Comparison of Accuracy Between ^{13}C- and ^{14}C-Urea Breath Testing: Is an Indeterminate-Results Category Still Needed?

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Helicobacter pylori infection is the leading cause of peptic ulcer disease. The purpose of this study was, first, to assess the difference in the distribution of negative versus positive results between the older ^{14}C-urea breath test and the newer ^{13}C-urea breath test and, second, to determine whether use of an indeterminate-results category is still meaningful and what type of results should trigger repeated testing. **Methods:** A retrospective survey was performed of all consecutive patients referred to our service for urea breath testing. We analyzed 562 patients who had undergone testing with ^{14}C-urea and 454 patients who had undergone testing with ^{13}C-urea. **Results:** In comparison with the wide distribution of negative ^{14}C results, negative ^{13}C results were distributed farther from the cutoff and were grouped more tightly around the mean negative value. Distribution analysis of the negative results for ^{13}C testing, compared with those for ^{14}C testing, revealed a statistically significant difference between the two. Within the ^{13}C group, only 1 patient could have been classified as having indeterminate results using the same indeterminate zone as was used for the ^{14}C group. This is significantly less frequent than what was found for the ^{14}C group. **Discussion:** Borderline-negative results do occur with ^{13}C-urea breath testing, although less frequently than with ^{14}C-urea breath testing, and we will be carefully monitoring differences falling between 3.0 and 3.5 %Δ. ^{13}C-urea breath testing is safe and simple for the patient and, in most cases, provides clearer positive or negative results for the clinician.

Key Words: breath test; helicobacter pylori; ^{13}C; ^{14}C; accuracy; distribution

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Infection with *Helicobacter pylori* is the leading cause of peptic ulcer disease. In developed countries, the prevalence ranges from 25% to 50%. It is also associated with gastric cancer and mucosa-associated lymphoid tissue lymphoma (*1*). Urea breath testing is based on production by *H. pylori* of urease, an enzyme that converts urea to ammonium and CO_2. A dose of urea labeled with an isotope of carbon, either ^{13}C or ^{14}C, is taken orally by the patient. In an infected patient, the urease activity in the mucosal layer of the stomach from the presence of *H. pylori* breaks down the labeled urea, and the converted labeled CO_2 diffuses to the epithelial cells, is carried in the bloodstream, and ultimately is exhaled by the lungs. A breath sample from the patient can be measured to determine the amount of labeled CO_2 exhaled and thus the presence or absence of *H. pylori* infection (*2*).

^{13}C is a nonradioactive isotope of carbon that is measured by isotope-ratio mass spectrometry. ^{14}C is a radioactive isotope of carbon that is measured by a scintillation counter. The radiation dose delivered by the standard ingested activity is estimated at less than 0.003 mSv (*2*), which is trivial when compared with the annual dose received from background radiation in Canada (1.8 mSv/y) and from routine radiologic studies (average of 5–30 mSv per study). Nevertheless, radiation mistrust is a nonissue with ^{13}C and may ease certain patients and physicians.

Because of a supply shortage of ^{14}C, we had to switch from ^{14}C- to ^{13}C-urea breath testing, and we decided to use a commercial kit (Helikit; Paladin Labs Inc.). The accuracy of this alternative is not questioned here, as proper analysis using biopsy-derived data as the gold standard was performed before commercialization (*3*). However, in our population we found some differences in the distribution of positive and negative results between the newer ^{13}C and older ^{14}C testing. We used to recall patients and repeat the testing when their results were too close to the cutoff. The commercial kit does not define an indeterminate-results category but only provides a cutoff for positivity.

In this study, we thus aimed to assess the difference in the distribution of negative versus positive results between the older ^{14}C test and the newer ^{13}C one. In addition, we sought to determine whether use of an indeterminate-results category could be meaningful and what type of results should trigger repeated testing in a given patient.

