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Accuracy of a Monoclonal Antibody-based Stool Antigen Test in the Diagnosis of *Helicobacter pylori* Infection

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Background: Recent availability of tests for *Helicobacter pylori* antigens in stool samples has provided potentially useful tools for epidemiological studies and clinical settings. The aim of this study was to evaluate a monoclonal antibody-based *H. pylori* antigen stool test in the primary diagnosis of *H. pylori* infection, and to study the test performance after patients were treated with lanzoprazole, and after eradication therapy. **Methods:** The study included 122 dyspeptic patients. At gastroscopy, biopsy specimens were obtained for culture and histology. Stool antigen and [^{14}C]-urea breath tests were performed concurrently. Positive culture alone or a positive [^{14}C]-urea breath test in combination with positive histology defined the reference standard. Forty-three Hp +ve patients were treated with lanzoprazole for 2 to 4 weeks, and stool antigen tests were performed on days 1 and 7 post-treatment. After eradication therapy, 32 patients were re-examined for *H. pylori* infection. **Results:** Prevalence of *H. pylori* was 44.3%. Sensitivity and specificity for the stool antigen test in the primary diagnosis of *H. pylori* infection were 98% and 94%, with positive and negative likelihood ratios of 16.7 and 0.02, respectively. All patients had positive stool tests immediately after lanzoprazole treatment, whereas 2 patients had negative stool tests after 7 days. Triple therapy rendered all patients stool test negative. **Conclusions:** The monoclonal antibody-based stool antigen test is an accurate tool in the primary diagnosis of *H. pylori* infection and after eradication therapy. Lanzoprazole treatment does not influence the clinical performance of the test.

Key words: Accuracy; antigens; diagnosis; enzyme-linked immunosorbent assay; *Helicobacter pylori*; monoclonal antibodies; sensitivity and specificity

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Helicobacter pylori is a major risk factor for gastric and duodenal ulcer (1, 2), and is in addition classified as a carcinogen (3–5). The presence of *H. pylori* infection can be detected by several methods, some of which require gastroscopy. These methods have different advantages and disadvantages (6–8). The patchy distribution of *H. pylori* infection in the gastric mucosa is a challenge to diagnostic methods based on biopsy, as obtaining too little material can lead to a false negative result. The culturing of *H. pylori* is difficult owing to slow growth and the demand for a microaerobic atmosphere, resulting in a low sensitivity. However, since there are expected to be no false positive results of culture, the specificity is close to 100% (9). Histology is highly observer dependent, although it is a good method in the hands of a trained pathologist (10). The rapid urease test is based on the urease activity of *H. pylori* in a biopsy sample. It is convenient because of the rapid test results, but the accuracy of the rapid urease test is not optimal (9). A major drawback of all invasive methods is that they are not suited to epidemiological studies, and may also be unsuitable for testing children.

Serology is not practical for the diagnosis of ongoing *H. pylori* infection, as the antibody level falls slowly after eradication, yielding false positive results and thus a low specificity (11, 12). The urea breath test has a high degree of accuracy whether [^{14}C]-based or [^{13}C]-based, and is in many studies considered to be the reference standard (13–15). However concerns over radiation may limit the use of [^{14}C]-urea, and the employment of the [^{13}C]-urea breath test requires expensive equipment for analysis.

In recent years a new diagnostic tool has been available; the detection of *H. pylori* antigen in stool samples. The main advantages are the non-invasive nature of this procedure, and the fact that the patient can obtain a stool sample at home and send it to the laboratory for analysis. The first commercially available stool test was based on polyclonal antibodies, and

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has been thoroughly evaluated with reports of a sensitivity in the range of 86%–100% (16, 17) and a specificity of 70%–100% (17, 18).

In this study we have evaluated a newly developed, monoclonal antibody-based, commercially available kit for detecting *H. pylori* antigen in faeces. It features a novel enzyme-linked immunosorbent assay (EIA) amplification technique, which, theoretically, allows the detection of smaller amounts of antigen compared to traditional EIA methods.

Treatment with acid-inhibiting agents is common among dyspeptic patients, even with proton pump inhibitors (PPIs), which are the most effective acid inhibitors (19). In general, PPI therapy is known to interfere with *H. pylori* diagnosis; it diminishes *H. pylori* load and enzyme activity, leading to lower sensitivity of both invasive tests and urea breath tests (20). The use of PPIs has been shown to affect the outcome of the polyclonal antibody-based *H. pylori* stool antigen test (21–23). However, there are no published data on the effect of PPI treatment on test results of monoclonal antibody-based tests.

