Prospective study to evaluate the number and the location of biopsies in rapid urease test for diagnosis of *Helicobacter Pylori*

Antoine Abou Rached,[1] Jowana Saba,[1] Cesar Yaghi,[2] Joyce Sanyour,[1] Ahmad El Hajjar,[1] Selim Abou Kheir[1]

[1]Division of Gastroenterology, School of Medicine, Lebanese University, Beirut; [2]Faculty of Medicine, Saint-Joseph University, Hôtel Dieu de France Hospital, Beirut, Lebanon

Abstract

Helicobacter pylori (*H. pylori*) can cause a wide variety of illnesses such as peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The diagnosis and eradication of *H. pylori* are crucial. The diagnosis of *H. pylori* is usually based on the rapid urease test (RUT) and gastric antral biopsy for histology. The aim of this study is to evaluate the numbers of needed biopsies and their location (antrum/fundus) to obtain optimal result for the diagnosis of *H. pylori*. Three hundred fifty consecutive patients were recruited, 210 fulfill the inclusion criteria and had nine gastric biopsies for the detection of *H. pylori* infection: two antral for the first RUT (RUT1), one antral and one fundic for the second (RUT2), one antral for the third (RUT3) and two antral with two fundic for histology (HES, Giemsa, PAS). The reading of the 3 types of RUT was performed at 1 hour, 3 hours and 24 hours and biopsies were read by two experienced pathologists not informed about the result of RUT. Results of RUT were considered positive if *H. pylori* was found on histology of at least one biopsy. The RUT1 at 1h, 3h and 24h has a sensitivity of 72%, 82% and 89% and a specificity of 100%, 99% and 87% respectively. The positive predictive value (PPV) was 100%, 99% and 85% respectively and the negative predictive value (NPV) of 81%, 87% and 90%. The RUT2 at 1h, 3h and 24h, respectively, had a sensitivity of 86%, 87% and 91% and a specificity of 99%, 97% and 90%. The PPV was 99%, 96% and 88% and NPV of 89%, 90%, 94%. The RUT3 at 1h, 3h and 24h, respectively, had a sensitivity of 70%, 74% and 84% and a specificity of 99%, 99% and 94%. The PPV was 99%, 99% and 92% and NPV of 79%, 81% and 87%. The best sensitivity and specificity were obtained for RUT1 read at 3h, for

RUT2 read 1h and 3h, and the RUT3 read at 24h. This study demonstrates that the best sensitivity and specificity of rapid test for urease is obtained when fundic plus antral biopsy specimens are used with a reading time at 3 hours.

Introduction

Helicobacter pylori (*H. pylori*), is a common infection. The prevalence is closely tied to socioeconomic conditions and, accordingly, this infection is more common in developing countries than in developed countries,[1] affecting 28% to 84% of subjects depending on the Population tested.[2] *H. pylori* is implicated in the development of duodenal or gastric ulcers, early gastric cancer, and gastric mucosa associated lymphoid tissue lymphomas and its eradication is recommended in the treatment of these diseases.[3-6]

Tests used to detect *H. pylori* are either invasive or non-invasive. These tests vary in their sensitivity and specificity. The rapid urease test (RUT) is suitable for diagnosis before treatment.[7-9]

The RUT is a rapid, cheap and simple test. False negative results can occur in patients with recent gastrointestinal bleeding, the use of PPI, H2 antagonists, antibiotics or bismuth-containing compounds.[8,10]

This study aimed to determine optimal results for RUT with respect to the number of biopsies needed, their location and the reading time for the diagnosis of *H. pylori*.

Materials and Methods

During a period of 6 months (from January 2015 till July 2015), 350 consecutive patients who presented for upper endoscopy at Saint Charles Hospital were recruited, after the approval of the ethical committee and signed informed consent.

Exclusion criteria were PPI and/or bismuth intake in the last 3 months, antibiotics intake in the last 6 months, active upper gastrointestinal bleeding and coagulation disorders contraindicating gastric biopsy. Of the 350 patients, 140 patients were excluded.

The most common indications were epigastric pain (82%), reflux (16%) and dyspepsia (13%).

