VIA MEDICA

ISSN 1506–9680

Original

Comparative diagnostic value of the breath test and the urine test with ^{14}C-urea in the detection of the Helicobacter pylori infection

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Abstract

Among 92 patients with chronic gastritis we conducted a synchronous diagnosis of the Helicobacter pylori (H. pylori) infection using a culture and a serological test (IFP), in conjunction with breath and urine tests involving ^{14}C-urea (BTU-C14 and UTU-C14). The infection was confirmed by isolation in 71 persons (77.2%), the presence of specific IgG in the blood serum was found in 75 (81.5%). In comparison, the BTU-C14 indicated a group of 77 people (83.7%) as infected, and the UTU-C14 a group of 76 (82.6%). In order to determine the diagnostic value (sensitivity, specificity and efficiency) of the latter tests, the results were compared with those of the culture and of the serological tests. It was found that the BTU-C14 test used showed a 100% sensitivity, a 89.5% specificity and a 97.9% efficiency. The UTU-C14 test showed a 100.0% sensitivity, a 94.4% specificity and a 98.9% efficiency in the detection of the H. pylori infection.

Key words: Helicobacter pylori, breath test with ^{14}C-urea, urine test with ^{14}C-urea, chronic gastritis

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Introduction

By the end of the 19th century attention was being drawn to the presence of spiral microorganisms in the stomachs of dogs, rats and cats. However, it was only in 1982 that we succeeded in confirming their occurrence in man with the isolation of a bacterium from the altered gastric mucosa. This was initially called a "Campylobacter-like organism", later Campylobacter pylori (pyloris) and, currently, Helicobacter pylori (H. pylori) [1, 2]. Simultaneously, many diseases of the upper part of the alimentary canal were linked with infection by the bacterium. The initiating role of H. pylori is recognised in the generation of chronic gastritis (Sydney System). There is increasing evidence pointing to its importance in ulcerous diseases in the duodenum and the stomach as well as in stomach cancer [1, 3–7].

There presently exist many methods for the detection of a H. pylori infection. These include A) microscopic analysis of direct specimens or of coloured histological specimens, B) culture, C) the urease test (CLO test), D) methods of molecular hybridisation of nucleic acids (PCR) and E) methods using isotopes, particularly the breath tests involving ^{14}C or ^{13}C-urea. In the former, the $^{14}CO_2$ in the air exhaled is measured with a liquid scintillation spectrometer whereas when using ^{13}C-urea, the measurement of $^{13}CO_2$ is made with a mass spectrometer. Another example of isotopic diagnosis of the H. pylori infection is the urine test, in which the activity of the carbon isotope is measured in the urine discharged after administration of ^{14}C-labelled urea [8–14].

Surprisingly, these qualitatively different isotopic methods (breath test and urine test) have rarely been compared in analysing the diagnostic values (sensitivity, specificity, efficiency) of the methods. The scarcity of such comparisons has led the present authors to detail their own results.

Material and methods

The analysis comprised a group of 92 persons, treated for the first time, in whom chronic gastritis had been confirmed through

endoscopic and histopathological tests. The group was made up of 56 men and 36 women aged 19 to 72 (average 40.1 years). All patients were included in an identical pattern of examinations aimed at detecting any H. pylori infection. These comprised:
— bacteriological culture;
— serological test;
— breath test with ^{14}C-urea;
— urine test with ^{14}C-urea.

The purpose of such a procedure was to determine the sensitivity, specificity and efficiency (accuracy) in the detection of a H. pylori infection of the urine test we applied. The sensitivity, i.e. the percentage of truly positive results among sick people, is: $[TP/(TP+FN)] \times 100\%$.

The specificity, i.e. the percentage of truly negative results among healthy people is given by: $[FP/(FP+TN)] \times 100\%$.

The efficiency, i.e. the percentage of truly positive and negative results among sick and healthy people is: $[TP/(TP+FN+FP+TN)] \times 100\%$.

The meanings of the quantities in these above expressions are: TP — number of sick persons among whom the testing method gave a positive result, i.e. truly positive; FN — number of sick persons among whom the testing method gave a negative result, i.e. falsely negative; TN — number of healthy persons among whom the testing method gave a negative result, i.e. truly negative; FP — number of healthy persons among whom the testing method gave a positive result, i.e. falsely positive.

