Application of *Helicobacter pylori* antigen test to evaluate gastric mucosa specimens

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ABSTRACT

Purpose: To investigate, whether the test documenting the presence of *Helicobacter pylori* (*H. pylori*) antigens in the gastric mucosa may be used as diagnostic test.

Materials and methods: Mucosal specimens taken from eighty-three patients during gastroscopic examination were subjected to rapid urease test (CLO test), histology, and *H. pylori* culture. The same biopsy specimens that had been evaluated in the CLO test or collected into the transport medium for bacterial culture were used to detect *H. pylori* antigens. An amplified immunoassay for the detection of *H. pylori* antigens in stool was used for gastric mucosa specimens. The sensitivity and specificity of the *H. pylori* antigen test were evaluated in relation to the results of each verifying test (CLO test, histology, culture) separately and to all 3 tests analysed together.

Results: The sensitivity and specificity of the *H. pylori* antigen test in relation to the CLO test, histological examination, and *H. pylori* culture were 85.4% and 90.5%, 76.1% and 83.4%, and 90.7% and 90.0% for specimens taken for the CLO test and 90.0% and 82.0%, 78.0% and 81.0%, and 93.0% and 88.0% for specimens taken for bacterial culture, respectively. The sensitivity and specificity of the antigen test in relation to all 3 verifying tests analysed together were 91.3% and 97.3% for specimens taken for the CLO test, and 91.7% and 97.1% for specimens taken for bacterial culture, respectively.

Conclusions: *H. pylori* antigen test in gastric mucosa specimens may be a sufficiently reliable source of information about stomach infection.

Key words: *Helicobacter pylori* antigens, immunoassay, gastric mucosa

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INTRODUCTION

For years, the urease tests [*Campylobacter*-like organism (CLO) test, urea breath test] have been the most commonly applied diagnostic test for *Helicobacter pylori* (*H. pylori*) infection. Their sensitivity and specificity can amount up to 90% [1-7]. This means that some patients have negative test result despite being infected, while others have a positive result when no infection exists. A false negative test result is found when the number of bacteria in the mucosal specimen is too small or their urease activity is too weak [1,8]. A false positive result of the urease test is most frequently associated with the presence of urease-positive bacteria other than *H. pylori* in the stomach; they usually originate from the oral cavity [9]. Such a phenomenon is most often observed in patient receiving long-term therapy with proton pump inhibitors [10,11]. Apart from the urease tests, there are a number of other methods used to diagnose stomach infection (histological examination, bacterial culture, molecular biology techniques) [2,3,12-19]. However, except for histological examination, other methods are not widely applied in clinical practice. In the last ten years, a test documenting the presence of bacterial antigens in the stool has been used for the diagnosis of *H. pylori* infection [7,20-22]. After being slightly adapted, this test has also found application in the evaluation of *H. pylori* infection of the oral cavity [23-26].

The aim of this study was to investigate whether the test for *H. pylori* antigens detection in stool may be used for detection of *H. pylori* antigens in biopsy specimens of the gastric mucosa.

MATERIALS AND METHODS

Study subjects

Eighty-three subjects, aged 20-79 years old, were included in the study (Table 1).

Table 1. Patient characteristics.

Age (years)	56.6 ± 13.9
Gender (M/F)	34 / 49
Smokers (%)	19 (22.9)
Drinkers (%)	18 (21.7)
Diagnosis Gastritis (%) Peptic ulcer disease (%)	 67 (80.7) 16 (19.3)

The inclusion criteria were good general condition (anamnesis, physical examination), no chronic or devastating diseases, no history of antibiotic therapy in the past month and no history of *H. pylori* eradication. The study was approved by the Ethics Committee for Research on Humans and Animals of Medical University of Białystok. Written consent was obtained from all subjects before study entry.