The remaining 210 patients underwent upper gastrointestinal endoscopies with 9 gastric biopsies. Four biopsies obtained respectively from the anterior and the posterior wall of the antrum and the fundus were addressed for histological examination; they were stained with H&E and PAS stain

and read by two experienced pathologists uninformed of the RUT results.

The five other biopsies were placed in the Pronto Dry® RUT kits (MIC France, Brignais, France). Two biopsies from the antrum obtained respectively from the posterior and the anterior wall were labeled *RUT1*, two biopsies obtained respectively from the posterior wall of the antrum and the fundus labeled *RUT2*, and finally one biopsy obtained from the posterior wall of the antrum Labeled *RUT3*. The reading of different RUTs was performed at the 1st, 3rd and 24th hour. A RUT was considered positive if the color changed from yellow to red, pink or orange. An unchanging yellow color indicated a negative result.

Statistical analysis

Categorical variables were compared using the chi-square or Fisher exact tests using SPSS v.20.0 (IBM Corp., Armonk, NY, USA). Sensitivity, specificity, negative and positive predictive values were calculated using SPSS 20.0 and Excel. Roc curves and Area under curve calculations were performed with SPSS 20.0.

Results

The demographic and clinical characteristics of the 210 patients included in this study are represented in Table 1. Major endoscopic findings were: gastritis (70%), bulbitis (20%) ulcer and normal finding in

16% each. Of the 210 patients who fulfill the inclusion criteria and underwent upper endoscopy, 6 were excluded for incomplete data, missing biopsy in 4 cases, and RUT results in 2 cases.

Concerning the histological examination, 96 patients out of 204 (47%) had positive evidence of *H. pylori*, whereas it was negative in 108 patients (53%).

As for the RUT, the test was positive in 104 patients (50%), negative in 104 patients (50%) regardless of the site of biopsies, the number of biopsies and the time of reading. Details about the RUT results are shown in Table 2. At 1 hour, RUT 2 showed best sensitivity (75%) followed by RUT 1 (70%) and RUT 3 (67%), with very high specificities of 99%, 100% and 99% respectively. At 3h, the RUT 3 had the same pattern of sensitivity (85%, 79% and 71% for respectively RUT2, RUT1, and RUT3) with high specificities of 96%, 99% and 99% respectively. At 24 hours, sensitivities were respectively 91%, 86%, and 81% for RUT 2, RUT 1, and RUT 3 but at the expense of a decreased specificity at 86%, 84% and 70% respectively for RUT2, RUT1 and RUT3. Results are shown in Table 3.

In Terms of PPV of the test, after 1 hour the PPV was 100%, 98% and 98% for RUT 1, RUT 2 and RUT 3 respectively. When

read after 3 hours, the PPV were 98%, 95% and 95% for RUT1, RUT 2 and RUT 3 respectively. But when the test interpretation is done after 24h, the PPV decreased slightly with the decrease in the specificity of the tests, with values of 83%, 85% and 92% for RUT1, RUT2 and RUT3 respectively (Table 3). Concerning the NPV of the different tests, the highest was for RUT 2 after 1 hour, 3 hours and 24 hours. The numbers are respectively for RUT 1, RUT 2 and RUT 3: 79%, 82% and 77% after 1 hour; 84%, 88% and 79% after 3 hours; 87%, 91% and 85% after 24 hours. This is comparable to the sensitivity with the highest for RUT 2 after 1hour, 3 hours and 24 hours. At this point, we have concluded that the RUT2 has the best sensitivity and specificity with high PPV and NPV. But which is better at 1h, 3h or 24h? Figure 1 shows Receiver Operating Characteristic (ROC) curves. These curves were constructed by computing the sensitivity and specificity and the area under the curve (AUC) is a measure of the accuracy of the test. The latter depends on how well the test separates the group being tested into those with and without the disease in question.

Table 4 represents the different AUC with their interval of confidence and p values.

Table 1. Demographic and clinical characteristics of patients (n=210).

	N (%)
Age	48.1±17.1 [13-94]
Male Gender	94 (44.8)
Previous Endoscopy	37 (17.6)
Previous *H. pylori* infection	17 (8.1)
Previous treatment for *H. pylori* infection	12 (5.7)
Current NSAID or Aspirin	20 (9.5)
Occasional alcohol intake	36 (17.1)
Smoking (on-going)	65 (31.0)

Table 2. Rapid urease test (RUT) results.

