Comparison of a monoclonal antigen stool test (Hp StAR) with the $^{13}$C-urea breath test (UBT) in monitoring Helicobacter pylori eradication therapy

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INTRODUCTION

Several non-invasive methods of testing for Helicobacter pylori ($H$. pylori) have been developed and routinely used in clinical practice. They are based on: (1) the detection of $^{13}$C- or $^{14}$C-labeled CO$_2$ in expired air as a result of $H$. pylori urease activity ($^{13}$C- or $^{14}$C-UBT)[1,2], (2) the detection of antibodies in serum (serology)[3], saliva[4], and urine[5], and (3) the detection of $H$. pylori antigens in stool[6]. Until now, the UBT has been considered to be the most reliable non-invasive test for the diagnosis of $H$. pylori infection with an overall accuracy approaching 95% in both untreated and treated patients[7]. However, concerns regarding the cost of $^{13}$C-urea, the radiological hazards of $^{14}$C-urea, and the local availability of either mass spectrometers or $\beta$-counters induced clinicians to search for new alternative tests. Serological tests have been extensively used in the past but the diagnostic accuracy is no longer adequate to justify their clinical use. Stool antigen tests have proven to be reliable in the diagnosis of $H$. pylori infection. In the previous years, mAb-based stool tests have been developed and introduced in clinical practice[8,9]. Sensitivity values ranged from 88% to 99% in post-treatment setting and from 89% to 100% in post-treatment setting[10]. Concerning the specificity, values ranged from 92% to 99% in post-treatment setting and from 95% to 100% in post-treatment setting[10]. The encouraging results obtained with mAb-based stool tests in post-treatment setting raise an important question: should the UBT be still considered on either clinical or economical ground as the most suitable non-invasive test for monitoring $H$. pylori infection after treatment? Aim of this prospective, controlled, single-center study was to evaluate the agreement between the UBT and the Hp StAR in post-treatment setting.

METHODS

Patients with discordant results on UBT and Hp STAR underwent endoscopy with biopsies for rapid urease test, culture, and histology to confirm $H$. pylori status.

RESULTS

Among 250 patients (50±14 years), 240 (96.0%) had concordant UBT and Hp StAR tests with a significant correlation between DOB and A values (R = 0.87; P<0.0001). The remaining 10 (4.0%) patients had discordant tests (positive Hp StAR and negative UBT) with the Hp STAR inaccurate in five cases (false positive) and UBT inaccurate in the other five cases (false negative). The “maximal expected” sensitivity, specificity, +PV, -PV, +LR, and -LR were 91%, 100%, 100%, 97.4%, $\infty$, and 8.2 respectively, for the UBT, and 100%, 97.4%, 91%, 100%, 38.8, and 0, respectively, for the Hp STAR. Overall accuracy for both tests was 98%.

CONCLUSION

Both the UBT and the Hp STAR are equally accurate in monitoring $H$. pylori infection. Nowadays, the choice of the “best” non-invasive $H$. pylori test in the post-treatment setting should be done not only in terms of diagnostic accuracy but also in view of cost and local facilities.

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MATERIALS AND METHODS

Consecutive outpatients, who were referred to our Center for non-invasively monitoring _H. pylori_ infection after a first-line or a second-line eradication treatment, were considered for recruitment into this study. Patients were prospectively interviewed on past medical history, smoking habits, and alcohol intake. Exclusion criteria were treatment with antibiotics, _H2_ receptor antagonists, bismuth, or PPI in the 4 wk preceding the study, previous gastric surgery, severe renal or liver diseases, and malabsorption syndromes. All patients gave their informed consent to participate and the study was approved by the Ethical Committee of our institution.

Study design

At 4-6 wk after completion of eradication therapy, patients underwent both ¹³C-UBT and antigen stool test. The UBT was performed with 75 mg ¹³C-urea. Breath samples were taken before and 30 min after ingestion of the urea. The ¹³C-enrichment in breath was determined by IRMS. The UBT was considered positive if the d-value over baseline (DOB) at 30 min was >5%. On the same day of UBT, patients collected stool from the toilet paper or bowl into an airtight container. Stool specimens were analyzed for _H. pylori_ antigen using a sandwich-type enzyme immunoassay (Hp StAR, DakoCytomation, Milan, Italy), as described by the manufacturer. The results were analyzed by spectrophotometry. The absorbance was read at 450 nm and expressed as optical density (A). Specimens with absorbance values >0.19 were considered positive. Patients with discordant tests did not undergo further investigation. Patients with discordant tests were endoscoped with multiple biopsies (three from antrum and three from corpus) for rapid urease test (Cp-test, Yamanouchi, Milan, Italy), culture (Biomerieux Pylori, BioMerieux, Marseille, France), and histology (H&E and Giemsa).