Sample collection

During the gastroscopic examination eight biopsy specimens of the gastric mucosa from the prepyloric area and gastric body were taken, one for CLO test, one for bacterial culture, and two for histological examination from each site. Gastric infection with *H. pylori* was evaluated by CLO test prepared in the Physiology Department of the Medical University of Białystok, according to the method of Marshall et al. [27]. The sensitivity and specificity of the test in relation to histological examination, culture, and stool test were 84.3% and 88.4%, 87.5% and 83.5%, and 75.4% and 87.5%, respectively. The test result was defined as positive if its colour changed from orange to pink within 2 hours. The biopsy specimens for histological examination were processed according to a standard procedure [17]. To detect *H. pylori* antigens in the gastric mucosa, the same biopsy specimens that had earlier been evaluated in the CLO test or collected into the transport medium for bacterial culture were used.

Sample processing

The specimens designed for bacterial culture were placed into the transport medium (Portagerm pylori, bioMerieux, Marcy I'Etoile, France) and then after homogenisation in 100 µL of saline were inoculated on selective Pylori agar (bioMerieux) and non-selective Columbia agar enriched with 5% sheep blood (bioMerieux) [28]. The remaining sample volume was supplemented with 100 µL of sample diluent, included in the *H. pylori* antigen detecting set for stool (Amplified IDEIA ™ Hp StAR ™, Oxoid, Ely, UK). Similarly, the specimens subjected to the CLO test (positive results within 2 hours) were homogenized in 100 µL of saline supplemented with 100 µL of the diluents, included in the *H. pylori* antigen detection set. All samples were vortexed for 30 seconds and centrifuged for 5 minutes at 5000 rpm. Fifty µL of supernatant was sampled for further analysis.

H. pylori antigen test performance

The laboratory procedures were performed according to the manufacturer's protocol attached to the test for *H. pylori* antigens detection in stool specimens (Amplified IDEIA ™ Hp StAR ™, Oxoid, Ely, UK). In brief, 50 µL of supernatant of gastric mucosa suspension as well as horseradish peroxidase labelled monoclonal antibodies were added in one step to the microwells of microtitration plate coated

by the manufacturer with monoclonal antibodies specific for *H. pylori*. During incubation, *H. pylori* antigens present in a sample bound to the antibodies located on the microplate and horseradish peroxidase, forming a sandwich complex. The microwells were washed with phosphate buffer to remove unbound antibody conjugate and then tetramethylbenzidine was added. Bound horseradish peroxidase oxidized tetramethylbenzidine to a blue coloured product. The reaction was stopped with sulphuric acid which changed the colour from blue to yellow. The intensity of the colour was determined spectrophotometrically.

Statistical analysis

The sensitivity and specificity of the test detecting *H. pylori* antigens in the endoscopically taken specimens of gastric mucosa were evaluated in relation to the results of each verifying test (CLO test, histological examination, culture), separately for the biopsy specimens used in the CLO test and those taken for culture. The calculation of sensitivity and specificity of the *H. pylori* antigen test was performed also in relation to 3 verifying tests analysed together. For this analysis, the result was defined as true positive, if at least one verifying test was positive, and false positive when all verifying tests were negative.

The result of the test was defined as true negative, if no more than one verifying test was positive and false negative, if more than one verifying test was positive. *H. pylori* antigen test sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy were calculated according to standard methods listed below.

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Specificity} = \frac{TN}{TN + FP}$$

$$\text{Positive predictive value} = \frac{TP}{TN + FN}$$

$$\text{Negative predictive value} = \frac{TN}{TN + FN}$$

$$\text{Accuracy} = \frac{TP + TN}{TP + FP + TN + FN}$$

(*Legend*: TP – true positive; FP – false positive; TN – true negative; FN – false negative)

RESULTS

In the group of 83 patients, we observed in only two subjects a difference in test results detecting *H. pylori* antigens in specimen's primary taken for the culture and the CLO test; the result was twice positive for specimens taken for culture, with a concomitant negative result in specimens taken for the CLO test. The sensitivity and specificity of the antigen test in relation to the CLO test, histological examination and culture in biopsy specimens taken for CLO test were 85.4% and 90.5%, 76.1% and 83.4%, and 90.7% and 90.0%, respectively. For the specimens taken for culture, the sensitivity and specificity were 90.0% and 82.0%, 78.0% and 81.0%, 93.0% and 88.0%, respectively.

The sensitivity and specificity of the antigen test in relation to all 3 verifying tests analysed together were, for the biopsy specimens taken for CLO test 91.3% and 97.3%, respectively, and for specimens taken for culture 91.7% and 97.1%, respectively (Table 3).

