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Evaluation of 3 different tests for the detection of stool antigens to confirm *Helicobacter pylori* eradication after treatment. A pilot study

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ABSTRACT

INTRODUCTION: Recently, several new diagnostic methods aimed to detect *Helicobacter pylori* stool antigens have been developed. Our aim was to evaluate the accuracy of 3 different stool tests to confirm *H. pylori* eradication.

PATIENTS AND METHODS: Twenty-six patients received *H. pylori* eradication treatment. Eradication was confirmed with ^{13}C-urea breath test 6-8 weeks later, when stool samples were analyzed by polyclonal (Premier-Platinum-HpSA™), monoclonal (Amplified-IDEIA™-HpStAR™), and rapid test (ImmunoCard-STAT-HpSA™).

RESULTS: *H. pylori* was eradicated in 85% of the cases. Sensitivity, specificity, positive predictive value and negative predictive value with the polyclonal test were: 25%, 91%, 33% and 87%. Corresponding results with the monoclonal test, using the cut-off point recommended by the manufacturer, were 100%, 46%, 25% and 100%. However, the best cut-off point in our study had 100% sensitivity and 91% specificity. The area under ROC curve for the polyclonal and the monoclonal tests was 0.65 and 0.95. Diagnostic accuracy with the rapid test was 75%, 90%, 60% and 95%.

CONCLUSION: Neither the polyclonal stool antigen test nor the rapid stool antigen test can be recommended to confirm *H. pylori* eradication after treatment. The monoclonal test has better diagnostic accuracy, although more studies are necessary to definitively recommend its use for the confirmation of *H. pylori* eradication success.

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EVALUACIÓN DE 3 PRUEBAS DIAGNÓSTICAS PARA LA DETECCIÓN DE ANTÍGENOS EN HECES A FIN DE CONFIRMAR LA ERRADICACIÓN DE *H. PYLORI* TRAS EL TRATAMIENTO. UN ESTUDIO PRELIMINAR

INTRODUCCIÓN: Recientemente se han desarrollado varios métodos diagnósticos nuevos dirigidos a la detección de antígenos de *Helicobacter pylori* en las heces. El objetivo de nuestro estudio ha sido la evaluación de la precisión de 3 pruebas distintas de detección de antígenos en heces para confirmar la erradicación de *H. pylori*.

PACIENTES Y MÉTODOS: Se administró tratamiento de erradicación de *H. pylori* a 26 pacientes. La erradicación se confirmó 6-8 semanas después mediante la prueba de urea marcada con ^{13}C en el aire espirado, con análisis de muestras de heces mediante una prueba policlonal (Premier-Platinum-HpSA™), una prueba monoclonal (Amplified-IDEIA®-HpStAR™) y una prueba rápida (ImmunoCard-STAT-HpSA™).

RESULTADOS: La erradicación de *H. pylori* se confirmó en el 85% de los casos. Los porcentajes correspondientes a la sensibilidad, la especificidad, el valor predictivo positivo y el valor predictivo negativo de la prueba policlonal fueron del 25, el 91, el 33 y el 87%. Los resultados correspondientes a la prueba monoclonal utilizando el umbral recomendado por el fabricante fueron del 100, el 46, el 25 y el 100%. No obstante, el mejor umbral considerado en nuestro estudio dio lugar a una sensibilidad del 100% y una especificidad del 95%. El área bajo la curva de rendimiento diagnóstico *(receiver operating characteristics)* respecto a las pruebas policlonal y monoclonal fue de 0,65 y 0,95, respectivamente. Los resultados correspondientes a la sensibilidad, la especificidad, el valor predictivo positivo y el valor predictivo negativo de la prueba rápida fueron del 75, el 90, el 60 y el 95%.

CONCLUSIÓN: Para la confirmación de la erradicación de *H. pylori* tras el tratamiento no se pueden recomendar la prueba policlonal ni la prueba rápida de detección de antígenos en heces. La prueba monoclonal muestra una precisión diagnóstica mayor, aunque son necesarios nuevos estudios para poder recomendar definitivamente su aplicación de cara a la confirmación de la erradicación de *H. pylori*.

INTRODUCTION

Helicobacter pylori infection plays a fundamental role in the development of several gastroduodenal diseases and, therefore, the diagnosis of the infection represents a clinically relevant chapter. The methods for the diagnosis of *H. pylori* infection are classically divided into invasive and non-invasive[1]. The former are based on the demonstration of the organism from gastric biopsy samples, therefore an endoscopy is needed. On the other hand, non-invasive methods, which require no endoscopic examination, are also available. Among non-invasive techniques, serology and urea breath test are the classically considered and the most widely used.