Endoscopic tests. Endoscopic tests were conducted using Olympus instruments, type XQ-10, Q-20, K-20. During the endoscopy, segments were taken from each person from places macroscopically altered. A part of the segments was used for histological tests, part for bacteriological tests (culture). The endoscopy was carried out each time with a sterilised instrument, i.e., each segment for bacteriological testing was taken with a different pair of sterilised biopsy pincets. The sample was then put into a separate vial with a vehicular base (Schedler's medium).

Bacteriological and serological examinations. The cultures were made on stable media (bioMerieux) in an atmosphere generated by a Generbox microairer (bioMerieux) 37°C for 3–4 days. To identify the micro-organisms culture we used:
— a microscopic examination of the specimens prepared from the culture (Gram method);
— a biochemical test (API identificator strips for Campylobacter, bioMerieux).

Serological examinations to detect specific IgG against H. pylori in the plasma were carried out with the IFP test (immunofluorescence assay) using bio-Whittaker methodology and reagents.

The results obtained were classified according to the bio-Whittaker criterion:
— 0–20 FSU (fluorescent units) — negative result (seronegative);
— above 20 FSU — positive result (seropositive).

Breath and urine test with carbon ^{14}C-urea (BTU and UTU). The examination started with taking a "background" specimen of exhaled air as well as a sample of urine from each patient. In the study we used ^{14}C urea prepared in vials with an activity of 7.4 MBq (200 μCi). The test was conducted on an empty stomach, starting with an oral intake of 92.5 kBq (2.5 μCi) of ^{14}C urea dissolved in 100 ml of a solution of physiological salt. The specimens of air exhaled were taken into special polyethylene bags of a capacity of 3 dm^3 after 2, 5, 10, 15, 20, 25 and 30 min from the administration of the isotope. Then, the content of each was passed through 3 cm^3 of a solution of hyamina (Hyamina hydroxide, Packard) with methanol (prepared in the proportion 1:2) with a supplement of 20 μl 1M phenolphthalein which absorbed exactly 1 mmol of CO_2 (0.012 g of carbon). A liquid scintillator (Insta-Fluor, Packard) was added. The activity of the samples was measured using a β-spectrometer (LKB Wallack 1217 "Rackbeta"), and the obtained results were expressed in terms of the number of impulses per minute (cmp — counts per minute). To convert them into Bq we used the standard activity: $STA_{BTU} = (0.020 \pm \pm 0.002)$ (Bq/cpm).

In interpreting the results we considered the highest value of Bq/mmol of $^{14}CO_2$ registered by the patient during the test, and the following criterion was adopted:
— to 5 Bq/mmol CO_2 — negative result (absence of H. pylori infection);
— above 5 Bq/mmol CO_2 — positive result (presence of H. pylori infection).

Urine samples were taken from each person during the first 24 hours after ingestion of the ^{14}C urea. From each volume of obtained urine (after previous precise mixing) we took 0.001 dcm^3 (1 ml) of urine into a graduated vessel containing 0.01 dcm^3 (10 ml) of scintillator (Pico-Fluor, Packard). The measurements were made on a liquid scintillation β-spectrometer (LKB Wallack 1217 "Rackbeta") and the results expressed in number of impulses (cpm). To convert them into Bq we used the standard activity: $STA_{UTU} = = (0.028 \pm 0.003)$ (Bq/cpm).

In the interpretation of the results we considered the highest value of activity (Bq/g urea) of urine samples registered in patients during the test, for which the following classification criterion was adopted:
— above 1.9 Bq/g urea negative result (absence of H. pylori infection);
— to 1.9 Bq/g urea positive result (presence of H. pylori infection).

Results

Among 92 patients with chronic gastritis, H. pylori infections were confirmed by culture in 71 and the presence of specific IgG in plasma was found in 75. The breath test indicated a group of 77 patients (83.7%) as infected with H. pylori and the urine test a group of 76 patients (82.6%).