The purpose of this study was to evaluate the monoclonal antibody-based *H. pylori* stool antigen test as compared to a reference standard in the detection of *H. pylori* infection both pre- and post-eradication, and also to evaluate the effect of PPI treatment on test performance.

Materials and Methods

Patients

Patients aged between 18 and 75 years with upper abdominal complaints referred to gastroscopy from primary care physicians and not previously examined or treated for *H. pylori* infection were eligible for the study. Exclusion criteria were the use of PPIs, H_2-antagonists, bismuth or antibiotics during the 4 weeks prior to examination, pregnancy, serious liver disease, prosthetic heart valves or grafts and serious illness in general.

The study took place at the outpatient clinic of the Dept. of Gastroenterology, University Hospital of Northern Norway, Tromsø, from October 2002 to October 2003. Patients were invited to participate in the study by letter in advance of their attendance at the clinic. The course of the study is presented in Fig. 1. About half of the invited patients were not enrolled, because of the presence of exclusion criteria, unwillingness to participate or they attended the clinic on a day that was too busy for inclusion. Of the invited patients, 131 were initially enrolled. Nine patients failed to provide a stool sample despite

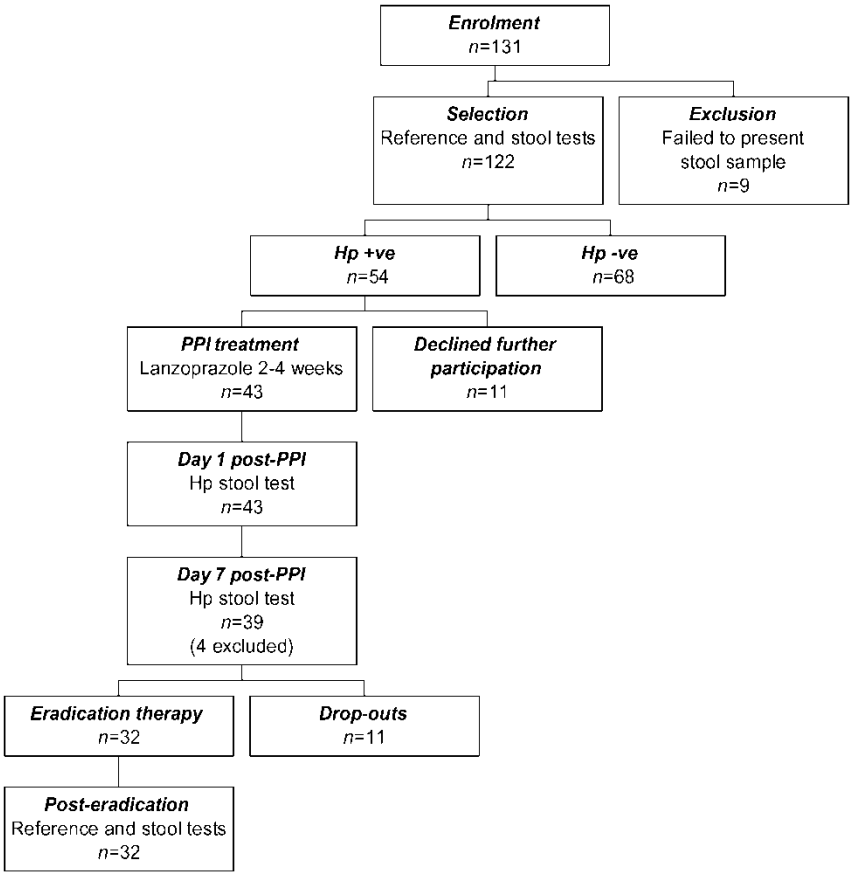

Fig. 1. Study design.

giving their consent, leaving 122 patients in the study (58 F (44%), 64 M, mean age 48 years, range 20–76).

Patients diagnosed with *H. pylori* infection ($n = 54$) were invited to participate in the second part of the study, concerning the effect of PPI treatment. Eleven patients declined further participation, leaving 43 Hp +ve patients, who were given lanzoprazole at 30 mg daily for 2 or 4 weeks (4 weeks if a peptic ulcer was diagnosed at endoscopy). They were instructed to obtain stool samples on day 1 and day 7 after the end of this treatment.

Finally, 32 patients were prescribed a 1-week treatment with 400 mg ranitidine bismuth citrate b.i.d., 250 mg clarithromycin b.i.d. and 500 mg metronidazole b.i.d., a regimen with a reported eradication rate of 97% (24). *H. pylori* antigen stool test, urea breath test and gastroscopy with biopsies taken for culture and histological examination were carried out between 1 and 2 months after triple therapy.

