Urea breath tests in the management of Helicobacter pylori infection

R P H Logan

Summary
The $^{13}$C-Urea breath test (UBT) is based on the simple principle that a solution of isotope-labelled urea will be rapidly hydrolysed by the abundantly expressed urease of *H pylori*. The released $^{13}$CO$_2$ is absorbed across the mucus layer to the gastric mucosa and hence, via the systemic circulation, excreted in the expired breath. Distribution of urea throughout the stomach prevents sampling error and allows semiquantitative assessments of the extent of *H pylori* infection.

Originally the $^{13}$C-UBT was complex, cumbersome and costly but, by simplifying the protocol and reducing the number of samples to be analysed, is now a much easier, quicker and cheaper test for detecting *H pylori*. Although mass spectrometry is needed for analysis of exhaled $^{13}$CO$_2$, the use of the stable isotope, which is completely safe, provides advantages over the $^{13}$C-UBT using radioactive $^{13}$C-urea, such that it can be used in women and children and a user's licence is not required. The widespread availability of scintigraphy for $^{14}$C-UBT using radioactive $^{14}$C-urea, which is completely safe, provides advantages that it can be used in women and children and a user's licence is not required. The widespread availability of scintigraphy for $^{14}$C-UBT using radioactive $^{14}$C-urea, which is completely safe, provides advantages that it can be used in women and children and a user's licence is not required. The widespread availability of scintigraphy for $^{14}$C-UBT using radioactive $^{14}$C-urea, which is completely safe, provides advantages that it can be used in women and children and a user's licence is not required. The widespread availability of scintigraphy for $^{14}$C-UBT using radioactive $^{14}$C-urea, which is completely safe, provides advantages that it can be used in women and children and a user's licence is not required. The widespread availability of scintigraphy for $^{14}$C-UBT using radioactive $^{14}$C-urea, which is completely safe, provides advantages that it can be used in women and children and a user's licence is not required. The widespread availability of scintigraphy for $^{14}$C-UBT using radioactive $^{14}$C-urea, which is completely safe, provides advantages.

Prior to the first description of the $^{13}$C-UBT, the diagnosis of *H pylori* infection had usually been established by histology, culture or biopsy urease test or non-invasively by serology. Detection of *H pylori* by ELISA serology reflects only previous exposure to *H pylori* and may not indicate active or current infection. In addition because antibody titres can take up to six months to fall after successful treatment, ELISA tests cannot readily be used to assess the efficacy of new treatment regimens or novel antimicrobial agents specifically developed for the treatment of *H pylori* infection. The development of the $^{14}$C-UBT as a simple, practical, highly accurate non-invasive test for *H pylori* infection is reflected in its increasingly important role in the management of dyspepsia. This review will consider some of the recent developments in the methodology and application of the $^{13}$C-UBT.

Principles of the $^{13}$C-UBT
The $^{13}$C-UBT exploits the copious amounts of urease produced by *H pylori* which hydrolyses urea to form ammonia and soluble carbon dioxide which is expired in the exhaled breath. Labelling of urea with either isotope allows the $^{13}$CO$_2$ to be detected in the expired breath. $^{13}$C is always measured as a ratio of $^{12}$C to $^{13}$C ($^{12}$C/$^{13}$C per mil), therefore the amount of excreted CO$_2$ does not need to be measured and a 10 ml tube of expired air suffices for analysis. When *H pylori* is present, the relative amount of $^{13}$CO$_2$ increases considerably, and often exceeds that in the calibration standard, which is why results of the $^{13}$C-UBT are expressed as the "excess" $^{12}$C/$^{13}$C per mil.

$^{14}$CO$_2$ is measured using scintigraphy which is simple and relatively cheap but may be more inconvenient, especially since its use is restricted in some European countries if stable isotopic equivalents are available. Although the radiation exposure of a single breath test is equivalent to only one day's background dose, patients must still have the risks explained. Some patients with chronic lung disease may be unable to provide sufficient CO$_2$ to change the colour of the trapping solution, in which case the $^{13}$C-UBT provides a useful alternative.