Most recently, a new non-invasive diagnostic test based on the detection of *H. pylori* stool antigens has been developed, and it has shown to be an accurate method for the detection of infection in non-treated patients[2]. However, the experience in the post-treatment setting, to confirm *H. pylori* eradication, is much more limited, and discouraging results have been reported in some studies[2]. After the first commercially available *H. pylori* stool antigen test, which used polyclonal antibodies to *H. pylori*, newer methods based on monoclonal antibodies have been developed, including an ELISA test and, even more recently, a rapid in-office test whose result is read after only 5 min. In summary, very limited experience exists with both the polyclonal ELISA and the monoclonal ELISA test in the confirmation of *H. pylori* eradication after treatment, and the rapid monoclonal immunochromatographic test has never been evaluated before in this setting. Therefore, our aim was to assess the accuracy of these 3 tests for the detection of stool antigens to evaluate *H. pylori* eradication success or failure after treatment.

PATIENTS AND METHODS

Patients

Twenty six *H. pylori*-positive patients (62% with peptic ulcer disease, and 38% with functional dyspepsia) were included in this pilot study. Exclusion criteria were: previous *H. pylori* eradication therapy administration, previous gastric surgery, and presence of associated conditions (hepatic, cardiorespiratory or renal diseases, diabetes treated with insulin, malignancies or coagulopathy). Informed consent was obtained from all the patients. All patients received *H. pylori* eradication treatment for 7 days with proton pump inhibitor (omeprazole, 20 mg/12 h), amoxicillin (1 g/12 h), and clarithromycin (500 mg/12 h).

Reference methods for the diagnosis of *H. pylori* infection

H. pylori eradication was defined as a negative ^{13}C-urea breath test (TAU-KIT®, Isomed, S.L., Madrid, Spain) 8 weeks after completing treatment. The standard protocol was performed with 4.2 g of citric acid (Citral *pylori*®) and 100 mg of ^{13}C-urea, as detailed in previous publications[3]. Breath samples were analyzed by means of isotope ratio mass spectrometer (ABCA, PDZ; Crew, Manchester, England). The result of ^{13}C-UBT was expressed as delta over baseline, values higher than 5 being considered positive.

Stool tests

At the time of breath test performance, the patient provided a stool sample. The samples were stored at –20 ºC until analysed by the 3 stool tests: *a)* polyclonal ELISA test (Premier Platinum HpSA™, Meridian, Cincinnati, OH, USA); *b)* monoclonal ELISA test (Amplified IDEIA™ HpStAR™, DAKO A/S, Denmark); *c)* and rapid monoclonal immunochromatographic test (ImmunoCard STAT! HpSA™, Meridian Bioscience Europe, Italy). All tests were performed in accordance with the manufacturer's protocol. The protocol for the first 2 tests has been detailed in previous publications[4-6]. For the novel rapid test, detailed protocol information is provided in the next section. All stool tests were performed without knowledge of the other stool tests results or the urea breath test result.

Rapid monoclonal immunochromatographic test

The ImmunoCard STAT! HpSA immunoassay is a rapid *in vitro* qualitative procedure, based on a lateral flow chromatography technique, for the detection of *H. pylori* antigens in human stool. The test utilizes a monoclonal anti-*H. pylori* antibody. Diluted patient samples are dispensed to the sample port of the test cassette and the appearance of a pink-red line in the reading window indicates a positive result after 5 min of incubation at room temperature. The stool specimen should be stored at 2 °C-8 °C until tested. The specimen should be tested as soon as possible, but may be held up to 72 h at 2 °C-8 °C prior to testing. Using the applicator stick of the diluent vial, a small portion (5-6 mm diameter) is transferred of thoroughly mixed stool into the sample diluent. The specimen is then emulsified using the applicator stick. The sample diluent vial containing the specimen should be held and the tip broken off. Four drops should be dispensed into the round window indicated by an arrow. Finally, the result should be read after exactly 5 min. The interpretation of results is as follows:

– Negative test result. Only one blue colored band (control line) appears across the central window of the device close to the letter C.
– Positive test result. In addition to the blue band (control line), a distinguishable pink-red band (test line) also appears across the central window of the device close to the letter T. Any pink-red line, even very weak, must be considered as a positive result.
– Any line or colour appearing after 5 min has no diagnostic value (nevertheless, we re-evaluated the test after 30 min, with the aim to evaluate concordance between the 5 and 30 min lecture).
– Invalid test result. The blue band (control line) is absent, with or without a visually detectable pink-red band (test line).