RUT positive	104 (50%)
RUT 1	
1h	68
3h	79
24h	100
RUT 2	
1h	73
3h	86
24h	104
RUT 3	
1h	67
3h	71
24h	102

Table 3. Sensitivity, specificity, negative and positive predictive value of the three rapid urease tests (RUTs).

	Sensitivity, %	Specificity, %	Positive predictive values, %	Positive predictive values, %	Accuracy
RUT 1 1h	70.7	100	100	79.3	0.862
RUT 2 1h	75.8	99.1	98.7	82.1	0.881
RUT 3 1h	67.7	99.1	98.5	77.5	0.843
RUT 1 3h	79.8	99.1	98.8	84.6	0.9
RUT 2 3h	85.8	96.4	95.5	88.4	0.914
RUT 3 3h	71.7	99.1	98.6	79.7	0.862
RUT 1 24h	86.9	84.7	83.5	87.9	0.857
RUT 2 24h	90.9	86.5	85.7	91.4	0.886
RUT 3 24h	81.8	70	92	85.2	0.881

RUT1: two biopsies from the antrum obtained respectively from the posterior and the anterior wall RUT2: two biopsies obtained respectively from the posterior wall of the antrum and the fundus RUT3: one biopsy obtained from the posterior wall of the antrum.

Table 4. Area under the curve (AUC) of the different RUT according to sites of biopsy and time of reading.

Test Result Variable(s)	AUC	Std. Error	P	95% Confidence Interval	
				Lower bound	Upper bound
RUT1 (2 antrum) read at 1h	0.854	0.029	<0.001	0.797	0.910
RUT1 (2 antrum) read at 3h	0.894	0.025	<0.001	0.845	0.944
RUT1 (2 antrum) read at 24h	0.858	0.028	<0.001	0.803	0.912
RUT2 (1 antrum + 1 fundus) read at 1h	0.874	0.027	<0.001	0.821	0.927
RUT2 (1 antrum + 1 fundus) read at 3h	0.911	0.023	<0.001	0.866	0.957
RUT2 (1 antrum + 1 fundus) read at 24h	0.887	0.025	<0.001	0.838	0.936
RUT3 (1 antrum) read at 1h	0.834	0.030	<0.001	0.774	0.893
RUT3 (1 antrum) read at 3h	0.854	0.029	<0.001	0.798	0.911
RUT3 (1 antrum) read at 24h	0.878	0.027	<0.001	0.826	0.930

The test result variable(s): Urease test 1 antrum + 1 fundus biopsies read at 1h, 3h, 24h; Urease test 2 antrum biopsies read at 1h, 3h, 24h, Urease test 1 antrum biopsy read at 1h, 3h, 24h. RUT 2 at 3h has at least one tie between the positive actual state group and the negative actual state group with the highest AUC and thus highest accuracy. Statistics may be biased.

Discussion

A strong association has been confirmed between *H. pylori* infection and dyspepsia, gastritis, peptic ulcer disease and gastric malignancies as it was defined as a class 1 carcinogen.[11-14]

H. pylori infection is so common and as many as 50% of the world population is infected with,[15] and a good mean of detection, with the best combination of simplicity, sensitivity, specificity, cost-effectiveness and rapid accurate results, is necessary to guarantee the correct treatment and preventing unnecessary further investigations for these patients or those with negative *H. pylori* gastric diseases. This guided us to look for an optimization of the widely used rapid urease test that combines all the characteristics to be an excellent confidential cheap and widely available test for the detection of *H. pylori*. Where is the best site to biopsy for the test? How many biopsies should we take? When to read and interpret the test for the optimum results? The answers to these questions remained ambiguous and little confusing for some gastroenterologists and others were unaware of their importance, with suboptimal test results, leading to treatment failure, increased resistance and costs.