Most of the validation studies and assessments of new methodologies for the UBT have been done using $^{13}$C-UBT, although some reports have also studied the $^{14}$C-UBT.

Introduction
Since the identification and subsequent isolation of *H pylori* in 1983 a considerable body of evidence has accumulated showing that *H pylori* is the principal cause of non-autoimmune gastritis and peptic ulcer. By 1994, the evidence was sufficiently strong for the National Institutes of Health (NIH) consensus conference in the United States to recommend eradication of *H pylori* infection to prevent ulcer recurrence in all patients with documented peptic ulcer disease. The observation that eradication of *H pylori* cures peptic ulcer disease and that screening for *H pylori* infection may improve the diagnostic yield of endoscopy is leading to a major re-evaluation of the optimal management of patients with dyspepsia.

Both tests are easy to perform with minimal opportunity for observer variation or methodological error; they are very sensitive and specific tests and provide a clinical "gold standard" against which the accuracy of other methods can be validated. The $^{13/14}$C-UBT exploits the copious amounts of urease produced by *H pylori* which hydrolyses urea to form ammonia and soluble carbon dioxide which is expired in the exhaled breath. Labelling of urea with either isotope allows the $^{13}$CO$_2$ to be detected in the expired breath. $^{13}$C is always measured as a ratio of $^{12}$C to $^{13}$C ($^{12}$C/$^{13}$C per mil), therefore the amount of excreted CO$_2$ does not need to be measured and a 10 ml tube of expired air suffices for analysis. When *H pylori* is present, the relative amount of $^{13}$CO$_2$ increases considerably, and often exceeds that in the calibration standard, which is why results of the $^{13}$C-UBT are expressed as the "excess" $^{12}$C/$^{13}$C per mil. $^{14}$CO$_2$ is measured using scintigraphy which is simple and relatively cheap but may be more inconvenient, especially since its use is restricted in some European countries if stable isotopic equivalents are available. Although the radiation exposure of a single breath test is equivalent to only one day's background dose, patients must still have the risks explained. Some patients with chronic lung disease may be unable to provide sufficient CO$_2$ to change the colour of the trapping solution, in which case the $^{13}$C-UBT provides a useful alternative. Most of the validation studies and assessments of new methodologies for the UBT have been done using $^{13}$C-UBT, although some reports have also studied the $^{14}$C-UBT.
Methodology

PATIENT RELATED FACTORS

Urease producing oropharyngeal bacteria may rarely cause false positive results if breath samples are taken within 10 minutes of urea administration; false negative $^{13}$C-UBT results will arise if tests are done in patients taking antibiotics, bismuth salts or more rarely taking proton pump inhibitors or sucralfate (which reduce the extent of antral H pylori infection). Patients should stop taking these drugs at least 10 days before undergoing a UBT.

Many $^{13}$C-UBT protocols require patients to fast for at least four hours but recently several groups have shown that fasting is no longer necessary if using the $^{13}$C-UBT and a protocol which includes a test meal. 5-7

UBT RELATED FACTORS

Test meal

The test meal delays gastric emptying to maximise the gastric residence time, and exposure of the organism, to labelled urea, thereby allowing the dose of isotope to be reduced and increasing the sensitivity of the test. Citric acid, which delays gastric emptying via pH, or nutrition supplements (for example, Pulmicore, or Calogen), which delay gastric emptying via lipid content, are often used as test meals for the UBT.

A citric acid test meal may decrease oral urease activity but at the same time increase the discrimination between positive and negative $^{13}$C-UBT values.8 9 The explanation for the increased excess $^{13}$CO$_2$ excretion in infected patients with citric acid is unclear. However the additional source of [H$^+$] contained in citric acid may enhance the extent of urea by providing a supplementary source of [H$^+$] for the generation of carbamate and ammonium.