Results of the rapid stool test were independently evaluated by 2 observers, who classified the results as positive, negative or indeterminate.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of the stool antigen tests were calculated, and the 95% confidence interval was provided. Comparisons between independent proportions were carried out by chi-square (χ^2) test. For homogeneity test regarding stool antigen methods in the same patients, McNemar statistic was used. Concordance for the rapid stool tests interpretation between the 2 observers was evaluated by the kappa statistic. The global yield of the stool antigen test to diagnose *H. pylori* infection was calculated using the area under the ROC (receiver operating characteristic) curve. The optimum cut-off point was evaluated by means of the sensitivity analysis (using contingency tables) and likelihood ratios.

RESULTS

Twenty six infected patients received *H. pylori* eradication therapy. Mean age (± standard deviation) was 55 ± 14 years, 42% were males, and 23% were smokers. *H. pylori* eradication was achieved in 85% of the cases and, therefore, prevalence of the infection post-treatment was of 15% (4 patients). Delta over baseline values of the ^{13}C-urea breath test in *H. pylori*-positive patients were: 93, 26, 22, and 57‰.

Sensitivity, specificity, positive predictive value and negative predictive value with the polyclonal ELISA test was: 1/4 (25%; 95% confidence interval [95% CI], 1-80%), 20/22 (91%; 95% CI, 72-97%), 1/3 (33%; 95% CI,

1-90%) and 20/23 (87%; 95% CI, 66-97%). Positive and negative likelihood ratios were 2.7 and 0.82, respectively. When the results of the polyclonal ELISA test were interpreted by visual reading instead of by spectrophotometry, results were only slightly better: sensitivity 2/4 (50%; 95% CI, 7-93%), specificity 20/22 (91%; 95% CI, 72-97%), positive predictive value 2/4 (50%; 95% CI, 7-93%), and negative predictive value 20/22 (91%; 95% CI, 72-97%). Positive and negative likelihood ratios were 5.5 and 0.55, respectively.

Corresponding results of sensitivity, specificity, positive predictive value and negative predictive value with the monoclonal ELISA test, using the cut-off point recommended by the manufacturer (optical density of 0.15), were: 4/4 (100%; 95% CI, 40-100%), 10/22 (46%; 95% CI, 24-68%), 4/16 (25%; 95% CI, 7-52%) and 10/10 (100%; 95% CI, 69-100%). Positive and negative likelihood ratios were 1.8 and 0. However, the best cut-off point in our study (optical density of 0.55) had 100% (4/4) sensitivity and 91% (20/22) specificity. Positive and negative likelihood ratios were 11.1 and 0, respectively. The area under the ROC curve for the polyclonal ELISA and the monoclonal ELISA test was, respectively, 0.65 and 0.95 ($p < 0.01$).

Diagnostic accuracy with the rapid monoclonal immunochromatographic test was: sensitivity 3/4 (75%; 95% CI, 19-99%), specificity 19/21 (90%; 95% CI, 71-97%), positive predictive value 3/5 (60%; 95% CI, 15-95%) and negative predictive value 19/20 (95%; 95% CI, 76-99%). Positive and negative likelihood ratios were 7.5 and 0.28, respectively. The rapid test was positive in 5 patients, negative in 20, and indeterminate in one. Concordance between the 2 observers was perfect (kappa = 1). Concordance between results obtained with the rapid test at 5 and 30 min was very poor (kappa = 0.27), due to a high number of false positives at 30 min.

DISCUSSION

The results of the present study show that the polyclonal ELISA stool test and the rapid monoclonal immunochromatographic test are insufficiently accurate for the confirmation of *H. pylori* eradication after therapy. However, monoclonal ELISA stool test has better diagnostic accuracy. The first commercially available *H. pylori* stool antigen test, Premier Platinum HpSA™ (Meridian Diagnostics), used polyclonal antibodies to *H. pylori* as capture antibodies, absorbed to microwells. More recently, a new stool antigen test, a quantitative enzyme immunoassay based on monoclonal –instead of polyclonal– antibodies, has been commercialized. The «old» Premier Platinum HpSA (Meridian) uses polyclonal antibodies obtained from intraperitoneal injection of *H. pylori* antigens to rabbits. This method obtains a profile of antibodies which is different in each animal, and this could generate, in theory, differences among diagnostic kits. In fact, considerable variability has been reported when several stool determinations with the polyclonal method have been performed in the same patients[7]; this, in turn, could explain remarkably differences among the different studies from the literature.