Concerning the best site to biopsy, very rare studies were done to help solving this question. Shou-Wu Lee encouraged two biopsies with one from antrum to minimize the false negative rate of rapid urease test in patients with gastric or duodenal ulcers.[16] Another study showed that the Upper Body Greater Curvature side is the most sensitive and specific biopsy site to detect *H. pylori* in gastric cancer patients due to less frequent atrophy and intestinal metaplasia than at the antrum or Upper Body Lesser Curvature side.[17] Concerning the optimum number of biopsies to take for the test, Li-Lin Lim *et al.* showed that the development of a positive Ultra-rapid urease test result is hastened by doubling the number of gastric biopsies, taking two instead of one biopsy is recommended to achieve an earlier positive RUT result.[18] Increasing the number of biopsies to more than two, three or four antral biopsy specimens, may increase the sensitivity given that this will probably increases the *H. pylori* load and therefore the amount of urease.[19]

The length of time it takes for rapid urease test still poses a problem to the endoscopists. Balancing the reading time length with the accuracy of the result of the test determines the ideal time that allows the physician to prescribe *H. pylori* eradication treatment to the patient without further delay. Since One hour was the optimal time to read the test in some studies and 24-hour latency could yield better accuracy in others.[20,21]

In our study, we worked on to make this clear. We studied the best site to biopsy from and the right time to read the test, fixing the number of biopsies to 2 specimens.

Our results suggested that combining tissue biopsies from the antrum and the fundus resulted in the highest sensitivity rate of *H. pylori* detection rather than using only antral or fundic mucosa specimens, whenever the test is read, with very high NPV and PPV (Mean values 91% and 94.3% respectively), guiding us to favor the use of fundic and antral mucosa specimens for RUT to give the best results in detecting *H. pylori*, as there may be an increased yield of detection when taking biopsy specimens from different sites of the stomach due to the differences in the geographical distribution and concentration of *H. pylori* (patchy nature of the disease).

In terms of specificity of the test, it was so high reaching 99-100% at 1h wherever was the site of biopsy. With time of reading, the specificity of the test decreases progressively but slightly to reach 87%, 90% and 92% for the antral specimens, the fundic plus antral and the fundic specimens respectively which are relatively high numbers. The decrease in specificity with time, and the increase in the rate of false positive results (though minimal) may be explained by the presence of other urease producing organisms such as Proteus mirabilis, *Citrobacter freundii*, *Klebsiella pneumonia*, *Enterobactor cloacae* and *Staphylococcus aureus*, which are unlikely to be present in sufficient concentration to produce a positive test unless given enough time and delaying the reading time to 24h to grow in the test media and give false positive results for the presence of *H. pylori*.[22]

The ROC curve demonstrated 3 h is the optimum time to interpret the RUT, using specimens of the antrum and the fundus, giving the best results when combining the sensitivity and specificity of *H. pylori* detection among the other two time intervals and among all the other tests whenever the time of reading is.

Conclusions

Until we have a more revolutionary RUT test that may shortens the reading interval, increase the sensitivity of detection even for special populations like those who are on PPI or antibiotics, with the smallest number of specimens, the optimum RUT results for *H. pylori* detection came when fundic plus antral biopsy specimens are used and delaying the time of test reading to 3 hours. On the other hand, patients excluded from this study, especially those under PPI treatment, RUT can be evaluated.
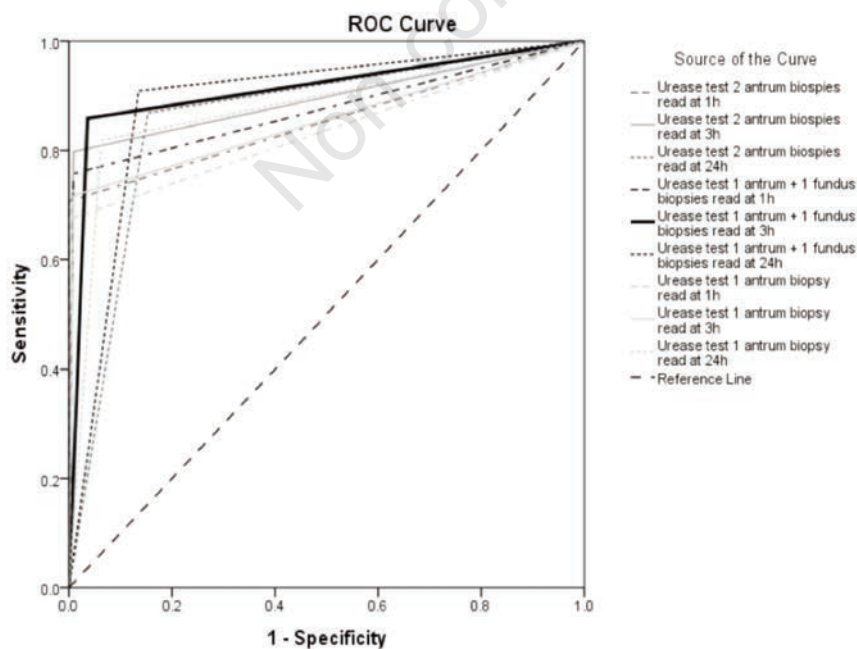

Figure 1. Comparison of ROC curves of the different tests.