A recent study suggested that citric acid, by increasing the extent of excess $^{13}$CO$_2$ excretion, was the optimal test meal for the $^{13}$C-UBT, but no results from uninfected patients were presented.10 In addition, the extent to which citric acid increased the excess $^{13}$CO$_2$ excretion was difficult to judge as the breath test values are reported as proportional values above control (baseline) excess $^{13}$CO$_2$ values. However any test meal which is proved to delay gastric emptying can be used providing it is palatable (the poor palatability of citric acid improves with orange juice), cheap, has a long shelf half-life, does not impair the dispersion of $^{13}$C-urea or contribute in itself to excess $^{13}$CO$_2$ excretion.

Quantity and formulation of urea

Although the amount of $^{13}$C-urea now used is less than half that originally proposed by Graham, the quantity of substrate must be sufficient to saturate the enzyme. The specific activity of $H$ pylori urease is similar to that of other bacteria but is produced over-abundantly and has a high binding affinity for urea.11 With purified $^{13}$C-urea, the urease is fully saturated at the dose of $^{13}$C-urea (100 mg) currently used, in contrast to $^{15}$C-urea which should be used with cold urea to ensure full saturation of the enzyme. Lower doses of $^{13}$C-urea (75 mg) have been incorporated into several commercial kits (many which may have been incompletely validated) but may be associated with false negative results (Perri F, personal communication).

Without a test meal, $^{13}$C-urea in solution rapidly empties from the stomach, often within 10 minutes and breath samples taken after this initial period may give false negative results.

Recent reports have shown that a test meal is not needed if $^{13}$C-urea is given in a capsule specially coated to disintegrate within minutes of entering the stomach. Consequently there is no $^{13}$C-urea hydrolysis by oropharyngeal urease, so that in uninfected patients, the small increase in excess $^{13}$CO$_2$ excretion normally seen after swallowing a solution of $^{13}$C-urea, is almost completely abolished.12 This may improve the accuracy of the test by reducing levels of excess $^{13}$CO$_2$ excretion in uninfected patients to almost zero. In addition without the hydrolysis from oropharyngeal urease, breath samples may be taken sooner, thereby reducing the time taken to perform the test. It is also possible that this approach will allow the quantity of isotope to be lowered, further reducing the overall cost of the test.

Measurement of $^{13}$CO$_2$ and analysis of results

The major disadvantage to the $^{13}$C-UBT is the cost of $^{13}$CO$_2$ analysis. Without the economies of scale and volume, purchase of a gas chromatography/isotope ratio mass spectrometry (GC/IRMS) unit is generally uneconomical, but samples can be sent for analysis by post to a commercial stable isotope laboratory. Although the cost of $^{13}$C detection by GC/IRMS has been falling, it is still the major factor limiting more widespread use of the $^{13}$C-UBT. Several alternative methods for the detection of $^{13}$CO$_2$ have recently been described, including the use of laser or infra-red spectroscopy.13-15 These new technologies are still at an early stage of development and studies of their accuracy are limited, but they promise to enhance considerably the use of the $^{13}$C-UBT, so that it can be done in most hospitals or specialist clinics.

The cut off value for the $^{13}$C-UBT was originally determined as 5.0 per mil based on the normal distribution of excess $^{13}$CO$_2$ values for $H$ pylori negative subjects who have never been infected. However, a recent re-analysis of data from several large clinical trials of $H$ pylori eradication have allowed construction of receiver operator characteristic curves (ROCs) to set the optimum cut off value according to sensitivity and specificity. On the basis of ROC analysis, a cut off value of 3.5 per mil excess $^{13}$CO$_2$ improves the sensitivity and specificity to 98.5% and 97.0%, respectively, although in clinical practice, and in contrast to the $^{13}$C-UBT, less than 0.1% of $^{13}$C-UBT results fall between 3.5–5 per mil.16 17 The accuracy of the $^{13}$C-UBT is supported by well designed studies which have shown the $^{13}$C-UBT to have a sensitivity and specificity of >95%. Less encouraging results reported in early studies
are likely to have arisen from the poor sensitivity of the endoscopic biopsy tests to which the 13C-UBT was compared. False positive results with the 13C-UBT are extremely rare and if found with an excess of 13CO2 excretion of >10 per mil should prompt a repeat assessment of patient’s status either at endoscopy or by repeating the 13C-UBT.

