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Short Communication

Evaluation of diagnostic accuracy of two rapid stool antigen tests using an immunochromatographic assay to detect *Helicobacter pylori*

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ABSTRACT

Objectives: The stool antigen assay for *H. pylori* infection diagnosis with monoclonal antibodies is a simple and recommended technique by the Maastricht V/Florence consensus report. Recently, Pylori K-Set K-1219 (Coris Bioconcept Sprl, Belgium) and HP-F23 (Symbiosys, Brazil) have been made commercially available in Brazil. Thus, the aim of this study was to evaluate the diagnostic accuracies of these two rapid stool antigen tests by immunochromatographic assays (index tests) for the clinical practice.

Design and methods: A total of 98 patients who underwent upper gastrointestinal endoscopy and ^{13}C-urea breath test entered the study. *H. pylori* infection status was defined by the combination of the rapid urease test and the ^{13}C-urea breath test (reference standard). Two observers who were aware of *H. pylori* status performed the reading of index tests. Diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value with 95% confidence intervals, positive likelihood ratio, negative likelihood ratio and kappa index measure of agreement) were determined.

Results: The index tests where in perfect agreement with the *H. pylori* status with kappa values of 0.87 for Pylori K-Set K-1219 and 0.92 for HP-F23. The sensitivity of HP-F23 was 97.9% (IC95%: 87.5–100) and specificity was 93.8% (IC95%; 84–97.2).The positive likelihood ratio was 15.8, and the negative likelihood ratio was 0.02. The Pylori K-Set K-1219 had a sensitivity of 87.7% (IC95%: 74.5–94.9) and a specificity of 100% (IC95%: 91.6–100); the positive likelihood ratio was ∞, and the negative likelihood ratio was 0.1. The test line on the cassette device of HP-F23 was stronger than of the Pylori K-Set K-1219.

Conclusion: The HP-F23 test performed better in clinical practice. Nonetheless, the ^{13}C-urea breath test is more reliable technique. Moreover, caution must be paid to the trace or clear pale test line readings that were observed in false positive and false negative results, leading to incorrect management of the patient.

1. Introduction

Helicobacter pylori (*H. pylori*) colonize the human stomach and are the etiological cause of chronic active gastritis that may evolve to peptic ulcers, atrophic gastritis, gastric adenocarcinoma and MALT (mucosa-associated lymphoid tissue) lymphoma. Hence, the cure of *H. pylori* infection is crucial to preventing these diseases [1]. Nonetheless, the diagnosis of *H. pylori* has been challenging, as there is no ideal gold standard technique [2]. Furthermore, the prior use of proton pump inhibitors and antibiotics that may alter the morphology of *H. pylori* to coccoid forms decreases the sensitivity of the *H. pylori* infection diagnosis [1,2].

The invasive tests are performed on gastric biopsies taken during gastrointestinal endoscopy: histological analysis stained by Giemsa, culture, urease, and genetic test by PCR [1–4]. However, not all patients can undergo endoscopic biopsies, in this condition the noninvasive tests are the tests of choice. Additionally, the noninvasive tests are indicated for post-treatment eradication control and a test-and-treat strategy. Among the non-invasive methods, serology, although highly sensitive and specific, demands prior local validation. Furthermore, it is not indicated for the eradication control [1]. The ^{13}C-urea breath test is the best choice for the confirmation of *H. pylori* eradication [1]. Nevertheless, in Brazil the ^{13}C-urea breath test is available in very restricted centers [5]. The stool antigen test with monoclonal antibodies may be an alternative; however, it is not as accurate as the ^{13}C-urea breath test [1]. Previous testing of the stool antigen test with polyclonal antibodies

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showed a sensitivity of 88% and a specificity of 87.5% [6], compared to the sensitivity and specificity of 100% for ^{13}C-urea breath test [5].

Recently, two kits of rapid stool antigen test with monoclonal antibodies by immunochromatographic assay were introduced in the Brazilian market. Thus, the aim of this study was to evaluate the diagnostic accuracy of these two rapid stool antigen tests with monoclonal antibodies by immunochromatographic assays (index tests) using the combination of the ^{13}C-urea breath test and the urease test as the reference standard.

2. Patients and methods

2.1. Patients

This study was approved by the local institutional Ethics Committee. All patients signed an informed consent form and were instructed to bring fresh stools on the same day of the urea breath test that was performed within a month after endoscopy. Anti-secretory drugs had to be discontinued at least 10 days and antibiotics for at least one month prior to endoscopy and until stool collection for the *H. pylori* antigen detection and the urea breath test. A total of 117 consecutive patients with dyspeptic symptoms, who underwent upper endoscopy from October 2015 to September 2016, were invited to participate. Seventeen patients did not bring fecal samples, and two who had undergone partial gastrectomy were excluded from the analysis. A total of 98 patients entered the study, and twenty-nine (29.6%) were men. The mean patient age was 54 years (range, 21–81 years). Twelve patients who had previous treatment for *H. pylori* eradication with the standard 7-day regimen of triple therapy (proton-pump inhibitor, amoxicillin and clarithromycin) were also included in the study.

2.2. Urease test

Antrum and corpus biopsies were placed into a homemade urease test according to a previously described technique [4].