Patient sample size

In the post-treatment setting, a sensitivity and specificity of 94% and 95% for the UBT and 95% and 98% for mAb-based stool tests have been reported[6]. Consequently, 4% and 1.2% of patients are expected to show a false positive or negative result on UBT, respectively, as compared to 1.6% and 1.0% of patients on stool test. In our study, the percentage of patients with discordant UBT and stool test was expected to range from 2.6% (5.2-2.6%) to 7.8% (5.2+2.6%) with a mean value of 5.2%. We assumed that at least 10 patients with discordant results would have been sufficient to evaluate the agreement between the UBT and the Hp StAR results. By taking into account a drop out rate of 20% (patients unwilling to undergo endoscopy), we decided that at least 13 patients with discordant results would have been evaluated. Therefore, the minimal sample size of patient population was 250 (13/5.2%).

Statistical analysis

Patients were considered as still infected if at least one of the biopsy-based tests was positive. Conversely, they were regarded as successfully eradicated if all the three tests were negative. Patients were sorted into two groups according to UBT and Hp StAR results: one group with concordant and the other with discordant results. Univariate analysis (t-test or χ²-test) was carried out between the two groups for age, sex, smoking, alcohol consumption, and type of treatment. A P-value less than 0.05 was considered statistically significant. Linear regression analysis was carried out in the group of patients with concordant results to search for a significant correlation between UBT and Hp StAR results. Logistic analysis was performed to identify the factors determining discordance of test results. Finally, the “maximal expected” values of sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and diagnostic accuracy were calculated for UBT and Hp StAR.

Funding

Stool kits were kindly provided by the manufacturer (DakoCytomation, Milan, Italy) who had no role in the collection, analysis and interpretation of the data. There was no other funding source.

RESULTS

Study population

Overall, 266 patients were asked to participate in this study but 16 (6%) refused to collect stool and were not included; 250 patients (50±14 years; range: 17-92 years; 113 males) were enrolled and none of them were excluded from the final analysis because of insufficient stool or breath collection. There were 75 (30%) smokers and 48 (19.3%) subjects reported alcohol consumption of more than 40 g daily. First-line eradication treatments were the “Maastricht therapy” (i.e., PPI with clarithromycin and either amoxicillin or metronidazole for 7 d) in 149 (59.6%) patients[11], and the 10-d sequential therapy proposed by Zallo et al. (i.e., PPI and amoxicillin for the first 5 d followed by PPI, clarithromycin, and tinidazole for the remaining 5 d) in 45 (18%) patients[12]. The quaduple therapy (i.e., PPI, bismuth citrate, tetracycline, and metronidazole for 7 d) was the second-line treatment used by the remaining 56 (22.4%) patients who were still _H. pylori_ positive after one course of the standard “Maastricht therapy”.

UBT and stool test results

UBT and Hp StAR were concordant in 240 (96%) patients (189 negative and 51 positive) and discordant in the remaining 10 (4%, Table 1). All 10 patients tested positive on Hp StAR and negative on UBT. All patients with discordant results agreed to undergo endoscopic examination. After endoscopy was done, 5 out of 10 (50%) patients were classified as _H pylori_ positive, and 5 (50%) _H pylori_ negative. Compared with the gold standard biopsy-based tests, the Hp StAR was inaccurate in five cases (five false positive) and the UBT in the other five cases (five false negative). No coccoid forms were detected in the five patients with negative UBT and positive Hp StAR.

The absolute values of sensitivity and specificity for UBT and Hp StAR were not calculated since all patients, including those with concordant UBT and Hp StAR tests, would have undergone endoscopy with multiple biopsies.
However, we extrapolated the “maximal expected” sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios (Table 2). The diagnostic accuracy for both tests was 98% (95%CI and 95.8–98.0%).

Both univariate and logistic analyses showed that patients with discordant results were not significantly different from those with concordant results as far as age, sex, smoking, alcohol consumption, and type of treatment were considered.

In patients with concordant results, a significant correlation (DOB = -0.42+14.9×A; R = 0.87, P<0.0001) was found between DOB and A values (Figure 1).

Table 2 Diagnostic accuracy of UBT and Hp StAR

|          | UBT            | Hp StAR     |
|----------|----------------|-------------|
| Sens=TP/(TP+FN) | 91.8 (87.4–91.8) | 100 (95.2–100) |
| Spec=TN/(TN+FP)  | 100 (98.6–100)   | 97.4 (96–97.4) |
| Positive predictive value | 100 (95.2–100) | 91.8 (87.4–91.8) |
| Negative predictive value | 97.4 (96–97.4) | 100 (98.6–100) |
| Positive likelihood ratio | (60.9–∞) | 38.8 (23.2–38.8) |
| Negative likelihood ratio | 8.2 (8.2–12.8) | 0 (0–0.05) |

DISCUSSION

The UBT and the Hp StAR test are currently considered to be the only reliable non-invasive tests for monitoring H pylori infection[13]. The diagnostic accuracy is reportedly high, although some doubt still exists in the post-treatment setting since non-uniform results on the diagnostic accuracy of the stool test have been reported[14,15]. In a previous work, we found that the UBT and the antigen stool test were discordant or indeterminate in nearly 8% of patients after treatment[16]. This finding was mainly due to a lower diagnostic accuracy of stool test vs the UBT with the ratio between false UBT and stool test results of 1:6. The polyclonal antibody of the stool test (Premier Platinum HpSA test, Meridian Diagnostics, Cincinnati, OH, USA) used in that study could have been responsible for this disappointing result.

The present study was designed to evaluate the discordance rate between UBT and stool test results in the post-treatment setting by using a mAb-based stool test. Our results show that the UBT and Hp StAR results are discordant in 4% of patients. Nevertheless, the overall diagnostic accuracy of UBT and Hp StAR is identical (i.e., 98%) with the ratio between false UBT and stool test results of 1:1. Interestingly, we found that the Hp StAR is more sensitive than the UBT in monitoring H pylori infection with a higher negative predictive value. This finding is extremely important to assess the true eradication of the bacterium after antibiotic treatment and to definitely reassure patients on treatment effectiveness. Since mAb-based techniques generally have a high specificity, it is unclear why false positive results on Hp StAR test occurred in the post-treatment setting. The principle on which non-invasive H pylori diagnostic tests rely are different: the UBT measures a metabolic function of the bacterium (i.e., its urease activity) in the stomach; the Hp StAR detects antigens shed from the microorganism in stool; biopsy-based tests detect the bacterium in gastric mucosal specimens. It would be tempting to speculate that - after eradication treatment - H pylori survive in very low concentration in extra-gastric “sanctuary sites” such as dental plaque[17], Merkel’s diverticulum[18] or rectum[19] persist in stool even when the other tests become negative. Another reason for the false positivity of the stool test might be due to the fact that it may take a few days for H pylori antigens to move from the stomach to the rectum and stay in the rectum for a few more days. Consequently while the UBT becomes negative, their stool could be still positive.

mAb stool tests are available as either laboratory-based or in-office-based[20] diagnostic tests. Both tests have

![Figure 1 Correlation between UBT and Hp StAR results.](image-url)
shown - at least in the post-treatment setting - a diagnostic accuracy comparable with the UBT. Moreover, in-office stool tests have also the advantage of being suitable for the use at the doctor’s office affording physicians to prescribe eradication therapy even during the same visit[20]. Consequently, we believe that the UBT should not be regarded as the most suitable non-invasive test for monitoring H pylori infection when compared with stool test. The choice of the best non-invasive H pylori test after treatment should be done not only in terms of diagnostic accuracy but also after careful evaluation of costs and local facilities. In this regard, monoclonal-based stool tests seem to be more cost-effective than the UBT. In fact, spectrophotometers are generally more available locally and cheaper than IRMS which also require appropriate technical setting to guarantee optimal 13C-measurements and accurate UBT results[21]. Moreover, for some patient subgroups, such as infants[22] elderly[23] or handicapped people, and in some specific clinical conditions, such as gastrointestinal bleeding[24] or severe obstructive respiratory diseases, the stool test seems to be more suitable than the UBT.

Another interesting finding of our work is the high significant correlation between UBT and stool test results. Since several investigators have shown a correlation between UBT results and intragastric bacterial load, it could be hypothesized that mAb-based stool test results give also a semiquantitative measure of bacterial load in the stomach[25]. Further studies comparing stool test results with histological data are warranted to confirm this finding.

In conclusion, both breath and mAb-based stool antigen tests are reliable non-invasive tests for monitoring H pylori infection. In comparison with the UBT, the Hp StAR seems to be more sensitive and cost-effective in the post-treatment setting and more appropriate in non-cooperating patients and in some clinical conditions such as gastrointestinal bleeding or difficult breath collection.

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