MATERIALS AND METHODS

A retrospective survey was performed of all consecutive patients referred to our service for ^{14}C-urea breath testing between 2005 and 2009 or for ^{13}C-urea breath testing between 2011 and 2013. The study was conducted at a university-affiliated hospital, after the local ethics commission had approved it and waived the requirement to obtain informed consent. We excluded 8 patients who had inadequate sampling results. The results had already been interpreted and were being used in the management of the referred patients. The collected data were stored in a password-protected spreadsheet on an

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encrypted drive. The personal information of the patients was discarded at the end of data collection.

Patient Preparation

Patient preparation was relatively simple (Appendix A), although a thorough analysis of the medications taken by each patient was necessary to minimize the risk of false-negative results (*2*). The patients had been instructed to stop taking antibiotics and bismuth-containing products for 1 mo before the test, proton pump inhibitors and sucralfate for 2 wk, and H_2 blockers and over-the-counter antacids for 24 h. They had also been asked to fast for at least 6 h and to refrain from smoking for at least 2 h.

Test Protocol

Helikit comes with a plastic cup containing 75 mg of ^{13}C-urea, citric acid, flavor enhancers, and stabilizers; 2 Exetainer tubes (Labco Limited) with colored labels and screw caps; straws; and a holder for transport of the tubes. Using a straw, fasting patients exhale fully into the baseline tube, which is then capped and labeled. The drink is prepared by adding 75 mL of tap water to the powder in the cup, which is shaken gently to dissolve the contents. The patient then consumes the drink and, after 30 min, provides another breath sample. This second tube is capped and labeled.

The analyses were performed on a Finnigan MAT 252 isotope-ratio mass spectrometer (Thermo Electron Corp.). The raw data were produced in units of difference per thousand, which refers to ^{13}C content relative to the Pee Dee Belemnite international standard, a measure of the ratio of the stable isotope ^{13}C to the stable isotope ^{12}C, reported in parts per thousand (%Δ). The difference from baseline refers to the difference per thousand between the baseline and postingestion samples.

Analysis and Cutoff

To standardize and allow comparison of the results distribution between the two tests, the value of each sample was divided by its own cutoff (S/CO), yielding negative results below 1.0 and positive results above 1.0 for each ^{13}C and ^{14}C test group.

Distribution was analyzed using Microsoft Excel formulas and analysis tools. Average and SD, as well as Student *t* and Wilcoxon testing, were used to assess the difference in distribution between positive and negative groups and between ^{13}C and ^{14}C cluster groups.

RESULTS

There were 562 patients who had undergone ^{14}C testing. Of those, the results for 366 (65.1%) were below the cutoff of 0.33 counts per second (cps) and thus were considered negative, and the results for 196 (34.9%) were positive.

The positive cutoff for ^{13}C testing was defined as a difference of greater than 3.5 %Δ. Of the 454 patients who had undergone ^{13}C testing, 335 (73.8%) had negative results and 119 (26.2%) had positive results.

Analysis of Negative Results

Of the 366 patients with negative ^{14}C results, the average was 0.0118 ± 0.0050 cps. Division by the 0.33-cps cutoff yielded an average of 0.357 ± 0.150 S/CO. Of the 335 patients with negative ^{13}C results, the average difference was 0.360 ± 0.293 %Δ. Division by the 3.5 %Δ cutoff yielded an average of 0.103 ± 0.084 S/CO.

Visual assessment of the distribution of results (Fig. 1) revealed that negative ^{13}C results were distributed farther from the cutoff (*y*-axis at 1.0 in S/CO standardization) and were grouped more tightly around the mean negative value, in contrast to negative ^{14}C results, which were more widely distributed although closer to the cutoff.

Unpaired *t* testing of the distribution of the negative ^{13}C results compared with the negative ^{14}C results revealed a statistically significant difference, with a *P* value of 1.68×10^{-70}. Similarly, Mann–Whitney–Wilcoxon analysis for nonparametric statistics yielded a *P* value of 9.67×10^{-65}, which was again highly significant.

Analysis of Negative Differences

Given that the results of ^{13}C testing were calculated from the subtraction of two successive measurements, 49 patients had a negative value for the difference. Although this is clearly below the cutoff of 3.5 %Δ for positivity, it raises the question of the validity of the measurements. These negative differences ranged from −0.01 to −1.89 %Δ. The lowest value (−1.89 %Δ) either was an outlier or fell within the elongated left tail of the negative value distribution as illustrated in Figure 2.