To determine the sensitivity, specificity and efficiency of the urine test used, we compared the results obtained with those of the culture and serological tests. For the patients with chronic gastritis free from a Helicobacter pylori infection we decided to consider those in whom negative results were obtained in the culture and serological tests. The remaining patients, those for whom either or both tests were positive, comprised the group taken as infected with the bacterium.

Adopting the above criterion, among 92 patients with chronic gastritis H. pylori was confirmed in 75; in the remaining 17 there was no infection (Table 1).

The results show that the BTU with ^{14}C-urea detects the H. pylori infection with a 100% sensitivity, a 89.5% specificity and

Table 1. Results of culture and of serological tests enabling a classification into H. pylori infected and non-infected patients

Culture	Serological test (IFP)	
	(–)	(+)
(–)	17	4
(+)	–	71

(–) The negative result of the culture and of the serological test means the absence of a H. pylori infection; (+) The positive result of both tests (culture and serological) or of at least one of them means the presence of a H. pylori infection

Table 2. Diagnostic value of the breath test with ^{14}C-urea in relation to the results of the reference tests (culture and serological)

Distribution of patients according to the reference tests (culture and serological)	Breath test (BTU-C14)	
	Infected by H. pylori	Non-infected by H. pylori
Infected by H. pylori	TP **75**	FN **0**
Non-infected by H. pylori	FP **2**	TN **17**

TP — result truly positive; FN — result falsely negative; FP — result falsely positive; TN — result truly negative; Sensitivity (75/75) × 100% = 100%; Specificity (17/19) × × 100% = 89.5%; Efficiency (92/94) × 100% = 97.9%

Table 3. Diagnostic value of the urine test with ^{14}C-urea in relation to the results of the reference tests (culture and serological)

Distribution of patients according to the reference tests (culture and serological)	Urine test (UTU-C14)	
	Infected by H. pylori	Non-infected by H. pylori
Infected by H. pylori	TP **75**	FN **0**
Non-infected by H. pylori	FP **1**	TN **17**

TP — result truly positive; FN — result falsely negative; FP — result falsely positive; TN — result truly negative; Sensitivity (75/75) × 100% = 100%; Specificity (17/19) × × 100% = 89.5%; Efficiency (92/94) × 100% = 97.9%

a 97.9% efficiency (Table 2). The UTU detects this infection with a 100.0% sensitivity, a 94.4% specificity and a 98.9% efficiency (Table 3).

Discussion

At present, nine types of Helicobacter type are differentiated. Some of them only live in animals, for instance Helicobacter mustelae in ferrets and Helicobacter felis in cats; others, such as Heliocbacter cinaedi, Helicobacter fennelliae, Helicobacter heimani, may also occur in the organs of humans. Amongst these bacteria, H. pylori clearly has the greatest importance for humans [1, 5].

Amongst the many diagnostic methods used to detect infection by that bacterium, tests using isotope-labelled urea are of increasing interest. Helicobacter pylori is a bacterium of high urease activity, so that the oral administration of ^{13}C or ^{14}C urea in presence of an infection of this bacterium in the gastric mucosa leads to an enhancement of the breakdown of that compound and the formation of radioactive CO_2:

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

The $^{14}CO_2$ is absorbed through the mucosa of the stomach and of the small bowel into the blood by which it is carried, inter alia, to the lungs and the kidneys and there excreted (Fig. 1) [15, 16].

It is reckoned that from 1.5 to 2.3 times as much of the isotope is eliminated from the organism through urine as through exhaled air. Our studies, as well as observations by other authors, indicate that measurement of the activity of the exhaled air and of excreted urine may be useful in diagnosing H. pylori [17–20].

However, the choice of test in the diagnosis of H. pylori infections is decided, among other criteria, by: the non-invasive nature of the technique, the possibility of obtaining the results quickly, the simplicity of any laboratory work and the need for high diagnostic reliability. We have found that both isotopic tests for the recognition of H. pylori infection satisfy the above conditions. For the same sensitivity, the UTU-C14 test has a slightly higher specificity and efficiency.

It might be argued that wider clinical use of the UTU-C14 technique is limited by the necessity for a 24-hour urine collection, but this would not be correct. It is sufficient to analyse the first sample of urine excreted by the patient after oral administration of the isotope to obtain fully reliable results.