References

1. Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin N Am 2000;29:559-78.
2. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. Helicobacter 2014;19:1-5.
3. Wang AY, Peura DA. The prevalence and incidence of Helicobacter pylori-associated peptic ulcer disease and upper gastrointestinal bleeding throughout the world. Gastrointest Endosc Clin North Am 2011;21:613-35.
4. McColl KE. Clinical practice. Helicobacter pylori infection. N Engl J Med 2010;362:1597-604.
5. Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut 2015;64:1353-67.
6. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 2007;56:772-81.
7. Megraud F. Advantages and disadvantages of current diagnostic tests for the detection of Helicobacter pylori. Scand J Gastroenterol Suppl 1996;215:57-62.
8. De Korwin JD. Advantages and limitations of diagnostic methods for *H. pylori* infection. Gastroenterol Clin Biol 2003;27:380-90.
9. Pajares-García JM. Diagnosis of Helicobacter pylori: invasive methods. Ital J Gastroenterol Hepatol 1998;30: S320-3.
10. Archimandritis A, Tzivras M, Sougioultzis S, et al. Rapid urease test is less sensitive than histology in diagnosing Helicobacter pylori infection in patients with non-variceal upper gastrointestinal bleeding. J Gastroenterol Hepatol 2000;15:369-73.
11. Maeda S, Mentis AF. Pathogenesis of Helicobacter pylori infection. Helicobacter 2007;12:10-4.
12. Mbulaiteye SM, Hisada M, El-Omar EM. Helicobacter pylori associated global gastric cancer burden. Front Biosci (Landmark Ed) 2009;141:490-504.
13. McGee DJ, Mobley HL. Pathogenesis of Helicobacter pylori infection. Curr Opin Gastroenterol 2000;16:24-31.
14. Vogiatzi P1, Cassone M, Luzzi I, et al. Helicobacter pylori as a class I carcinogen: physiopathology and management strategies. J Cell Biochem 2007;102:264-73.
15. Testerman TL, Morris J. Beyond the stomach: An updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. World J Gastroenterol 2014;20:12781-808.
16. Lee SW, Lee HC, Chi-Sen C, et al. The comparison of biopsy sites measured by rapid urease test for diagnosed of Helicobacter pylori infection between a population with gastric and duodenal ulcers. J Gastroenterol Hepatol Res 2012;1:226-9.
17. Kim CG, Choi IJ, Lee JY, et al. Biopsy site for detecting Helicobacter pylori infection in patients with gastric cancer. J Gastroenterol Hepatol 2009;24:469-74.
18. Lim LL, Ho KY, Ho B, Salto-Tellez M. Effect of biopsies on sensitivity and specificity of ultra-rapid urease test for detection of Helicobacter pylori infection: A prospective evaluation. World J Gastroenterol 2004;10:1907-10.
19. Siddique I, Al-Mekhaizeem K, Alateeqi N, et al. Diagnosis of Helicobacter pylori: improving the sensitivity of CLOtest by increasing the number of gastric antral biopsies. J Clin Gastroenterol 2008;42:356-60.
20. Pande PR, Karki BB, Pande R, Khatri R. Evaluation of locally made rapid urease test for diagnosis of Helicobacter Pylori. PMJN 2009;9:50-3.
21. Ho KY, Kang JY, Lim TP, et al. The effect of test duration on the sensitivity and specificity of ultra-rapid urease test for the detection of Helicobacter pylori infection. Aust N Z J Med 1998;28:615-9.
22. Osaki T, Mabe K, Hanawa T, et al. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of Helicobacter pylori infection. J Med Microbiol 2008;57:814-9.