13C urea breath test for Helicobacter pylori: Determination of the optimal cut-off point in a Canadian community population

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AIM: To determine the test characteristics and the optimal cut-off point for the 13C urea breath test (13C UBT) in a Canadian community laboratory setting.

METHODS: Of 2232 patients (mean age ± SD: 51±21 years, 56% female) who completed a 13C UBT, 1209 were tested to evaluate the primary diagnosis of Helicobacter pylori infection and 1023 were tested for confirmation of eradication following treatment. Cluster analysis was performed on the 13C UBT data to determine the optimal cut-off point and the risk of false-positive and false-negative results. Additionally, 176 patients underwent endoscopic biopsy to allow validation of the sensitivity and specificity of the 13C UBT against histology and microbiology using the calculated cut-off point.

RESULTS: The calculated cut-off points were 3.09 δ‰ for the whole study population (n=2232), 3.09 δ‰ for the diagnosis group (n=1209) and 2.88 δ‰ for the post-treatment group (n=1023). When replacing the calculated cut-off points by a practical cut-off point of 3.0 δ‰, the risk of false-positive and false-negative results was lower than 2.3%. The 13C UBT showed 100% sensitivity and 98.5% specificity compared with histology and microbiology (n=176) for the diagnosis of active H pylori infection.

CONCLUSIONS: The 13C UBT is an accurate, noninvasive test for the diagnosis of H pylori infection and for confirmation of cure after eradication therapy. The present study confirms the validity of a cut-off point of 3.0 δ‰ for the 13C UBT when used in a large Canadian community population according to a standard protocol.

Key Words: 13C urea breath test; Cut-off point; Helicobacter pylori

Helicobacter pylori infection is one of the most common human infections worldwide (1). This organism has been shown to infect over 50% of the world’s population, with a prevalence of 20% to 40% in the Canadian population and up to 80% in developing countries (2-5).

H pylori is the primary cause of gastritis and peptic ulcer disease, and has been associated with gastric lymphoma and adenocarcinoma (6,7). Since the discovery of its pivotal role in many human gastrointestinal pathologies, several diagnostic tests, both invasive and noninvasive, have been developed. Recently, it has been recommended that subjects younger than 45 years of age with abdominal discomfort should undergo a noninvasive and rapid diagnosis of H pylori infection, followed by pharmacological treatment if the test is positive (8). The 13C urea breath test (13C UBT), with a specificity of 98% and a sensitivity of 97%, allows the diagnosis of an active H pylori infection without the need of costly and invasive endoscopic testing (9). Thus, the 13C UBT has become the noninvasive
test of choice in many jurisdictions for diagnosis and confirmation of eradication following treatment, as recommended by a number of clinical guidelines (8,10,11). The 13C UBT also has the advantage of assessing the global presence of Helicobacter pylori throughout the stomach, whereas endoscopy-based tests are limited to focal assessments (at the site of biopsy) with the consequent risk of false-negative tests due to sampling errors (12).

The 13C UBT detects the presence of gastric H pylori urease, which hydrolyzes orally administered 13C-labelled urea (a stable isotope) and produces ammonia and 13C-labelled carbon dioxide (13CO2). The 13CO2 diffuses into the blood and is excreted by the lungs; therefore it can be detected in the breath using various methods. To distinguish between positive and negative results, a diagnostic cut-off value is defined at a specified time point after ingestion of a substrate. This cut-off point is generally determined by comparison with the ‘gold standard’ diagnostic technique (usually, histology and culture) in the affected population (13). However, there is still controversy about the value of the best cut-off point. The 13C UBT value may, for example, be affected by sociodemographic factors, concomitant medication and bacterial and host factors (14-18), leading to the possibility that the optimal cut-off point could be quite variable. Thus, before a test such as this is widely adopted, a comprehensive reassessment of the cut-off point is needed in the appropriate populations. Despite the need to validate the clinical performance of the 13C UBT in a Canadian population, until now, there have been no large-scale data available for this purpose. Validation of the 13C UBT’s performance in a community setting is essential before it is adopted as part of a primary care-based ‘test and treat’ strategy for dyspepsia management (19).

The objective of the present study was to determine the optimal cut-off value for the 13C UBT by cluster analysis in a large dataset from Canadian patients who had undergone testing in community laboratories, and to support it by independent validation against histology and culture in a Canadian setting using the same study protocol.