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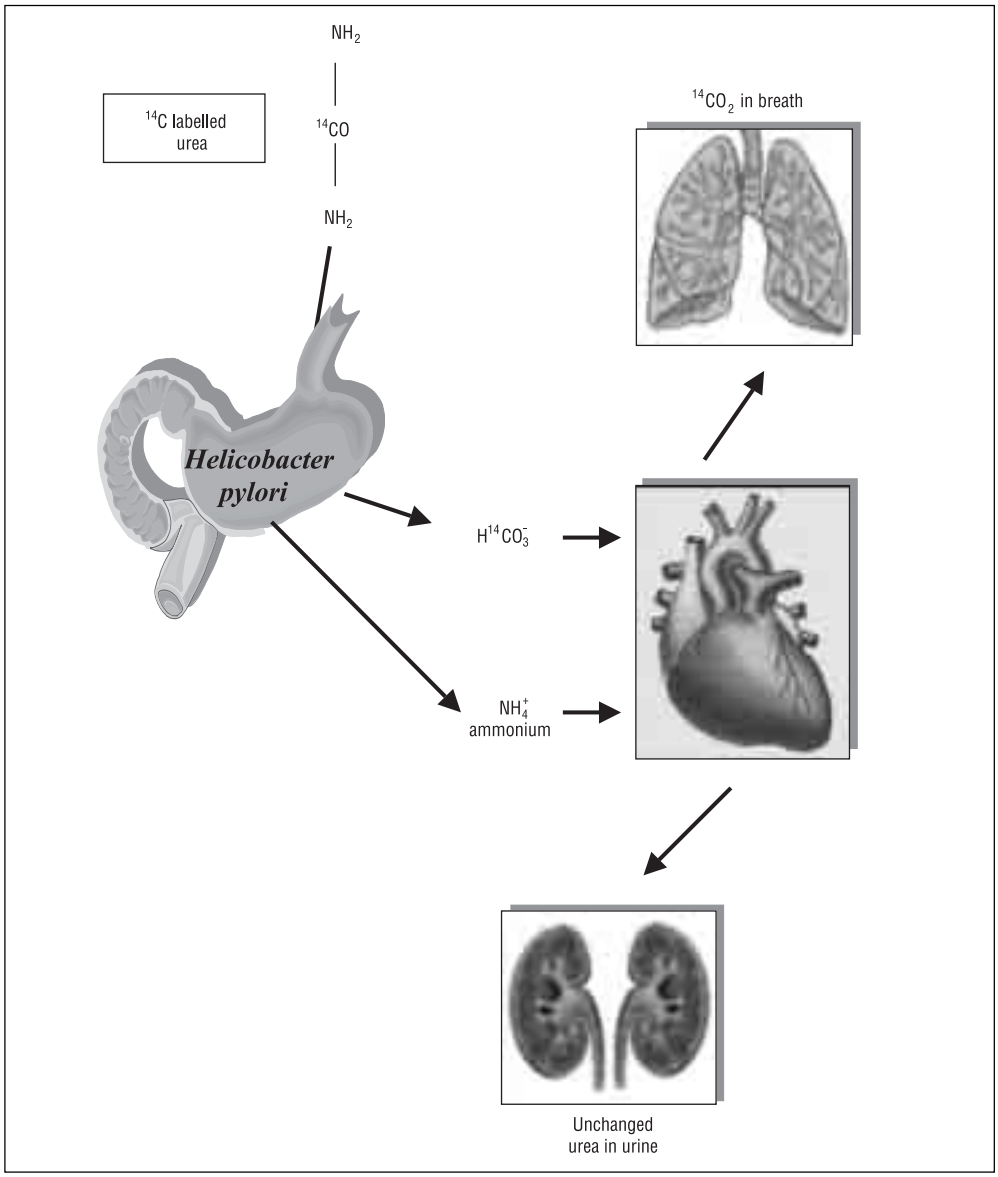

Figure 1. Basic ways of elimination from the organism of the radioactive ^{14}C after oral administration of $^{14}CO(NH_2)_2$ [16, 19].

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