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## **The Place and Role of Serologic Methods in Detecting *Helicobacter Pylori* Infection**

Serologic Methods for *Helicobacter Pylori* Infection

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## **ABSTRACT**

*The aim of the study was to determine the place and role of serologic methods in detecting Helicobacter pylori (H. pylori) infection, on the basis of estimated enzyme-linked immunosorbent assay (ELISA) and complement fixation test (CFT) sensitivity and specificity. A total of 549 patients were included in the study. ELISA and CFT as serologic methods were compared with invasive methods (rapid urease test – CLO test, culture, histology). The sensitivity of serologic methods was above 90%, and their specificity was around 80%. Study results confirmed the value, reliability and usefulness of serologic methods in the detection of H. pylori infection.*

**Key words:** H. pylori, serology tests, sensitivity, specificity

## Introduction

*Helicobacter pylori* (*H. pylori*), a bacterium that marked the 20<sup>th</sup> century, is the most common etiologic factor of peptic ulcer, especially in duodenum<sup>1-6</sup>. It is associated with non-cardiac carcinoma of the stomach (diffuse and intestinal type)<sup>7</sup>, and its association with some extraintestinal diseases has also been postulated<sup>8-10</sup>. Diagnostic methods for the detection of *H. pylori* infection are divided into two groups: invasive and noninvasive<sup>11</sup>. All invasive methods are based on endoscopy with biopsy samples of gastric mucosa obtained for direct (histology and culture) or indirect (rapid urease test) diagnosis.

Rapid urease test or CLO test has a sensitivity of 90%-95% and specificity of 98%. In 90% of patients with negative CLO test gastric mucosa is usually unchanged. However, 5%-10% of tested samples can be CLO negative because of inadequate number of the bacteria present in the sample<sup>12-14</sup>.

Histology is a rapid, reliable and reproducible method. This method can also be used to determine the morphological characteristics of gastritis. The sensitivity and specificity of the method are around 95%<sup>13,15,16</sup>. Culture requires a gastric mucosa biopsy sample; however, at least two samples (antrum/corpus) are needed due to uneven colonization of gastric mucosa. This is particularly important on taking samples for the control of *H. pylori* eradication. The sensitivity of culture is 90%-95% and specificity around 100%<sup>13,17</sup>. In addition to identifying the strain of *H. pylori*, molecular methods are used to determine the genes responsible for different factors of virulence<sup>6,13</sup>.

Noninvasive methods are based on the detection of urease activity (urea breath test), presence of specific antibodies in serum and/or saliva of infected person (serology), and in recent time on antigen detection in stool.

Urea breath test detects the presence of *H. pylori* in stomach by detecting the *H. pylori* urease. This test has a high sensitivity and specificity (95%-98% both)<sup>13,18,19</sup>. Urea breath test is usually used to prove *H. pylori* eradication at 4 weeks of antimicrobial therapy completion.

*H. pylori* induces inflammatory reactions in gastric mucosa, thus activating specific humoral immunity response, which in turn results in the production of specific IgM, IgA and IgG antibodies. Specific IgM antibodies are produced in a minority of infected persons. They are specific but difficult to detect. The sensitivity of tests for the detection of specific IgA antibodies, which are bound to the surface of the bacteria and prevent their adhesion to the cells, is 60%-80%. Specific IgG antibodies, subclasses IgG1, IgG2 and IgG4, are most commonly present in the serum of infected individuals. The tests used for their detection have a high sensitivity (94%) and specificity (98%), and are most commonly used in the diagnosis of *H. pylori* infection. During the course of infection, the levels of antibodies are insignificantly changed<sup>20,21</sup>. Different serologic tests are used to detect *H. pylori* infection: agglutination, latex agglutination, passive hemagglutination, complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA) and immunoblot test<sup>22</sup>. Serologic diagnosis has a special place in epidemiological studies<sup>23</sup>.

Since recently, immunoenzyme procedures have been used for direct detection of *H. pylori* antigen in stool sample. These procedures are used to detect active infection as

well as its eradication. The procedure sensitivity is 80%-90% and specificity around 100%<sup>17-23</sup>.

The aim of this study was to determine the place and role of serologic methods in the diagnosis of *H. pylori* infection, on the basis of estimated ELISA and CFT sensitivity and specificity.

## Materials and Methods

The study was performed during the 1994-2002 period at Merkur University Hospital and Prison Infirmary in Zagreb, and included 549 patients (Table 1): 436 patients (M/F 250/186, mean age 53.4 years) regularly attending Endoscopy Laboratory, Merkur University Hospital, and 113 patients (M/F 102/11, mean age 41.9 years) from Prison Infirmary. All patients suffered pain in the upper abdomen with dyspeptic symptoms. Prior to entering the study, the patients signed the informed consent form for gastroscopy. The study design was approved by the Hospital Ethics Committee.

