Use of the ^{14}C breath test in the treatment of *Helicobacter pylori*

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AJ RAE, A BELZBERG, IGM CLEATOR, M CAGLAR. Use of the ^{14}C breath test in the treatment of *Helicobacter pylori*. Can J Gastroenterol 1995;9(4): 191-194. Fifty-two patients with gastric disorders referred to the same physician over the course of one year were endoscoped, biopsied and given a ^{14}C breath test to identify *Helicobacter pylori*. Sensitivity, specificity and accuracy of the ^{14}C breath test were found to be 83%, 89% and 87%, respectively, when taking biopsy results as the 'gold standard'. These figures rose to 85%, 93% and 89%, respectively, when the first five cases were not included, representing a 'learning curve' associated with the development of cut-off levels for the breath test. In five cases, persons were biopsied and given a breath test at least one month later, making 10 comparisons possible. Biopsy and breath test corresponded in nine of 10 comparisons (90%). In two of four false negatives (^{14}C breath test negative but biopsy positive) only scant numbers of helicobacter organisms were found. In one of three false positives (^{14}C breath test positive but biopsy negative) acute inflammation of the duodenal biopsied material was detected. Also, double eradication therapy (omeprazole 20 mg bid and amoxicillin trihydrate 1000 mg bid), administered in all three false positive cases, was followed by ^{14}C breath testing six weeks later, which indicated normal scores or absence of the organism.

Key Words: *Breath test, Duodenal ulcer, Gastric ulcer, Helicobacter pylori*

Test respiratoire avec marquage au ^{14}C pour le traitement de *Helicobacter pylori*

RÉSUMÉ : Cinquante-deux patients atteints de troubles gastriques et adressés au même médecin au cours d'une année ont subi une endoscopie, une biopsie et un test respiratoire avec marquage au ^{14}C afin d'identifier *Helicobacter pylori*. La sensibilité, la spécificité et la précision de cette épreuve ont été évaluées à 83 %, 89 % et 87 %, respectivement sur la base de résultats de biopsies utilisées comme étalon or. Ces chiffres se sont élevés à 85 %, 93 % et 89 % respectivement lorsque les cinq premiers cas ont été exclus, puisqu'ils représentaient la courbe d'apprentissage associée à la détermination des taux-seuils pour les tests respiratoires. Dans cinq cas, les sujets ont subi une biopsie et un test respiratoire plus d'un mois plus tard, ce qui rendait possible une comparaison à 10. La biopsie et le test respiratoire correspondaient chez 9 des 10 cas comparés (90 %). Dans deux des quatre

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THE PRESENCE OF *HELICOBACTER pylori* is strongly associated with gastric and duodenal ulcers; studies have shown this organism to be found in up to 100% of patients displaying gastric and duodenal ulcers not induced by nonsteroidal anti-inflammatory drugs (1,2). Application of antibiotic and acid-blocking pharmaceuticals aim at eradicating the organism and, if successful, result in an extremely high cure rate (3).

Diagnosis of *H pylori* infection involves endoscopy and biopsies of antral tissue. The organism can be visualized by the pathologist using a hematoxylin and eosin or acridine orange stain. Due to the patchiness of the gastric inflammation and the organism, several biopsies are usually required. Presently, biopsy represents the 'gold standard' for *H pylori* detection, although noninvasive testing (breath, saliva, blood) will undoubtedly become as accurate as biopsy.

^{14}C breath test: The ^{14}C urea breath test is based on the intense urease activity of the *H pylori* bacterium. Detection of *H pylori* is based on the liberation of ^{14}C carbon dioxide following oral administration of ^{14}C urea.

Unlike serology tests for *H pylori*, the ^{14}C breath test can be used a month after eradication therapy for confirmation of treatment efficacy. In terms of ease of use, noninvasiveness, cost per test and usefulness in follow-up, the ^{14}C breath test represents a

cas faussement négatifs (test respiratoire avec marquage au ^{14}C négatif, mais biopsie positive), seul un faible nombre d'organismes *Helicobacter* ont été isolés. Chez l'un des trois faux positifs (test respiratoire avec marquage au ^{14}C positif, mais biopsie négative), une inflammation aiguë du matériel duodénal soumis à la biopsie a été décelée. Également, la double thérapie (par oméprazole 20 mg bid et trihydrate d'amoxicilline 1 000 mg bid) administrée aux trois faux positifs a été suivie de tests respiratoires avec marquage au ^{14}C trois semaines plus tard, qui indiquaient des résultats normaux, ou l'absence de l'organisme pathogène.

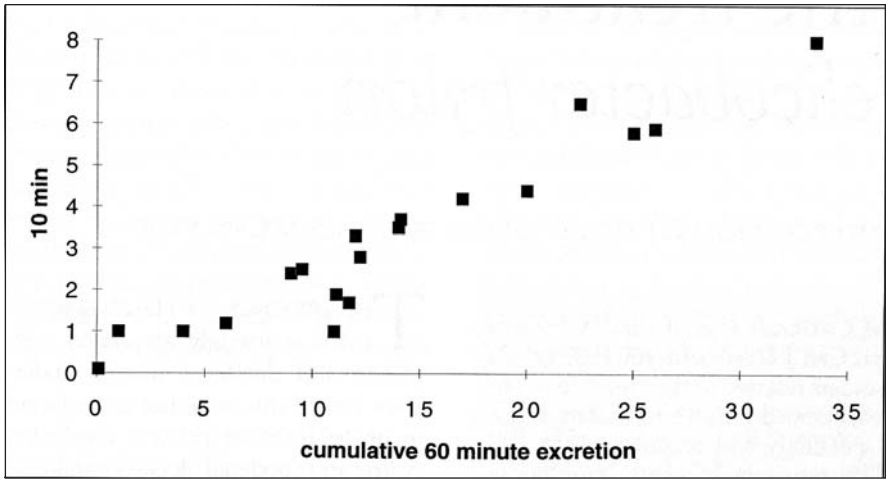

Figure 1) *Plot of cumulative ^{14}C excreted over 60 mins compared with a 10 min ^{14}C excre- tion (r^2=0.915)*

TABLE 1
Results of ^{14}C breath tests

	Biopsy positive	Biopsy negative
^{14}C test positive	20	3
^{14}C test negative	4	25

TABLE 2
Results of ^{14}C breath tests when the first five cases were removed

	Biopsy positive	Biopsy negative
^{14}C test positive	17	2
^{14}C test negative	3	25

very promising approach. Two initial European validation studies have reported sensitivity/specificity figures of 90.2%/ 83.8% (4) and 94%/89% (5), respectively.

PATIENTS AND METHODS

A retrospective study was conducted to investigate the validity of a ^{14}C breath test developed for the diagnosis and follow-up of *H pylori*-infected persons entering the authors' gastrointestinal clinic. All patients who were endoscoped by the same physician and possessed records of simultaneous biopsy and breath test from April 1993 to March 1994 were studied.

The department of Nuclear Medicine at St Paul's Hospital, upon validation using normal controls, determined a positive test as greater than 0.3% at 10 mins or greater than 2.0% at 60 mins.

Biopsy was chosen as the 'gold standard' against which the ^{14}C breath test was compared. Two gastric biopsies were taken, as well as biopsies from the duodenum if indicated. Patients already taking antacids, H_2 blockers or omeprazole were not excluded. Double (omeprazole 20 mg bid and amoxicillin trihydrate 1000 mg bid) or triple therapy (bismuth subsalicylate 5 mL qid, metronidazole 500 mg qid and tetracycline 250 mg by mouth daily for six weeks) had not yet been applied to this patient population.

The noninvasive breath test began with each patient blowing through a tube into a vial of carbon dioxide trapping agent (benzethonium hydroxide) to give baseline values for future comparisons. All patients had fasted before the procedure and brushing of teeth was not done. Afterwards, 370 kBq of ^{14}C-labelled urea in 30 mL of water was ingested by patients (radiation exposure is approximately 1/50 that of a chest x-ray). Breath samples were taken at 10, 15, 20, 30, 45 and 60 mins. A pH marker turned from pink to clear when a sufficient breath sample (0.5 mmol carbon dioxide) had been obtained. The key values for beta-counting are those obtained at 10 mins and the total ^{14}C- labelled carbon dioxide for 1 h.

Measurements were adjusted according to the weight, but not height, of each patient. A person's test was considered normal if the counted value was less than 0.3% at 10 mins or less than 2% at 60 mins. This test was based on the notable urease activity of *H pylori*. Finally, the results of a 60 min cumulative collection were compared with those of a modified 10 min collection.

RESULTS

^{14}C breath tests were conducted by the same physician, in conjunction with endoscopy and biopsy, in 52 cases. The pathology department performed hematoxylin and eosin staining and was blinded to the results of endoscopy and ^{14}C breath tests. Excellent correlation existed between the cumulative 60 mins and 10 mins sample collections (r^2=0.915) (Figure 1). In three cases a ^{14}C test positive result and negative biopsy were obtained. In four other cases a ^{14}C test negative result and positive biopsy were determined. Sensitivity, specificity, reliability and positive predictive value were 83%, 89%, 87% and 87%, respectively (Table 1). However, when the first five cases were removed, these values increased to 85% (sensitivity), 93% (specificity) and 89% (reliability) (Table 2). In five cases, patients were once again biopsied and given a breath test (at least one month later). Biopsy and breath test corresponded in nine of 10 comparisons (90%). In two of four false negatives (^{14}C breath test negative but biopsy positive) only scant numbers of

H pylori organisms were found. The authors believe that these results were simply due to a low number of *H pylori* organisms producing minimal extracellular urease rather than due to sampling error. In one of three false positives (^{14}C breath test positive but biopsy negative) acute inflammation of the duodenal biopsied material was detected (Table 3). Also, double eradication therapy, administered in all three of these false positive cases, was followed by ^{14}C breath testing six weeks later, which indicated normal scores or absence of the organism.

Finally, five patients in whom 'double therapy' did not work and for whom only ^{14}C breath testing was used in follow-up were examined. In all cases repeat ^{14}C breath tests were positive and values showed little change from earlier measurements (Table 4).

TABLE 3
False negative and false positive results further examined

Patient	Biopsy result	Breath result	Pathology
A	Positive	0.3%/1.7%/negative	No inflammatory infiltrate, scant H pylori in superficial gastric pits
B	Negative	0.5%/5.0%/positive	Mild nonspecific inflammatory infiltrate, no H pylori found
C	Positive	0.2%/1.0%/negative	Nonspecific inflammation, scant H pylori in mucosa
D	Negative	0.9%/6.8%/positive	Mild nonspecific inflammation, no H pylori
E	Negative	0.7%/6.03%/positive	Acute inflammatory changes in duodenum
F	Positive	0.1%/0.3%/negative	Mild, chronic inflammation
G	Positive	0.25%/1.0%/negative	Mild, active, chronic, superficial gastritis

All results are from initial biopsy and breath test performed at time zero

TABLE 4
Results of five patients in whom 'double therapy' did not work and for whom only ^{14}C breath testing was used in follow-up

Patient	First ^{14}C test (at 10/60 mins)	Second ^{14}C test (at 10/60 mins)
1	6.0%/24.66%	4.7%/15.8%
2	10.7%/39.2%	7.2%/24%
3	3.3%/14.9%	1.6%/9.5%
4	3.6%/12.1%	2.5%/9.5%
5	4%/20%	5%/25.2%

DISCUSSION

Values obtained in our study correspond well with those established in other 'validation' trials. Interestingly, five of seven noncorresponding breath tests and biopsies could be clearly explained and reveal a pitfall of comparing breath tests with biopsy. As earlier noted, biopsy sampling is crucial because inflammation and presence of *H pylori* are patchy. Hansing et al (6) noted a lower *H pylori* infection rate in their study but attributed this to several factors, including number and site of biopsies. In the same study, pathological analyses revealed that *H pylori* organisms were commonly identified in both the antrum and body, although corresponding neutrophilic inflammatory compounds, as well as mucus degeneration and depletion, "were more common and more severe in the antrum" (6). In our validation study we made several observations that might indicate a higher sensitivity and specificity for the ^{14}C breath test. One of three false positives (breath test positive but biopsy negative) displayed acute inflammation of duodenal biopsied material. Because *H pylori* is associated with almost all duodenal ulcers (7) and because double therapy was applied in this case and followed by a negative breath test, this case may have been a legitimate positive. Also, in the two other false positive cases double eradication therapy was applied and followed by ^{14}C breath testing six weeks later; each test at that time indicated normal scores or absence of the organism. Additionally, in two of four false negatives (breath test negative but biopsy positive) only scant numbers of helicobacter organisms were found and no inflammatory infiltrate was observed. Pathological investigation indicated basically normal tissue, and thus the question arises whether such persons should be treated in this instance.

Finally, one other argument may exist for a higher reliability of the ^{14}C breath test. We looked at five additional patients in whom 'double therapy' did not work and for whom only 14C breath testing was used for follow-up. In all cases repeat ^{14}C breath tests were positive and values showed little change from earlier measurements (Table 4). The correlation between scores seems quite close and reveals only a slight diminishing of *H pylori* infestation. Again, these observations are meant only to be descriptive and require a larger sample size for validation. We included them, however, to indicate the intrinsic difficulty of comparing the ^{14}C breath test solely with biopsy, and suggest that more accurate noninvasive diagnostic tests for *H pylori* may overtake the biopsy as gold standard in the future.

We conclude that ^{14}C testing has been a welcome addition to the standard gastrointestinal work-up at our institution and we believe it to be the noninvasive test of choice at this time. Because the correlation between cumulative 60 min collection and 10 min collection is excellent, the possibility of reduced test times will likely increase patient acceptance without reducing accuracy. Saliva and blood testing have not yet come of age, although new refinements may improve the questionable reliability or time-dependent accuracy they presently display (8-10). (In a separate study we are investigating the utility of the saliva test that measures immunoglobu-

lin G antibodies to *H pylori* by ELISA and have found it to possess a sensitivity no greater than 80% [unpublished data].)

As for blood tests, improvements in reliability still are outweighed by the dilemma of seroconversion, ie, how long after anti-*H pylori* treatment can the test be administered to check for eradication? In contrast, the ^{14}C breath test represents a reliable and safe alternative (11). The ^{14}C test displayed a 87% (n=52)/89% (n=47) accuracy in this study, and this figure may have been higher than reported previously due to the biopsy sites chosen. We recommend the ^{14}C breath test, particularly for follow-up of *H pylori* infection therapy in limited cases, no sooner than one month after double therapy is finished. This ability to follow-up without worrying about seroconversion provides a strong argument for this test versus other noninvasive tests. Additionally, centres may perform these tests and have them analyzed at a more equipped centre, a further argument for the ease of use of this test even in poorly equipped centres.

Clearly the use of this test must be delineated according to clinical indication. Does a patient with a proven duodenal ulcer require any test? Probably not if *H pylori* is responsible, as in nearly 100% of these cases. Gastric ulcer requires endoscopy to rule out cancer. Persons with gastritis and nonulcer dyspepsia initially should be endoscoped. So what is the place of the ^{14}C breath test? Perhaps the most obvious use presently is in the follow-up of clinically healed duodenal ulcer and of clinically established (upon endoscopic evaluation) gastritis, replacing re-endoscopy. On the other hand, ^{14}C follow-up of gastric lesions cannot be supported because of cancer risk.

CONCLUSIONS

We recommend the ^{14}C test for follow-up of a limited population (clinically healed duodenal ulcer and follow-up of clinically established gastritis) already endoscoped and receiving *H pylori* eradication therapy. Retesting must be performed at least one month after therapy has ended. Until blood and saliva tests prove more reliable, we view this form of *H pylori* detection as a superior noninvasive test.

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