**PATIENTS AND METHODS**

The protocol of the present study was approved by the McMaster University Research Ethics Board. Results of 13C UBT performed on 2232 patients (mean age ± SD: 51±21 years, 56% female) were analyzed. Among them, 1209 patients (mean age: 49±17 years, 54% female) were tested to evaluate the primary diagnosis of H pylori infection (diagnosis group), and 1023 patients (mean age: 53±25 years, 57% female) were tested for confirmation of eradication following treatment (post-treatment group). Samples were collected through community laboratories (MDH Diagnostic Services, Toronto, Ontario) and analyzed at the McMaster University Medical Centre, Hamilton, Ontario. An additional 176 patients from the McMaster University Medical Centre had both 13C UBT and endoscopic biopsies to determine their H pylori status.

Using a standardized questionnaire, patients were asked about their use of antibiotics and acid-suppressive treatment during the four weeks before testing. Exclusion criteria included the use of proton pump inhibitors, bismuth compounds or antibiotics within 14 days before the 13C UBT. The 13C UBT was performed after an overnight fast by obtaining two breath samples, one before (T0) and the second 30 min after (T30) oral administration of 75 mg 13C-labelled urea (Helikit, Isotechnika Diagnostics, Canada) in 100 mL of citric acid solution. The samples were obtained by having patients gently exhale through a plastic straw, with its distal tip placed at the bottom of a 10 mL glass tube. The tube was sealed with a stopper immediately after patient exhalation. All samples were analyzed by a gas isotope ratio mass spectrometer (BreathMAT, Finnigan MAT GmbH, Germany). The difference between values at 30 min and at baseline (T30-T0) is expressed as delta (δ) over baseline (DOB, ‰).

Data were examined visually by plotting the logarithmic transformation of measurements taken from 13C UBT. The distribution of the natural logarithms (logn) of the DOB values for each breath sample test interval identified two normal subpopulations that were considered to represent H pylori-positive and H pylori-negative patients. The normal distributions of the positive and negative populations allowed cluster analysis to be performed on these data to determine the minimal interclass variance, and thus, the logn (T30-T0) value, which best separated the presumed H pylori-negative and H pylori-positive populations. Thereafter, the parameters (mean DOB [‰], and SD) for the H pylori-negative and H pylori-positive populations were established. The cut-off value was calculated as the point equidistant between the mean DOB values of the H pylori-negative and H pylori-positive populations. This was the basis for determining the probability of an H pylori-negative patient producing a 13C UBT result above the cut-off point (ie, a false-positive result), and the probability of an H pylori-positive patient producing a 13C UBT result below the cut-off point (ie, a false-negative result). This analysis was performed on the whole patient group and on the subgroups (primary diagnosis and confirmation of cure after eradication therapy).

To compare the 13C UBT with histology and microbiology, upper gastrointestinal endoscopy was performed on 176 patients before the 13C UBT was administered. During an examination, three biopsy specimens each from the gastric antrum and corpus were obtained for histology and bacterial culture. Silver staining (Warthin-Starry) was used to identify H pylori in the specimens. Culture samples were inoculated in Brucella blood agar, Mueller-Hinton sheep blood agar and egg yolk emulsion; the plates were incubated at 35°C for four days. H pylori growth was confirmed by Gram-staining, a rapid urea test, an oxidase test and a wet preparation for motility. Negative cultures were reincubated and examined seven to 10 days later. H pylori status was determined when both methods (histology and culture) produced concordant results. Sensitivity, specificity and positive and negative predictive values for the 13C UBT were calculated using the optimal cut-off point.

**RESULTS**

**Cut-off point determined by cluster analysis**

Data were examined visually by plotting the logarithmic transformation of the DOB values obtained from 2232 13C UBT results. It was evident that the normal distributions of the DOB values could describe two distinct classes of results: H pylori-negative and H pylori-positive populations (Figure 1). From this, the means and SDs of the logn (T30-T0) for the presumed subpopulations were calculated. The point equidistant between the mean values of the subpopulations was determined to be an appropriate cut-off point. The calculated cut-off point for the whole study population was 3.09 ± 2SD. The distances (normal deviate) between the cut-off point and the means of the negative and positive H pylori distributions were calculated. The normal deviates were found to be greater than 2 SD (normal deviate = 2.28 SD), indicating that by comparison with the table of proportions of the normal curve,
the proportions of *H. pylori*-negative and *H. pylori*-positive populations producing a \(^{13}\)C UBT result greater or smaller than the cut-off point were always lower than 2.3%. Thus, the risk of a false-positive or a false-negative result from the \(^{13}\)C UBT for a diagnosis of *H. pylori* infection was lower than 2.3% when using the cut-off point 3.0 \(\delta\)‰. Using a practical cut-off point of 3.0 \(\delta\)‰, the corresponding normal deviates for the *H. pylori*-negative and *H. pylori*-positive distributions were still greater than 2 SD; therefore, according to the table of the proportions of the normal curve, the risks of error were still lower than 2.3%. Using a cut-off point of 3.0 \(\delta\)‰, 73.3% of the population studied was negative and 26.7% was positive for *H. pylori* infection.