2.3. ^{13}C-urea breath

The test was performed using the capsule prepared by the Pharmacy Division of the Hospital das Clinicas, of 50 mg of ^{13}C-urea (Euriso-top®, Saint-Aubin, Île-de-France, France), which was ingested with orange juice, as previously described [5]. The 50 mg capsule of ^{13}C-urea was formulated with 170 mg of citric acid and was ingested with orange juice (pH = 3) to acidify the stomach milieu, inducing the urease synthesis by *H. pylori*, and enhancing the sensitivity of the urea breath test [7]. Breath samples were collected before and after ingestion of the capsule. Breath samples were analyzed by infrared spectroscopy (IRIS DOC, Wagner Analysen – Technik, Bremen, Germany). A DOB (Delta over baseline-value) ≥ 4.0% was considered positive for *H. pylori* infection.

2.4. Stool antigen test by immunochromatographic assay with monoclonal antibodies

Patients were instructed to store the formed stools at 4–16 °C until the arrival at the laboratory where were immediately processed. Specimens were tested using two stool antigen tests (**Pylori K-Set K-1219**, Coris Bioconcept Sprl, Belgium, commercialized by Serion, São Paulo, Brazil and **HP-F23**, *H. pylori* antigens rapid test device for feces, Symbiosys, São Paulo, commercialized by Vyttra, São Paulo, Brazil) according to the manufacturer's instructions. The results were based on the appearance of colored lines across the central window of the cassette; two lines, C (control) and T (test), indicated a positive test, while only one line in C indicated a negative result. A pale-colored line in T was also considered positive. Two observers who were aware of *H. pylori* status performed independent readings of the tests after a 10-minute incubation period (following the manufacturer's instruction) and at 20 min. Both tests were performed simultaneously with the fresh fecal samples. The tests were repeated on fecal samples collected after some months in one untreated patient of *H. pylori* positive group and in one of *H. pylori* negative group. Some tests were randomly performed twice by other technicians of the lab.

2.5. H. pylori status

The *H. pylori* status was based on the combination of the results of the urease test and the ^{13}C-urea breath test to ascertain the *H. pylori*-positive and the *H. pylori*-negative groups. The patients with no concordant results between urease and urea breath test were ineligible for the study.

2.6. Statistical analysis

The statistical analysis was performed with SPSS software version 15.0 for Windows (Chicago, Illinois, USA) and software R version 3.1.2 (R Core Team, R Foundation for Statistical Computing Vienna, Austria, http://www.R-project.org). The sample size was calculated with G Power software (G * Power: Statistical Power Analyses for Windows), with an allocation ratio of N2/N1 = 1 (53% prevalence of *H. pylori* infection in the studied population [8]) with α error probability of 0.05 and a power (1-β error probability) = 0.95. For a total sample size of 98 patients (49 *H. pylori* positive and 49 *H. pylori* negative), the actual power was 0.95, and the actual α was 0.029.

Sensitivity, specificity, positive predictive value, negative predictive value, accuracy and kappa index measure of agreement with 95% confidence interval (95%CI) were calculated using *H. pylori* status as the gold standard by Fisher's exact test. Positive likelihood ratios were calculated as sensitivity/1-specificity and negative likelihood ratios were calculated as 1-sensitivity/specificity. A value of $p < 0.05$ was considered significant. The STARD 2015 was followed for reporting the results [9].

3. Results

The endoscopic findings were as follows: normal or minor changes (51; 52%), gastroduodenal erosions (24; 24.5%), peptic ulcers (14; 14.3%) esophagitis (2; 2%), and 7 (7.1%) presented other conditions (hiatal hernias, angiodysplasia, Barrett's esophagus, gastric polyposis and gastric heterotopias).

Forty-nine of the 98 patients eligible for the study were *H. pylori* negative, and 49 were positive. The reading of the HP-F23 test was easier than the Pilori K-Set K-1219, with a stronger colored test line, and the test line of both tests was stronger at the 20-minute reading. The trace test line and a clear pale test line were observed in both false negative and false positive (one eradication control patient) results with both kits. The tests performed twice by other technicians of the lab and those collected on different days had the same results. The STARD flow diagram is depicted in Fig. 1.

In the *H. pylori*-positive group, the Pylori K-Set K-1219 (Coris Bioconcept Sprl, Belgium), 43 patients were positive (sensitivity 87.7%; 95%CI: 74.5–94.9), and six were false negative. In the *H. pylori*-negative group, 49 were negative (specificity 100%; 95%CI: 91.6–100), showing a kappa index of 0.87 (95%CI: 0.78–0.97). The positive predictive value was 100% (95%CI: 89.3–100), the negative predictive value was 89% (95%CI: 78.2–94.7), and the accuracy was 93.8% (95%CI: 86.9–97.3) and the positive likelihood ratio was ∞, and the negative likelihood ratio was 0.1 (as shown in Table 1).

The kit HP-F23 (Symbiosys, Brazil) detected *H. pylori* antigen in 48 of the 49 positive patients (a sensitivity 97.9%; 95%CI: 87.5–100) and in one false-negative patient. In the *H. pylori*-negative group, 46 presented negative results (specificity 93.8%; 95%CI: 84–97.2), and three were false positive, showing a kappa index = 0.92 (95%CI: