eliminate uncertain results, we had intentionally set strict criteria for H. pylori positivity; thus, 6.3% of cases (see Table 1) remained in the gray zone. Although not fulfilling our criteria for H. pylori positivity, some of these cases (having two or three test positive results) most probably were H. pylori infected. In four of the cases with a positive serology also UBT and RUT were positive, showing that serology did not add any additional information to other non-invasive tests in confusing cases in our group of patients.

Even if the cases with the serology results in the gray zone would be classified as serology-positive, this would not provide any superiority of serology if compared to the other tests. Moreover, the combined IgA and IgG antibody testing that was used in our study might have given an additional advantage over the standard IgG antibody tests [29]. Also the requirement for local validation of serology tests [4, 14] was not an issue for us, because the particular test-system has been extensively validated in our region, and all the enrolled patients were of Caucasian origin recruited in a single centre.

The limitation of our study was that the relatively small number of patients with atrophy did not allow the further subdivision of this group within subgroups with either corpus or antral atrophy; the number of patients having higher

### Table IV. Performances of the five invasive and noninvasive diagnostic tests for H. pylori detection in the group without atrophy

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Overall accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid urease test</td>
<td>81% (43/53)</td>
<td>100% (35/35)</td>
<td>100% (43/43)</td>
<td>78% (35/45)</td>
<td>89% (78/88)</td>
</tr>
<tr>
<td>Histology</td>
<td>100% (53/53)</td>
<td>100% (35/35)</td>
<td>100% (53/53)</td>
<td>100% (35/35)</td>
<td>100% (88/88)</td>
</tr>
<tr>
<td>13C urea breath test</td>
<td>96% (51/53)</td>
<td>100% (35/35)</td>
<td>100%(51/51)</td>
<td>95% (35/37)</td>
<td>98% (86/88)</td>
</tr>
<tr>
<td>Serology</td>
<td>96% (51/53)</td>
<td>74% (26/35)</td>
<td>85% (51/60)</td>
<td>93% (26/28)</td>
<td>88% (77/88)</td>
</tr>
<tr>
<td>Culture</td>
<td>100% (53/53)</td>
<td>100% (35/35)</td>
<td>100% (53/53)</td>
<td>100% (35/35)</td>
<td>100% (88/88)</td>
</tr>
</tbody>
</table>

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atrophy grades was quite small. The standard evaluation included most of the routinely used tests.

We cannot deny that the performance of biopsy-based tests as well as UBT could possibly be worse in the group of patients with advanced atrophy that was not seen in our study due to specific enrolment criteria. Our results do not support the use of serology as the single *H. pylori* test in the presence of atrophy. Therefore the rational approach would be to run UBT or a biopsy-based test plus serology in the presence of atrophy, as previously recommended [9]. Such an approach was even more strongly supported in the recent German guideline update [30].

**Conclusion**

Histology, UBT and culture were the best performing tests for diagnosis of *H. pylori* infection in our cohort of patients with atrophic gastritis. We cannot recommend serology as a singular diagnostic test in patients with gastric atrophy, while a reasonable combination of serology with histology, UBT or culture might be used to diagnose *H. pylori*.

**Conflicts of interest**

None to declare.

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