Overall, the distribution of negative results for ^{13}C testing appeared to follow a somewhat peaked curve around the 0.36 ± 0.47 mean. However, this curve was skewed to the right, with a calculated skewness of 2.67, indicating that most of the negative results were grouped to the left of the mean negative value and that the right tail was longer than the left tail. The kurtosis was rather elevated, with a calculated value of 13.49, indicating that as compared with a bell-shaped distribution, the central peak was higher and sharper and its tails longer. This could explain the negative results, as the values fell within the long left tail of the negative-results distribution.

The Indeterminate-Results Zone

For ^{14}C testing, results that fell between 0.30 and 0.33 cps were classified as indeterminate. This corresponds to an interval of 0.90–1.00 S/CO. Of the 562 patients who underwent ^{14}C testing, 8 (1.42%) were classified as having indeterminate results.

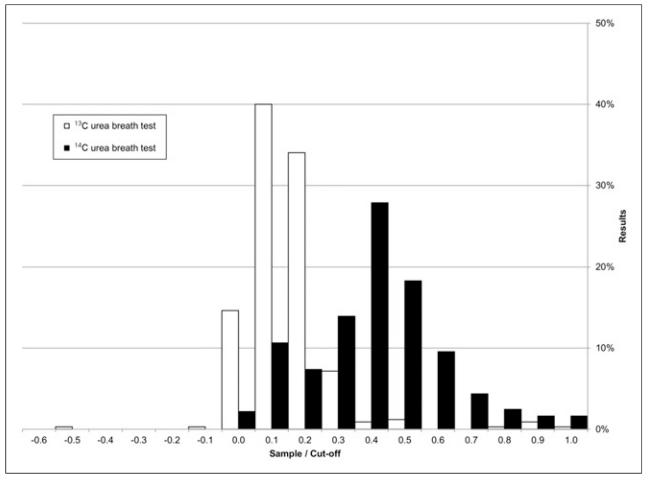

FIGURE 1. Distribution of negative results (S/CO < 1.0) for ^{13}C- and ^{14}C-urea breath testing.

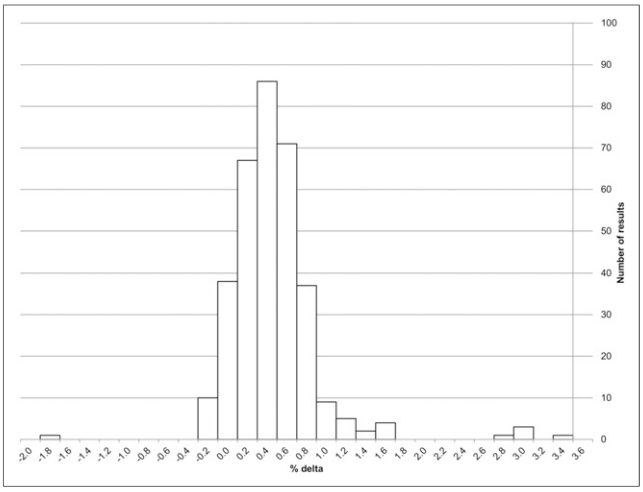

FIGURE 2. Distribution of negative results for ^{13}C-urea breath testing.

Of the 454 patients who underwent ^{13}C testing, only 1 (0.22%) could have been classified as having indeterminate results using the same range of 0.90–1.00 S/CO. This corresponds to a difference interval of 3.0–3.5 %Δ.

The difference in distribution between indeterminate and determinate (negative and positive) results for ^{13}C testing was statistically significant, with a 2-tailed *P* value of 0.0480 (Fischer exact test).

Analysis of Positive Results

Of the 196 patients with positive ^{14}C results (Fig. 3), the average was 0.300 ± 0.172 cps, with a corresponding 5.210 ± 0.172 S/CO. Of the 119 patients with negative ^{13}C results, the average difference was 20.658 ± 10.359 %Δ, and thus 5.920 ± 2.960 S/CO.

