¹³C urea breath testing to diagnose *Helicobacter pylori* infection in children

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C Deslandres. ¹³C urea breath testing to diagnose *Helicobacter pylori* infection in children. *Can J Gastroenterol* 1999;13(7): 567-750. The causal relationship between *Helicobacter pylori* colonization of the gastric mucosa and gastritis has been proven. Endoscopy and subsequent histological examination of antral biopsies have been regarded as the gold standard for diagnosing *H pylori* gastritis. The ¹³C urea breath test is a noninvasive test with a high specificity and sensitivity for *H pylori* colonization. Increasingly, it is becoming an important tool for use in diagnosing *H pylori* infection in pediatric populations. This test is particularly well suited for epidemiological studies evaluating reinfection rates, spontaneous clearance of infection and eradication rates after therapy. However, few groups have validated the test in the pediatric age group. The testing protocol has not yet been standardized. Variables include fasting state, dose of urea labelled with ¹³C, delta cutoff level of ¹³C carbon dioxide, choice of test meal and timing of collection of expired breath samples. Further studies are urgently needed to evaluate critically the impact of *H pylori* infection in children. The ¹³C urea breath test should prove very useful in such prospective studies.

Key Words: ¹³C urea breath test, *Helicobacter pylori*, Pediatrics

Test respiratoire à l'urée marquée par le ¹³C pour diagnostiquer une infection à *Helicobacter pylori* chez les enfants

RÉSUMÉ : La rapport de causalité entre la colonisation de la muqueuse gastrique avec *Helicobacter pylori* et la gastrite a été prouvé. L'endoscopie suivie d'un examen histologique des biopsies de l'antré sont considérés comme la méthode de référence pour diagnostiquer la gastrite à *H. pylori*. Le test respiratoire à l'urée marquée par le ¹³C est un test non effractif démontrant une spécificité et une sensibilité élevées pour la colonisation à *H. pylori*. Ce test est un outil de plus en plus important pour poser un diagnostic de colonisation à *H. pylori* dans la population pédiatrique. Il est particulièrement bien adapté aux études épidémiologiques évaluant les taux de réinfection, la disparition spontanée de l'infection et les taux d'éradication après un traitement. Cependant, peu de chercheurs ont validité le test chez les enfants. Le protocole d'application du test n'est toujours pas normalisé. Les variables comprennent l'état de jeûne, la dose d'urée marquée par le ¹³C, le seuil delta du gaz carbonique marqué par le ¹³C, le choix du repas du test et le moment du recueil des échantillons de gaz expiré. Il faut de toute urgence mener d'autres études pour évaluer de façon critique l'impact de l'infection à *H. pylori* chez les enfants. Le test respiratoire à l'urée marquée par le ¹³C devrait s'avérer très utile dans ces études prospectives.

The causal relationship between *Helicobacter pylori* colonization of the gastric mucosa and gastritis is well accepted. Endoscopy and subsequent histological examination of antral biopsies have been regarded as the gold standard for diagnosing *H pylori* gastritis. The ¹³C urea breath test (UBT) is an attractive diagnostic alternative that offers numerous advantages. It is highly reliable, safe, nonradioactive, noninvasive and independent of expired air volumes.

The ¹³C urea breath test is based on the production of urease by the organism. In 1954, Kornberg et al (1) showed that antibiotics destroy gastric urease in cats and thereby concluded that urease was bacterial in origin. In 1983, Warren and Marshall (2) discovered *H pylori* in humans. In 1987,

Graham et al (3) described the first breath test specifically designed to detect *H pylori* by using urea labelled with the stable isotope ¹³C. The principle of the ¹³C UBT is very simple. When *H pylori* is present in the stomach, relatively large amounts of urease are produced. This enzyme then hydrolyzes labelled urea given orally, thereby liberating ¹³C carbon dioxide in the bloodstream, which is then recovered in expired air.