This study and the presentation of the results are designed to adhere to the guidelines in the STARD statement (25).

Tests for H. pylori

Biopsy specimens were obtained from the antrum and body of the stomach during gastroscopy (one each for culture and two each for histology). The tissue specimens for histological examination were fixed in 10% formaldehyde solution and embedded in paraffin according to standard procedures, and subsequently cut into 3-μm sections and stained with haematoxylin/eosin and Alcian Yellow/toluidine blue. Biopsy specimens for microbiological analysis were immediately put on a special transport medium (Portagerm pylori, bioMérieux, France), and cultured on Columbia horse-blood agar (Oxoid, UK) with *Helicobacter pylori* Selective Supplement (Oxoid, UK) in a microaerobic atmosphere for at least 9 days.

[^{14}C]-urea breath test was performed according to previously published protocols (26). Results were expressed as recovery of [^{14}C]-CO_2 in percent of ingested [^{14}C] during sampling at 10, 20 and 30 min after administration. A cut-off value of 1.86% was used.

Stool samples were obtained within a few days after gastroscopy (0–2 days), with the patients taken off all antibiotics or acid-inhibiting medication. The evaluated stool test was Amplified IDEIA Hp StAR (DakoCytomation Norden, Denmark), previously distributed as FemtoLab *H. pylori*. This test is based on a new amplifying enzyme immunoassay technique, using a monoclonal *H. pylori* antibody, and is processed as a sandwich EIA. Optical density was read by spectrophotometer. The test is qualitative, and the numeric results for optical density were converted to positive or negative test results, in accordance with the manufacturer's instructions. The patients sent stool samples (1–2 mL) by mail, and the samples were frozen at −70 °C immediately upon arrival. Tests were processed at intervals of less than 3 months. Every stool sample was analysed in two wells on the same microtitre plate.

The reference standard for Hp +ve patients was defined as a positive culture alone or a combination of a positive [^{14}C]-urea breath test and positive histology. Stool test and reference tests were all performed blinded to the other test results.

Table I. Performance of the *H. pylori* antigen stool test compared to the reference standard in the primary diagnosis of *H. pylori* infection

	Reference standard		
	Hp +ve	Hp −ve	Total
Stool test +ve	53	4	57
Stool test −ve	1	64	65
Total	54	68	122

Statistical methods

Measurements of test performance were calculated using SPSS statistical software (SPSS Inc., Chicago, Ill., USA). Positive likelihood ratio is calculated as sensitivity/(1–specificity) and negative likelihood ratio as (1–sensitivity)/specificity.

Ethical considerations

The local Regional Committee for Medical Research Ethics approved the study. License to register patients participating in the study was granted by the Norwegian Data Inspectorate. Each subject gave written informed consent.

Results

Among 122 patients who were eligible for analysis, 54 were *H. pylori* positive according to the reference standard (Table I). Prevalence of *H. pylori* infection was thus 44.3% in the study population. Sensitivity of the stool test was 98% and specificity was 94%. Likelihood ratio for a positive test result was 16.7, and likelihood ratio for negative test result 0.02. Positive and negative predictive values were 93% and 98%, respectively. At gastroscopy, we found a peptic ulcer prevalence of 7%, all ulcers non-bleeding, and no other major gastric pathology.

Results for stool tests for the 43 Hp +ve patients that received PPIs are listed in Table II. Three patients misunderstood the instructions and obtained the second stool sample after having taken the prescribed triple therapy for eradication of *H. pylori*. The second stool samples from these patients

Table II. Performance of the *H. pylori* antigen stool test after PPI treatment

	Post-PPI treatment stool test			
	Day 1		Day 7	
Hp +ve patients	($n = 43$) Hp +ve	Hp −ve	($n = 39$) Hp +ve	Hp −ve
43	43	0	37	2

PPI = proton-pump inhibitor.

Table III. Performance of the *H. pylori* antigen stool test after triple therapy

	Reference standard		
	Hp +ve	Hp −ve	Total
Stool test +ve	0	0	0
Stool test −ve	0	32	32
Total	0	32	32

were excluded from the analysis. One patient did not send the second stool sample after termination of lanzoprazole treatment.

Among the 32 patients who had triple therapy, all were Hp −ve both by stool test and reference standard at follow-up. The eradication rate for the used regimen was thus 100%. Specificity of the test after eradication was 100%. Statement of sensitivity is futile, as the prevalence of disease after eradication was 0% (see Table III).