H. pylori antigens detection in gastric mucosa specimens was the most consistent with bacterial culture, and the least consistent with histological examination (Table 2).

Table 2. Sensitivity, specificity, positive and negative predictive values, and accuracy of *H. pylori (H.p.)* antigen test for gastric specimens versus the CLO test, histology and culture.

	***H.p.* antigens vs CLO test**		***H.p.* antigens vs histology**		***H.p.* antigens vs culture**	
	A	B	A	B	A	B
Sensitivity	85.4	90.0	76.1	78.0	90.7	93.0
Specificity	90.5	82.0	83.4	81.0	90.0	88.0
Positive predictive value	89.7	81.4	85.4	83.7	90.7	93.0
Negative predictive values	86.4	90.0	75.5	75.0	90.0	87.0
Accuracy	87.9	85.5	79.5	79.5	90.3	90.4

A- CLO test samples; B- culture samples

Table 3. Sensitivity, specificity, positive and negative predictive values of *H. pylori* antigen test for gastric specimens versus 3 other diagnostic tests (CLO test, histology and culture).

	CLO test samples	**Culture samples**
Sensitivity	91.3	91.7
Specificity	97.3	97.1
Positive predictive values	97.7	97.8
Negative predictive values	90.0	89.5
Accuracy	94.0	94.0

DISCUSSION

The results of the current study indicate that the evaluation of *H. pylori* antigens in the endoscopically taken biopsy specimens is a relatively good diagnostic tool. However, the cost of a single examination in Poland is higher than a CLO test, but comparable with a histological examination, urea breath test or culture. The time required to obtain the test result is comparable with that of the CLO test or urea breath test, but shorter than histological examination or culture. The probability of obtaining a false positive result is small, as *H. pylori* belongs to dominating flora in the stomach and other bacteria present within the gastric mucosa should not be responsible for a positive test result. The probability of obtaining a false negative result is not large as well, because the presence of as low as 100 *H. pylori* bacteria is sufficient to obtain a positive test result [23]; when two gastric mucosa biopsy specimens from the same patient were analysed together, the number of *H. pylori* bacteria was much larger than 100 [29]. One biopsy specimen of the gastric mucosa is probably sufficient to perform the test, however, in our study we used two specimens, one from the antrum and another one from the gastric body, because some patients participating in the study were under therapy with proton pump inhibitors and the distribution of bacteria in such patients is different than in untreated patients [4,18].

Since currently used methods for detecting stomach infection with *H. pylori* are encumbered with certain error, the new diagnostic techniques are still being developed and tested worldwide. The novelty of our study lies in the fact that the immunologic test formerly used for detection of *H. pylori* antigens in feces may be used more widely than the manufacturer designed. The evaluation of *H. pylori* antigens in gastric mucosal specimens, which were earlier used in a CLO test or taken for bacterial culture, enables the extension of diagnosis of *H. pylori* gastric infection without the need of having additional biopsy specimens. According to the results of current study, the evaluation of *H. pylori* antigens, both in the biopsy specimens taken for CLO test or culture, is encumbered with similar error. In the case of antigen detection in specimens taken exclusively for this aim, a small per cent decrease in sensitivity and specificity of this test in relation to verifying tests must be taken into account; this is suggested in our study on the base of slightly lower sensitivity and specificity of the *H. pylori* antigen test in relation to the histological examination. It cannot be ruled out that the test evaluating the presence of *H. pylori* antigens in the endoscopically taken specimens of the gastric mucosa might be used on equal terms with other tests diagnosing the stomach infection, both before and after eradication therapy. However, it is rather unlikely that it could compete with the urea breath test, CLO test or histological examination. We hope that in selected cases, detection of *H. pylori* antigens will answer the question whether increased urease activity in the gastric mucosa is related to the presence of *H. pylori* or other urease-positive bacteria.

CONCLUSIONS

Taking into account a relatively low accessibility to *H. pylori* bacteria culture from the biopsy specimens of the gastric mucosa (only selected microbiology laboratories in Poland provide such service), the test evaluating the presence of the

bacterial antigens may become a sufficiently reliable source of information concerning stomach infection.

Conflicts of interest

None declared

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