As a noninvasive test, the ¹³C UBT is ideally suited for use in the pediatric age group. We and others have shown the ¹³C UBT to have a high specificity and sensitivity in school aged children and adolescents (4). The ¹³C UBT is well suited for use in epidemiological studies of *H pylori* in-

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TABLE 1
Comparison of ^{13}C urea breath test validation studies in children and adolescents

Authors (reference)	Sensitivity/specificity	Test meal	Gold standard	^{13}C urea dose (mg)	Cutoff level ($\delta^{13}\text{C}\text{‰}$)	Fasting	Time sample collection (mins)
Rowland et al (19)	100%/97.6%	None	Culture of biopsy or histology	50<50 kg 75>50 kg	3.5	>2 h	30
Kalach et al (27)	100%/98.3%	Citric acid	Culture of biopsy	75	3.44	Overnight	40
Vandenplas et al (24)	96%/93%	Ice cream	Culture of biopsy	2/kg (100 mg maximum)	2.5	≥ 6 h	10, 20, 40, 60
Kato et al (25)	100%/96.3%	None	Culture of biopsy or histology	3/kg (100 mg maximum)	4.5	>2 h	10, 20, 30, 40
Delvin et al (4)	100%/100%	Citric acid	Histology	50<15 kg 75>15 kg	3.0	Overnight	30

$\delta^{13}\text{C}\text{‰}$ Delta ^{13}C /million

fection. It may also become an important diagnostic tool in the assessment of children with nonspecific, recurrent abdominal pain.

Most studies that have used the ^{13}C UBT in children have been published since 1997. These studies have evaluated the prevalence of *H pylori* infection in different pediatric populations (5-14) and spontaneous clearance of infection in preschoolers (7,15,16), as well as the success of eradication after treatment (4,17-19).

Few studies have validated the test for use in the pediatric age group (Table 1).

METHODOLOGIC CONSIDERATIONS

Investigators have compared the accuracy of histological examination for *H pylori* with that of ^{13}C UBT. However, the ^{13}C UBT has not been standardized among various study centres. Difference in testing variables include presence of a fasting state, dose of urea labelled with ^{13}C , delta cutoff level of ^{13}C carbon dioxide, use of a variation of test meals to delay gastric emptying and the time of collection of breath samples.

Fasting: The duration of withholding food has varied from 2 h to overnight. Rowland et al (19) demonstrated that eating just before the ^{13}C UBT decreases the sensitivity of the test to only 50%. They also showed that tests carried out after an overnight fast and those undertaken between 1 and 2 h after a meal are both highly sensitive and specific. However, the mean excess of delta ^{13}C carbon dioxide over baseline values is lower in fed than in fasting patients. This finding implies that food in the stomach reduces the interaction between *H pylori* urease and the urea substrate.

Test meal: Food has been used to slow gastric emptying to enable the ^{13}C urea to be in sufficient contact with the urease enzyme of the organism. Tests meals employed have varied from none to citric acid to ice cream. Dominguez-Munoz et al (20) studied different test meals in adults (0.1 N citric acid solution, semiliquid fatty meal and semiliquid meal) and showed 100% specificity of the ^{13}C UBT for all test meals, with identical sensitivities. However, the citric acid solution gave higher ^{13}C carbon dioxide delta peak values. Use of a citric acid test meal increases the separation of

excess delta ^{13}C carbon dioxide values between *H pylori* infected and uninfected patients, thereby making the test more robust, with fewer test results subject to misclassification (4). This benefit must be weighed against the poor taste of citrate, which can reduce compliance, particularly in young children. Patients ingesting a fatty meal have a higher intragastric pH than those taking citric acid. The *H pylori* urease enzyme is likely to be more active at the lower pH obtained with citric acid.

Cutoff values: ^{13}C carbon dioxide is naturally present in expired air. In patients with a positive breath test result, the level of ^{13}C carbon dioxide may increase by only one part/1000. Therefore, the most appropriate technique to measure ^{13}C carbon dioxide is mass spectrometry, which can detect particles of a given molecular weight with extreme precision.

The amount of ^{13}C carbon dioxide expired in the breath varies according to the quantity of ^{12}C carbon dioxide expired. Results are generally expressed, therefore, as a ratio of ^{13}C to ^{12}C . The amount of ^{13}C carbon dioxide in breath varies according to the subject's diet. Individuals eating a ^{13}C -rich diet (as contained in corn and other maize products) have a higher level of basal ^{13}C carbon dioxide excretion. The measurement of ^{13}C enrichment based on the ^{13}C to ^{12}C ratio is independent of the volume of expired air. In principle, it is, therefore, a much more reliable test in pediatric patients than is the ^{14}C UBT, which depends on expired air volume in a fixed time frame (21,22).