The manufacturer claims that a single test is sufficient when using the monoclonal antibody-based stool test. In this study every stool sample was tested twice on the same microtitre plate. Three out of 204 double tests showed disagreement, which renders an observed agreement between tests on the same stool sample of 99%. The optical density values of the tests showing disagreement were close to the cut-off limit, as opposed to most of the remaining test results, which were clearly above or below this value (data not shown).

Discussion

The monoclonal antibody-based *H. pylori* antigen stool test has a high specificity and sensitivity. This is in accordance with previous studies on this test, with reports of sensitivity and specificity in the range of 88.5%–98% and 93.8%–99%, respectively (23, 27, 28). These studies have, however, not addressed the effect of PPIs on test performance. In this study we show that short-term use of lanzoprazole does not affect test outcome. The finding of 2 negative tests out of 39 (5%) on day 7 after the end of PPI treatment is within the limitations of the test. Patients with dyspeptic complaints often use acid-suppressing drugs prior to gastroscopy. At our hospital, patients are instructed not to use any such drugs in the week prior to examination; a severe challenge to many of them. This method may present a diagnostic tool for patients that are not able to discontinue PPI therapy. However, as the patients tested here had used PPIs for no more than 4 weeks, we still would recommend the discontinuation of PPI treatment for most patients prior to testing for *H. pylori*.

We have given the likelihood ratios, as they incorporate both the sensitivity and specificity of a test, in addition to being unaffected by prevalence of the target disease. A positive likelihood ratio of 16.7 states that the odds that a positive test is from an Hp +ve patient are 16.7 times higher than that it originates from an Hp −ve patient; likewise, a negative likelihood ratio of 0.02 states that the odds are 1:50 that the test comes from an Hp +ve patient.

About half of the invited patients were not enrolled in the study, the main reason for this being the presence of exclusion criteria, especially the use of acid-inhibiting drugs prior to examination. Some eligible patients were unwilling to provide consent; some were not enrolled despite being invited by letter, as they attended the outpatient clinic on a day that was too busy for them to be included. The exclusion of patients for the latter reason was purely coincidental, and should not give rise to selection bias.

Instructions to the patients turned out to be somewhat inadequate. Three patients misunderstood the protocol and provided the second stool sample after PPI treatment not on day 7, but later, after having taken triple therapy. We had, on the other hand, expected the patients to be reluctant to deal with stool samples, but this proved to be a minor problem.

In our region it is an established practice to refer dyspeptic patients for gastroscopy regardless of age, and the severity of disease related to *H. pylori* infection was not surprisingly low in the study population. Thus spectrum bias can be expected to be of less significance in the interpretation of the test results.

It is important to remember that, in this study, the test is limited to assessing *H. pylori* infection, and not disease. We carried out the study in order to evaluate a test primarily for use in epidemiological studies, where a non-invasive test with high diagnostic performance is required for several reasons. It would seem that the monoclonal antibody-based *H. pylori* antigen stool test has both the non-invasiveness needed, as well as being a test that performs adequately for this purpose. However, this test is also probably quite adequate for diagnosis in a clinical setting.

The European *Helicobacter pylori* Study Group has recommended a ‘test and treat’ approach to adult dyspeptic patients under the age of 45, without particular risk factors or alarm symptoms (29). This approach is still controversial, and has not been generally adapted in Norway. There are still some unanswered questions; such as, for example, how such an approach will have an influence on ecology, as well as on long-term outcome for the patients treated. However, in the ‘test and treat’ approach, the stool test seems very well suited, because of the high degree of accuracy and its non-invasive character. The accuracy of the monoclonal *H. pylori* stool test makes it a useful tool to assess infection status after eradication therapy, comparable with that of the urea breath test. This is in accordance with previous reports (30). In our study, however, the stool test is measured against the same reference standard as in the primary diagnosis of *H. pylori*.

The clinician is too often confronted with test results of indeterminate values when using a diagnostic tool. The stool test in this study differed from this, as we found most values to be far from the cut-off value of the test.

We conclude that the monoclonal antibody-based *H. pylori* antigen stool test is a reliable and convenient instrument in the

primary diagnosis of *H. pylori* infection. Single testing, as recommended by the manufacturer, is a safe approach and will reduce the costs of the test. We could not find any evidence to show that short-term treatment with PPIs diminishes the value of the test, but recommend caution when using the test on patients receiving PPI treatment.

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