On the other hand, the test based on monoclonal antibodies may have greater reproducibility of test results. In this respect, some studies have reported excellent results despite the fact that the test was performed in 3 different laboratories using 2 different production lots[8]. In a recent systematic review of the stool antigen test, overall results of the studies evaluating (pre-treatment) the monoclonal technique were better than those obtained with the polyclonal method[2]. Of special interest are those studies that compare the monoclonal and polyclonal method in the same protocol. Thus, although some of these studies have obtained similar results with both techniques[7,9], others have reported a tendency for better results[10] or a clear advantage with the monoclonal method[6,11,12].

In the post-treatment setting, relatively low accuracy was reported in some studies with the polyclonal ELISA stool antigen test; thus, mean sensitivity calculated from 33 studies evaluating polyclonal stool antigen test 4-8 weeks after finishing therapy was of only 84%[2], and even worse results were achieved in our study. When the results of the polyclonal ELISA test were interpreted by visual reading instead of by spectrophotometry, results were relatively similar, which coincides with other studies where the concordance of *H. pylori* stool antigen test interpreted by these 2 methods has been very high[13-15].

However, better results were achieved with the monoclonal ELISA test in our experience, specially when the best cut-off point in our study was considered, with 100% sensitivity and 91% specificity. In this respect, the selection of the appropriate cut-off point for the stool antigen test is still a matter of debate, as several studies have found that the manufacturer's recommendations do not always coincide with the best cut-off point calculated by statistical methods, and it needs to be clarified which factors influence it (for example, pre- *versus* post-treatment setting). It should be stressed, however, that the use of the cut-off point arising from a study as a reflection of the performance accuracy of the test in the general population may flaw the results, and therefore it will be necessary to confirm the encouraging results of this cut-off point in a new sample of patients. Nevertheless, the area under the ROC curve, which globally evaluates the yield of all the cut-off points of the monoclonal ELISA test, was 0.95, which indicates an excellent diagnostic performance (better than the 0.65 obtained for the polyclonal test). These excellent results are in agreement with those reported in the few studies from the literature evaluating the monoclonal ELISA technique 4-8 weeks post-treatment, which achieved mean 95% sensitivity and mean 97% specificity[2]. Furthermore, the differences between positive and negative results obtained with the monoclonal ELISA test are generally greater in comparison to the polyclonal ELISA test and therefore the monoclonal test allows a better distinction between infected and non-infected patients[9,10]. Thus, in contrast with the polyclonal test, no grey zone seems to be necessary when the monoclonal test is used.

The rapid monoclonal immunochromatographic stool test is currently incorporated in a device which integrates sampling, processing and analysis in one test unit allowing for simple and hygienic handling. Although limited experience exists on this new method, encouraging results have been reported in patients without prior treatment of *H. pylori*[16-19]. As the results with this novel office-test are available in approximately 5 min, it has the advantage of being suitable for the use at the doctor's office (making possible the prescription of *H. pylori* eradication therapy, if necessary, during the same visit). Although it has been suggested that a methodological limitation is the sometimes very low intensity of the bands on the test device, resulting in difficulties to interpret the results, concordance between the 2 observers in our study was perfect (kappa = 1). However, the disappointing results obtained in our study (with a sensitivity of only 75%, and a positive predictive value of only 60%), which represents the first experience evaluating this rapid monoclonal immunochromatographic stool test in the post-treatment setting, suggests that this test may not be accurate to confirm *H. pylori* eradication.

Finally, several advantages of the stool antigen determination should be underlined[2]. The *H. pylori* stool antigen test does not need an endoscopy, is easy and simple to perform, rapid (the test provides results in approximately 2 h with the ELISA stool test, and in only 5 min with the immunochromatographic test), only one stool specimen is required (instead of 2 breath samples with urea breath test), and it does not require a technician or nurse. The stool sample can be stored at 2 °C -8 °C for up to 3 days, or indefinitely at –20 °C before the test; this makes it possible to collect multiple samples over several days or weeks, which is valuable in a small hospital with a low number of patients, to be tested in one session, thus reducing the cost. Lastly, preliminary studies using decision analysis suggest that stool antigen test is associated with a high cost-effectiveness ratio for the diagnosis of *H. pylori* infection[2]. On the other hand, as any diagnostic method, stool antigen test has also some disadvantages[2]. In particular, collection of stools may be a disagreeable task for some patients, and it remains to be seen whether patients will be more or less willing to provide a stool or breath sample for *H. pylori* testing; we must not forget that ease of specimen acquisition is likely to affect the patient's compliance.

In summary, the results of the present pilot study, although including a low number of patients, suggest that neither the polyclonal ELISA stool antigen test nor the rapid monoclonal immunochromatographic stool antigen test can be recommended to confirm *H. pylori* eradication after treatment. The monoclonal ELISA test has better diagnostic accuracy, although more studies are necessary to definitively recommend its use for the confirmation of *H. pylori* eradication. Therefore, future studies will need to demonstrate whether stool antigen tests are equally reliable than ^{13}C-urea breath test before the generalized use of these tests are recommended in the post-treatment setting.