Results of the ^{13}C UBT are given as ^{13}C enrichment, which is expressed as the delta ^{13}C /million ($\delta^{13}\text{C}\text{‰}$), or delta ^{13}C carbon dioxide excretion/million at 30 mins compared with delta ^{13}C carbon dioxide excretion at baseline:

$$\delta^{13}\text{C}\text{‰} = \frac{{}^{13}\text{C}/{}^{12}\text{C sample} - {}^{13}\text{C}/{}^{12}\text{C PDB} \times 10^3}{{}^{13}\text{C}/{}^{12}\text{C PDB}}$$

where PDB is the Pee Dee Belemnite unit.

Mion et al (23) defined a cutoff value of 3 $\delta^{13}\text{C}\text{‰}$. We used the same cutoff level (4) and found it to be both highly specific and highly sensitive (100%). Vandenplas et al (24) reported a false positive rate of 17% using a cutoff level of 2.5 $\delta^{13}\text{C}\text{‰}$, whereas Rowland et al (19) observed 100% sen-

sitivity and 97.6% specificity at 30 mins, using a cutoff level of 3.5 $\delta^{13}\text{C}\%$.

Collection of samples: The time at which breath samples are collected after ingestion of ¹³C urea varies among the published studies. Rowland et al (19) found that samples obtained at 15 mins are less specific than those performed at 30 mins. This difference probably occurs because of the interference with the test by urease-producing bacteria in the oral cavity. Similarly, if specimens are obtained at 60 mins after ingestion, there is a significant loss in specificity (19).

ACCURACY OF ¹³C UBT

The ¹³C UBT has not been standardized among the various pediatric studies. Table 1 compares the differences in sensitivities and specificities reported to date.

Specificity and sensitivity were high in each study. Vandenplas et al (24) obtained a higher rate of false-positive results (17%) when using a cutoff value of 2.5 $\delta^{13}\text{C}\%$ and culture of the organism as the gold standard. The high number of false-positive results suggests that the cutoff value of 2.5 $\delta^{13}\text{C}\%$ employed is too low.

Test meal: Rowland et al (19) found that a test meal is not necessary in children. Six infected children were given ice cream and a cola drink immediately before the ¹³C UBT. There were three false negative results at 30 mins when a cutoff value of 3.5 $\delta^{13}\text{C}\%$ was used. Vandenplas et al (24) also reported reduced sensitivity of the test when using ice cream as a test meal.

Fasting: Interestingly, Rowland et al (19) showed no differences in sensitivity and specificity between ¹³C UBT carried out after an overnight fast and tests undertaken between 1 and 2 h after ingestion of a test meal.

Reinfection rate: Using the ¹³C UBT, Kato et al (25) studied the rate of reinfection after eradication therapy against *H pylori*. Twenty-three successfully treated patients (biopsy negative one to two months after the completion of eradication therapy) remained free of infection 12 to 19 months after therapy. Thereafter, the reinfection rate was 2.4%/patient-year. In contrast, Rowland et al (26) have shown

that reinfection is rare in children older than five years of age (1.35%/patient-year), whereas it occurs frequently in pre-school children under five years of age (60.6%/patient-year).

SUMMARY

The ¹³C UBT is very attractive as a diagnostic test because it is highly accurate, easy to perform, noninvasive and non-radioactive. Unfortunately, it cannot be performed in many centres because mass spectrometry is not widely available. However, the stability of ¹³C carbon dioxide offers the advantage of being able to send breath samples over long distances. A 12-month delay in delivery of a sample to the laboratory does not modify the results of the ¹³C UBT (unpublished data, JL Brazier). Extremes in temperature in either the summer or winter also do not influence test results (unpublished data, JL Brazier). Ten replicates can be measured from one 10 mL breath sample collected into a vacuum tube. However, standardization of the ¹³C UBT among reference laboratories is necessary and should become an important priority.

In a recent pediatric study (4), 12 (15%) of 79 patients tested were infected with *H pylori*, as shown by both the ¹³C UBT and histological examination. Among these 12 children, nine were first-generation Canadians born to immigrant parents, eight of 12 received triple therapy and seven of these were retested by ¹³C UBT one month or more after treatment. At reassessment, four of seven were ¹³C UBT-negative after therapy – findings comparable with those reported by Rowland et al (19). Thus, the ¹³C UBT may be used for the diagnosis of *H pylori* infection, as well as for the evaluation of eradication after therapy.

CONCLUSIONS

Further study is needed to evaluate the impact of *H pylori* infection in children. The long term results of treatment remain uncertain, as do the epidemiological aspects of this infection in various pediatric populations. The ¹³C UBT will prove to be very helpful as an accurate and reliable diagnostic tool in these prospective studies.

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