TABLE 1.  
PATIENT GENERAL CHARACTERISTICS

	N	Mean age ( $\bar{x}$ ) (yrs)
Merkur University Hospital patients	436	53.4
- men	250	53.2
- women	186	54.1
Prison Infirmary patients	113	41.9
- men	102	42.2
- women	11	40.0
<b>Total</b>	<b>549</b>	<b>51.8</b>

N-number of patients

Study patients underwent clinical examination and gastroscopy. During gastroscopy 7 histological samples of gastric mucosa were obtained (3 from the corpus and 4 from the antrum). One sample was taken for rapid urease test (CLO test, Delta West, Bentley, Western Australia), two samples were obtained for culture (Skirrow agar, Mueller-Hinton agar, E-test), and four samples for histology (Giemsa modified technique and Warthin-Starry stains; Sydney classification system of gastritis).

Patients were included into *H. pylori* positive group if the result of histology and urease test and in some cases of culture were positive for *H. pylori*. The *H. pylori* negative group included patients in whom histology, urease test and culture were negative. Histology<sup>16</sup>,



urease test<sup>14</sup> and culture for *H. pylori*<sup>17</sup> were done according to the previously described methodology. The patients who had been taking any kind of antibiotic therapy or a combination of antisecretory and antibiotic therapy for one month before endoscopy were excluded from the study.

Serum samples were tested with commercial ELISA (Eurospital, Trieste, Italy) and CFT (Institute Virion, Zurich, Switzerland). The tests were performed according to the manufacturer's instructions. Borderline test values were established in line with the manufacturer's instructions, to interpret the results obtained.

ELISA: each serum sample diluted 1:200 was applied onto a microtiter plate with previously bound *H. pylori* antigen. The antigen-antibody complex was proven by sheep antihuman IgG antibodies labeled with alkaline phosphatase and incubated with chromogen substrate. The substrate absorption was determined by ELISA reader (Multiscan, Titertek, MCC/340, Finland). An index of IgG antibodies equal or higher than 40% was considered as a positive result.

CFT: complement fixation antibodies (IgM, IgG) were proven by *H. pylori* strain Lior type 1. Each serum sample was diluted with a 1:10 Veronal buffer and incubated for 30 minutes at 56 °C to inactivate the complement present in the serum. Then serum sample as well as positive and negative serum controls were diluted from 1:10 to 1:160, edging certain dilution of antigen and complement. The test included controls to detect anticomplementary activity in each sample tested as well as control for the complement used (0.5, 1.0, 1.5 and 2.0 units of complement). The result of CFT was assessed on the basis of hemolysis inhibition. The inhibition of 50% or more was considered positive,

indicating the presence of antibodies in the respective dilution. Antibody titer of less than 1:30 was considered negative.

To determine the specificity of the serologic methods used we tested sera of 227 patients with pain in the upper abdomen, free from dyspeptic symptoms and without *H. pylori* in the gastric mucosa biopsy samples (histology, rapid urease test, cultures were negative) (Table 2).

### *Statistics*

The  $\chi^2$  test for dependent and independent samples, and the test of proportions were used. Statistical analysis was done by use of the Microstat software. Statistical significance was set at  $p < 0.05$ .

## Results

### *Sensitivity and specificity of ELISA and CFT*

The sensitivity of ELISA and CFT was evaluated by testing serum samples of 276 patients with dyspeptic symptoms. Patients underwent gastroscopy, and *H. pylori* was detected in biopsy samples by culture, CLO test and histology. The sensitivity of serologic methods was above 90%, i.e. 94.9% for ELISA and 93.1% for CFT (Table 2).

The specificity of ELISA and CFT was assessed by testing serum samples of 227 patients free from dyspeptic symptoms and without *H. pylori* detected in biopsy samples of gastric mucosa (histology, rapid urease test, cultures were negative). The specificity of serologic methods was around 80%, i.e. 80.1% for ELISA and 78.4% for CFT (Table 2).

TABLE 2.  
EVALUATION OF SENSITIVITY AND SPECIFICITY OF ELISA AND CFT

	N	ELISA		CFT		Sensitivity		Specificity	
		n +	n -	n +	n -	ELISA (%)	CFT (%)	ELISA (%)	CFT (%)
<i>H. pylori</i> (+)	276	262	14	257	19	94.9	93.1	-	-
<i>H. pylori</i> (-)	227	45	182	49	178	-	-	80.1	78.4

N - total number of tested patients, (+) - *H. pylori* positive patients, (-) - *H. pylori* negative patients, ELISA - enzyme-linked immunosorbent assay, CFT - complement fixation test

### *Evaluation of invasive and noninvasive serologic methods in patients with dyspeptic symptoms*

On the basis of gastroscopy findings, 549 patients were divided into two groups: group 1 including patients without endoscopically verified ulcer and/or ulcer scar (168 patients with nonulcer dyspepsia), and group 2 including patients with ulcer and/or ulcer scar (381 patients).