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REFERENCES

1. Vaira D, Holton J, Menegatti M, et al. Review article: invasive and non-invasive tests for *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2000;14 Suppl 3:13-22.
2. Gisbert JP, Pajares JM. Stool antigen test for the diagnosis of *Helicobacter pylori* infection: a systematic review. Helicobacter. 2004;9:347-68.
3. Gisbert JP, Vázquez MA, Jiménez I, et al. 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection before treatment: is citric acid necessary? Dig Liver Dis. 2000;32:20-4.
4. Vaira D, Vakil N, Menegatti M, et al. The stool antigen test for detection of *Helicobacter pylori* after eradication therapy. Ann Intern Med. 2002;136:280-7.
5. Gisbert JP, Cabrera M, Pajares JM. Test de antígeno en heces para el diagnóstico de la infección por *Helicobacter pylori* y para la confirmación de su erradicación tras el tratamientoMed Clin (Barc). 2002;118:401-4.
6. Leodolter A, Peitz U, Ebert MP, Agha-Amiri K, Malfertheiner P. Comparison of two enzyme immunoassays for the assessment of *Helicobacter pylori* status in stool specimens after eradication therapy. Am J Gastroenterol. 2002;97:1682-6.
7. Makristathis A, Barousch W, Pasching E, et al. Two enzyme immunoassays and PCR for detection of *Helicobacter pylori* in stool specimens from pediatric patients before and after eradication therapy. J Clin Microbiol. 2000;38:3710-4.
8. Koletzko S, Konstantopoulos N, Bosman D, et al. Evaluation of a novel monoclonal enzyme immunoassay for detection of *Helicobacter pylori* antigen in stool from children. Gut. 2003;52: 804-6.
9. Agha-Amiri K, Peitz U, Mainz D, Kahl S, Leodolter A, Malfertheiner P. A novel immunoassay based on monoclonal antibodies for the detection of *Helicobacter pylori* antigens in human stool. Z Gastroenterol. 2001;39:555-60.
10. Chisholm SA, Watson CL, Teare EL, Saverymuttu S, Owen RJ. Non-invasive diagnosis of *Helicobacter pylori* infection in South-east England: a comparative evaluation of two commercial kits, HpSA and HpStAR, for stool antigen detection. Gut. 2002;51 Suppl 2:A107.
11. Malfertheiner P, Axon A, O'Morain C, et al. Evaluation of a new *Helicobacter pylori* catalase based stool antigen test (FemtoLab H. pylori CNX): an European Multicenter Study. Gastroenterology. 2002;122 Suppl 1:1316.
12. Vijola L, Oksanen A, Löfgren T, et al. Preliminary result of two stool antigen tests for detection of *Helicobacter pylori* in adults. Gut. 2002;51 Suppl 2:A112.
13. Yu FJ, Wu DC, Kuo CH, et al. Diagnosis of *Helicobacter pylori* infection by stool antigen test in southern Taiwan. Kaohsiung J Med Sci. 2001;17:344-50.
14. Oderda G, Rapa A, Marinello D, Ronchi B, Zavallone A. Usefulness of *Helicobacter pylori* stool antigen test to monitor response to eradication treatment in children. Aliment Pharmacol Ther. 2001;15:203-6.
15. Sarker SA, Bardhan PK, Rahaman M, Beglinger C. Usefulness of the *Helicobacer pylori* stool antigen test for detection of *Helicobacter pylori* infection in young Banglasdeshi children. Gastroenterology. 2003;124 Suppl 1:1231.
16. Calvet X, Salceda F, Sanfeliu I, et al. Evaluación de un nuevo test rápido para la detección de *Helicobacter pylori* en heces. Med Clin (Barc). 2002;118:126-9.
17. Calvet X, Quesada M, Sanfeliu I, et al. Evaluación de un test rápido (Inmmuno-Card-STAT-HpSA®) para la detección de *Helicobacter pylori*. Gastroenterol Hepatol. 2003;26:531-4.
18. Wong WM, Lam SK, Tong TSM, et al. Evaluation of a rapid stool antigen test for the diagnosis of *Helicobacer pylori* infection in Chinese patients. Chin J Dig Dis. 2003;4:132-5.
19. Wu IC, Ke HL, Lo YC, et al. Evaluation of a newly developed office-based stool test for detecting *Helicobacter pylori*: an extensive pilot study. Hepatogastroenterology. 2003;50:1761-5.