Applications of the 13C/14C-UBT
Both the 13C-UBT and 14C-UBT can be used to screen dyspeptic patients prior to endoscopy and to assess the efficacy of H pylori eradication therapy. However the 13C-UBT can also be used to detect infection in children, to measure suppression and clearance of infection in phase I and II trials, for epidemiological research and as a near patient test in primary care.

Screening before Endoscopy
Several studies have suggested that non-invasive tests for H pylori can be used to screen young dyspeptic patients prior to endoscopy, although the most appropriate subsequent management strategy for these patients is as yet unclear and may vary from country to country.1 10 11 However a study from Glasgow which used the 13C-UBT to screen dyspeptic patients, found the prevalence of peptic ulcer in infected patients was 59% with a positive predictive value high enough for it alone to be recommended as a screening test for ulcer disease.20

Assessing Eradication of Infection
The 13C-UBT is the best method of following patients in whom eradication of H pylori is being attempted. The test can clearly identify patients successfully treated and detect those in whom treatment has not been successful more easily and at an earlier stage than other tests for H pylori. Because eradication of H pylori is associated with resolution of histological gastritis and prevention of relapse of duodenal ulcer, the 13C-UBT can be used as the sole method of follow up.

Using well validated protocols the sensitivity and specificity of the 13C-UBT 4–6 weeks after the end of therapy has been found to be >95% and 96% respectively. There are fewer longer follow up studies, although, those using the European Standard 13C-UBT protocol have shown >99% concordance between a 13C-UBT and biopsy based tests at one year after treatment.21 Unfortunately other 13C-UBT studies from the United States with apparently minor protocol variations resulted in poorer sensitivity and specificity values when assessing eradication.

Applications of the 14C-UBT
Assessing Suppression and Recurrence of H pylori Infection
The 14C-UBT, as a semiquantitative measure of H pylori, can be used to monitor the suppression of H pylori by the fall in excretion of 14CO2. In single dose or short term treatment studies the extent of suppression can allow differences between anti-H pylori drugs to be rapidly and easily assessed. By performing serial 14C-UBTs the rate of recurrence of H pylori infection after different treatments can also be determined—for example, showing that, regardless of the preceding length of treatment with bismuth, H pylori infection recurs within days of finishing therapy.22

13C-UBT in Children
The 13C-UBT is ideally suited for the determination of H pylori status in children, although until very recently there was a paucity of adequate data to validate its routine use.23 24 As the endogenous CO2 excretion by small children is much less than in adults, less urea is required and for children below the age of 8. Adult breath sampling is feasible for children over 3 years, but a mask may be required to collect expired breath samples from younger children.

Epidemiological Research
A very important advantage of the 13C-UBT over the 14C-UBT is that it can be used for epidemiological studies, particularly in children, where in contrast to serological methods, the 13C-UBT detects active infection, rather than previous exposure. Recently several studies have been performed using the 13C-UBT in an attempt to document possible routes of transmission of H pylori between children.25 26

Conclusions
The 13C-UBT and 14C-UBT are very accurate tests for detecting H pylori infection with a sensitivity and specificity better than many other tests for H pylori. The 13C-UBT detects much lower levels of H pylori infection and by assessing the entire gastric mucosa avoids the risks of sampling error. The 13C-UBT is a practical and readily available test providing a “gold standard” against which other tests for H pylori can be compared. The recent development of encapsulated 13C urea and cheaper methods of 13CO2 analysis allow the duration of the test and costs to be reduced, and may herald a more widespread clinical application of this useful test.


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Notes