In all patients, biopsy samples of gastric mucosa were tested for the presence of *H. pylori* (culture, CLO test, histology). Serum samples were tested by ELISA and CFT to detect specific antibodies against *H. pylori*. Results obtained in patient sera by use of invasive and noninvasive methods and their evaluation are shown in Table 3.

TABLE 3.  
COMPARISON OF ENDOSCOPY FINDINGS WITH RESULTS OF SEROLOGIC AND INVASIVE METHODS IN STUDY PATIENTS

Endoscopy finding	N	ELISA n (%)	CFT n (%)	Histology n (%)	CLO N (%)	Culture n (%)
Non-ulcer dyspepsia	168	142 (84.5)	127 (75.5)	134 (79.7)	126 (75.0)	65 (38.6)
Ulcer (scar)	381	365 (95.8)	354 (92.9)	341 (89.5)	323 (84.7)	244 (64.0)
Total	549	507 (92.3) <sup>*/**</sup>	481 (87.6) <sup>***</sup>	475 (86.5)	449 (81.7) <sup>*</sup>	309 (56.2) <sup>**/**</sup>

N - total number of patients, ELISA - enzyme-linked immunosorbent assay, CFT - complement fixation test, CLO - rapid urease test, \* p<0.05, \*\* p<0.001, \*\*\* p<0.001

A statistically significant difference between ELISA and invasive methods was only recorded in the group of patients with ulcer (scar) ( $\chi^2=6.45$ , p=0.09), however, only at a 90% level. Comparison of CFT and invasive methods showed no statistically significant difference in either group of patients ( $\chi^2=6.02$ , ns). Comparison of ELISA and CFT results with the results of each individual invasive method produced a statistically significant difference in both groups of patients only between positive ELISA results and positive culture results ( $\chi^2=4.57$ , p<0.05). Proportion testing showed a statistically higher number of *H. pylori* infection detected in the group with ulcer (scar) by both serologic and invasive methods: ELISA (Z=4.59, p<0.001), CFT (Z=5.70, p<0.001), histology (Z=3.09, p<0.001), rapid urease test (Z=2.7, p<0.005) and culture (Z=5.23, p<0.001).

On analysis of overall results obtained by serologic and invasive methods (Table 3) using the test of proportions, there was no statistically significant difference between ELISA

and CFT ( $Z=0.82$ , ns), or between ELISA and histology ( $Z=1.02$ , ns). However, ELISA showed a statistically significantly higher sensitivity than either urease test ( $Z=1.9$ ,  $p<0.05$ ) or culture ( $Z=7.27$ ,  $p<0.001$ ). CFT was statistically significantly more sensitive only compared with culture ( $Z=6.36$ ,  $p<0.001$ ), whereas the sensitivity of histology ( $Z=0.19$ , ns) and urease ( $Z=1.06$ , ns) yielded no statistically significant difference.

## Discussion

A variety of methods have been used in the diagnosis of *H. pylori* infection. Most of the methods are invasive because they require gastroscopy to obtain biopsy samples of gastric mucosa for further analysis and detection of *H. pylori* infection. Culture is necessary to test for antimicrobial susceptibilities. The other group of methods are noninvasive because they do not require gastroscopy and *H. pylori* infection can be detected by the presence of antibodies in serum samples (serology), by the presence of labeled CO<sub>2</sub> in exhaled breath upon ingestion of labeled urea, and by the bacterial urease activity (urea breath test).

ELISA is most widely used in the detection (qualitative) and measurement (quantitative) of the level of specific antibodies in serum samples. The previously used non-purified antigens have been replaced by purified products of urease and/or proteins of great molecular mass extracted from glycine. Immunoblot (Western blot) has recently been used as the method of choice for evaluation of immunity response against different *H. pylori* antigens (VacA, CagA). Antibodies against these antigens indicate an increased risk of ulcer and gastric adenocarcinoma, and are used as a confirmation test for the results obtained by other serologic methods<sup>24-26</sup>. ELISA detects the presence of individual classes of specific antibodies and can also determine the level of these antibodies in serum samples. ELISA tests for the detection of IgG antibodies have a more than 90% sensitivity and specificity<sup>27</sup>. The sensitivity of serologic methods used in the present study was more than 90%. The sensitivity of ELISA was 94.9%, exceeding the sensitivity of CFT of 93.1%. The specificity was slightly lower: 80.1% for ELISA and 78.4% for

CFT, which is consistent with the results reported elsewhere for commercial serologic procedures<sup>28,29</sup>.