These calculations were also performed for the diagnosis and post-treatment groups. The calculated cut-off point for the \(^{13}\)C UBT was 3.09 \(\delta\)‰ for the diagnosis group and 2.89 \(\delta\)‰ for the post-treatment group (Figure 2). Replacing the cut-off points obtained in the two groups with 3.0 \(\delta\)‰ (as determined for the whole population), the normal deviates for the *H. pylori*-negative and *H. pylori*-positive distributions in both groups were greater than 2 SD in all cases, which is again indicative of a risk lower than 2.3% for false-positive or false-negative results. Overall, 29.4% of patients were positive for *H. pylori* infection in the diagnosis group and 22.4% were positive in the post-treatment group. There was no difference between women and men when a cut-off point of 3.0 \(\delta\)‰ was used; the risk of error was lower than 2.3% in both groups.

A cut-off point was also determined for different age groups. Although DOB values increased with age (Figure 3), using a cut-off point of 3.0 \(\delta\)‰ showed a risk of error lower than 2.3%.

**Validation of \(^{13}\)C UBT by histology and microbiology**

In 176 patients, the \(^{13}\)C UBT results at a cut-off point of 3.0 \(\delta\)‰ were compared with histology and culture (considered to be the gold standard). In this analysis, the \(^{13}\)C UBT showed a sensitivity of 100% and a specificity of 98.5% for the diagnosis of *H. pylori*. Based on an *H. pylori* incidence of 20% to 40% in the Canadian population (16), the positive predictive value of the \(^{13}\)C UBT would be 94.5% to 97.9% and the negative predictive value would be 100% (Figure 4).

**DISCUSSION**

The \(^{13}\)C UBT is now widely used to document *H. pylori* infection (20). The test has been recommended as the preferred method for epidemiological studies and for screening patients with dyspeptic symptoms (19). Because it is a test for active...
infection, it is also recognized as the best noninvasive test to assess the efficacy of anti-*H. pylori* treatments (21,22).

Considering the increasing application of the $^{13}$C UBT and the fact that different test meals, fasting states, nationalities, bacterial and host factors, and concomitant medication use may affect this test, we assessed a large set of Canadian patients with dyspeptic symptoms to determine the cut-off point for the $^{13}$C UBT in community practice (14-18,23-25).

Using cluster analysis, we found that the optimal cut-off point for the $^{13}$C UBT for our population was 3.09 $\delta\%$, which showed 100% sensitivity and 98.5% specificity compared with histology and culture. This is a lower cut-off point than that reported in earlier studies ($T_{30-T_{0}}=5.0 \delta\%$) (26,27), but it has been confirmed by others in comparison with histology (28) and by cluster analysis in a large set of data (29). The risk of false-negative or false-positive results was less than 2.3% when a practical cut-off point of 3.0 $\delta\%$ was used, a more than adequate efficacy for a noninvasive test. The risk of error was less than 2.3% when the same cut-off point was used in the diagnosis and post-treatment groups (Table 1).

**CONCLUSION**

The $^{13}$C UBT is an accurate, noninvasive test for the diagnosis of *H. pylori* infection and for the confirmation of cure after eradication therapy. The present study confirms the validity of a cut-off point of 3.0 $\delta\%$ for a Canadian community population using a standard protocol. Thus, the $^{13}$C UBT is a practical and accurate test on which to base a 'test and treat' strategy for the management of dyspepsia in community practice in Canada. These data provide further support for making the $^{13}$C UBT more widely available in community practice because it is convenient, accurate and likely more cost-effective than endoscopy or *H. pylori* serology (30).

**ACKNOWLEDGEMENTS:** The authors thank Dr Fiona Smail and Pamela Lyn for their invaluable assistance. The work of author Markad Kamath was supported by the DeGroote Foundation and the Natural Sciences and Engineering Research Council of Canada (NSERC).

**REFERENCES**