Unpaired *t* testing of the distribution of positive ^{13}C results compared with positive ^{14}C results revealed a statistically significant difference, with a *P* value of 2.15×10^{-7}. Wilcoxon analysis for nonparametric statistics yielded a *P* value of 9.05×10^{-5}, which was again statistically significant.

DISCUSSION

In their extensive and particularly well-written review (*4*), Gisbert and Pajares highlighted that "A unique and generally proposed cut-off level is not possible because it has to be adapted to different factors, such as the test meal, the dose and type of urea, or the pre-/post-treatment setting." The commercial kit we are using has a defined difference cutoff of 3.5 %Δ for positivity, which is inside the 2–5 %Δ range in which most urea breath test results tend to cluster (*5,6*). The aim of this study was to assess the difference in the distribution of negative and positive results between the older ^{14}C test and the newer ^{13}C test. We determined that negative results were distributed significantly farther from the cutoff for ^{13}C testing than for ^{14}C testing. The distribution of negative results was more closely grouped around the mean negative value, with, however, a longer left tail, potentially explaining why some values were negative as the result of the subtraction between baseline and post-ingestion breath samples.

In addition, we sought to determine whether use of an indeterminate-results category could be meaningful and what type of results should trigger repeated testing in a given patient. Borderline-negative results do occur with ^{13}C testing, although less frequently than with ^{14}C testing, and we will be carefully monitoring differences falling between 3.0 and 3.5 %Δ.

Many authors have advocated use of a gray zone of indeterminate results to account for the inherent variation in measurement technique. Again, the definition of this indeterminate zone has varied between authors, but a fairly small number of patients have generally fallen into it (*7*). Caution is advised when the test is being performed to confirm eradication of *H. pylori.* If infection persists, a lower bacterial density may decrease the test response, and using a lower cutoff in such cases may improve detection of residual infection and reduce false-negative results (*8,9*).

CONCLUSION

^{13}C-urea breath testing is accurate for detecting *H. pylori* infection. It is safe and simple for the patient, usually provides clearly positive or negative results for the clinician, and thus is the noninvasive test of choice in this clinical setting.

The interpreter is always advised to exercise caution to minimize false-positive and -negative results. Use of an indeterminate zone of result values may help the interpreter improve the diagnostic accuracy of the test.

APPENDIX A: PREPARATION FOR ^{13}C UREA BREATH TESTING

The patient should have no contraindications to the test, should fast from liquids and solids for 6 h beforehand, should refrain from smoking for 2 h beforehand, and should stop taking the following medications:

Oral or intravenous antibiotics for 30 d beforehand (antiviral and antifungal agents need not be stopped).

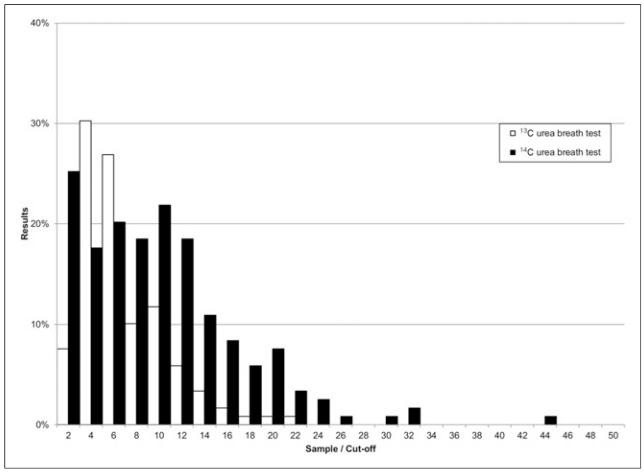

FIGURE 3. Distribution of positive results (S/CO > 1.0) for ^{13}C- and ^{14}C-urea breath testing.

Bismuth for 30 d beforehand.

Sucralfate for 14 d beforehand.

Proton pump inhibitors for 14 d beforehand.
- Omeprazole
- Lansoprazole
- Dexlansoprazole
- Rabeprazole
- Pantoprazole
- Esomeprazole

H_2 blockers for 24 h beforehand.
- Cimetidine
- Ranitidine
- Famotidine
- Nizatidine

Over-the-counter antacids for 24 h beforehand.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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