The sensitivity and specificity are important parameters which show the purpose of using serologic methods in the diagnosis of *H. pylori* and evaluation of the methods employed. Different values of the sensitivity and specificity reported from various studies could be explained by the use of different normal values and "standard" methods. Some studies employed only one noninvasive method (culture, histology or rapid urease test) as a standard method, whereas others employed a combination of two or more methods. In our study, we chose histology, culture and rapid urease test as standard methods.

The sensitivity and specificity of the superior serologic tests are the same as the sensitivity and specificity of urea breath test<sup>30</sup>. The sensitivity of serologic tests is slightly higher than the sensitivity of invasive methods<sup>31,32</sup>, as confirmed by our results. In the group of patients with ulcer and/or ulcer scar, a statistically significant difference was recorded in the detection of infection between ELISA (at 90% level) and invasive methods. The difference in sensitivity between serologic and invasive methods may be caused by difficulty in obtaining biopsy material due to the poorly visible site of *H. pylori* colonization on the gastric mucosa<sup>32-36</sup> and the effect of antibacterial therapy. Some authors<sup>37,38</sup> emphasize a disproportion between the grade of infection and the degree of immune response, pointing to inter-individual differences in the immune response to infection. There are literature reports on cases of *H. pylori* infection detected by invasive methods yet not accompanied by corresponding antibody levels, which results from a weak or absent response of the immune system<sup>39,40</sup>. In atrophic gastritis, serology may be the only tool to detect *H. pylori* infection<sup>41-43</sup>. In addition, invasive tests do not perform

well in patients with bleeding ulcers<sup>44-46</sup>. It should be noted that the sensitivity and specificity of serologic methods are reduced in persons above 60 years of age as the result of weak immune response<sup>47</sup>.

The incidence of *H. pylori* negative "nonspecific gastritis" is higher in the elderly, which may be due to the small number of bacteria present in gastric mucosa, previous infection treated with antibiotics, gastric mucosa atrophy, and gastritis of other etiology (autoimmune gastritis, prolonged therapy with nonsteroidal anti-inflammatory drugs). Like serologic methods, histologic methods also are less reliable in detecting *H. pylori* infection in the elderly. Tests for antibody detection in saliva samples have a lower sensitivity and specificity than tests for the detection of serum antibodies<sup>48,49</sup>.

In spite of these shortcomings associated with serologic methods, simultaneous usage of a serologic method with one or more invasive methods will significantly increase the overall sensitivity of the diagnostic work-up. This is important in patients with clinical signs of severe infection and in those aged >45, who are at a higher risk of developing serious complications. Some authors suggest that patients younger than 45 without alarming symptoms can be screened for the presence of infection using only serologic methods<sup>50</sup>. Today, the recommendation is to use more methods for detecting *H. pylori* infection because all known methods yield some 5%-10% of false positive or false negative results<sup>51</sup>.

Our results showed the use of serologic methods in the detection of *H. pylori* infection (primary infection) with commercial CFT and ELISA tests to be helpful, reliable and fully justified. Commercial products were evaluated by testing the sera from a selected patient population. The sensitivity, specificity and reference values were determined, as



they differ from population to population. Three standard methods, i.e. histology, culture and urease test, were used on evaluation of the serologic method sensitivity and specificity. The sensitivity of serologic methods exceeded 90%; however, ELISA showed higher sensitivity and specificity than CFT (94.9% vs 93.1% and 80.1% vs 78.4%, respectively).

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## **MJESTO I ULOGA SEROLOŠKIH METODA U UTVRĐIVANJU INFEKCIJE *HELICOBACTER PYLORI***

### **SAŽETAK**

Cilj nam je bio na temelju utvrđenih vrijednosti osjetljivosti i specifičnosti metoda ELISA (imunoenzimski test) i reakcije vezanja komplementa odrediti mjesto i značenje seroloških metoda u otkrivanju infekcije *Helicobacter pylori* (*H. pylori*). U ispitivanje je bilo uključeno 549 bolesnika, a navedene serološke metode su uspoređene s invazivnim metodama (CLO test, izolacija, histološki pregled). Osjetljivost seroloških metoda premašila je 90%, dok je specifičnost bila približno 80%. Ovim radom je dokazana vrijednost, pouzdanost i opravdanost uporabe seroloških postupaka u otkrivanju infekcije *H. pylori*.