Urea breath test : a diagnostic tool in the management of *Helicobacter pylori*-related gastrointestinal diseases

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Abstract

The urea breath test (UBT) is generally considered as a simple, non-invasive and accurate test to demonstrate *Helicobacter pylori* (*H. pylori*) infection. The principle of the test is simple. The orally given urea, isotopically labelled with ^{14}C or ^{13}C, is hydrolysed by the enzyme urease of *H. pylori* and $*CO_2$ is expired in breath. Although the radiation exposure is negligible ($3*10^{-6}$ Sv), the test with the stable isotope ^{13}C should be preferred. Since the first description of the test in 1987 many refinements have been described. Most studies reported sensitivity and specificity figures between 95-100% for both. A uniform test protocol with regard to the test meal, the appropriate ^{13}C-urea dose, the number of breath samples to be taken, ... would be ideal. But today, it is better to strive for a validation and a determination of cut off values for each protocol as such.

The main indication for UBT is the confirmation of successful eradication. To avoid false negative results, testing should be performed 4 to 6 weeks after the end of treatment and 5 days after the end of acid suppressive drugs. The test is also an ideal tool to check for infection when an ulcer is found at endoscopy, but biopsy specimens can't be taken because of anticoagulant treatment. Mostly serology is the first choice to perform epidemiological studies, but UBT is a good alternative and moreover it gives an idea of the presence of active infection.

The role of non-invasive tests, i.e. UBT and serology, in primary diagnosis of *H. pylori* is more controversial. Questions such as who will perform the test (general practitioner or gastroenterologist), what is the age limit, how to organise the follow up, what is the cost-benefit, ... still remain. All these questions need a further evaluation in terms of its influence upon clinical decision making not only in general, but also more specific for the Belgian situation.

***In conclusion* :**

1. **The ^{13}C-urea breath test is a very accurate, non-invasive test to diagnose gastric *H. pylori* colonisation in adults and children.**
2. **If local protocols are validated and appropriate cut off values are determined, general standardisation of methodology isn't necessary.**
3. **The ^{13}C-urea breath test is the ideal diagnostic tool to monitor eradication therapy in patients with complicated duodenal ulcers, gastric ulcers, Malt lymphomas, poor compliance and to perform large epidemiological studies.**
4. **The role of the ^{13}C-urea breath test in the clinical decision making prior endoscopy remains controversial.**

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Key words : *Helicobacter pylori*, *C-UBT.

Introduction

The principles of the $^{14}CO_2$ breath test were introduced by Schwabe *et al.* (1) in the 1960s, when large quantities of substrates with radioactive isotopes became available, including ^{14}C, and measuring facilities were developed. Breath tests were greatly simplified by Abt and von Schuching (2) who showed that man in rest produces roughly constant amount of CO_2 per unit of time, so that interval breath sampling can be used for estimating $^{14}CO_2$ output. In 1973 Lacroix *et al.* (3) introduced the use of naturally ^{13}C-enriched enriched substrates in breath test technology. An example of breath test technology was the urea breath test (*C-UBT) reported by Graham *et al.* (4) in 1987. This test was designed to identify the presence of urease activity in the gastrointestinal tract. *Helicobacter pylori* (*H. pylori*) is a potent urease-producing gastric pathogen, and therefore a positive urea breath test can generally be equated with the presence of an *H. pylori* infection.

All breath tests are based on the administration of a substrate with a functional group containing a carbon atom with either the radioactive (^{14}C) or the stable isotope (^{13}C) of carbon. The functional group is enzymatically cleaved and the cleaved portion undergoes further metabolism to $^{14}CO_2$ or $^{13}CO_2$. Applied to the urea breath test the principle is that, in the presence of the enzyme urease, orally administered urea is hydrolysed to CO_2 and ammonia. If urea is labelled with either radioactive ^{14}C or the stable isotope ^{13}C, it can be detected in the breath as labelled CO_2. In this way, the $^{14/13}CO_2$ excretion is a very reliable parameter to demonstrate the presence of *H. pylori* in the stomach, given urease relates to the rate limiting step in the whole process.

Between 1987 and now, 10 years after the first report, an increasing amount of papers have been published on methodological aspects of *C-urea breath tests. An update seems necessary.

Generally, the *C-UBT is accepted as a reliable means of assessing *H. pylori* eradication in adults and children. It can also be used for epidemiological studies to diagnose *H. pylori* colonisation in different age groups. In this paper we will focus on the indications to assess eradication and the possible role of *C-UBT in the primary management of dyspepsia.

An update on *C-UBT methodology

Helicobacter pylori infection is associated with a wide range of gastroduodenal diseases. Since its identification in 1982 (5), overwhelming evidence has implicated *Helicobacter pylori* as an etiologic component of chronic gastritis, peptic ulcer, and gastric cancer. It has been regarded by the International Agency for Research on Cancer Working Group of the World

Health Organisation as a definite human carcinogen. Therefore there is need for simple and accurate diagnostic tests. Most methods used to diagnose *H. pylori* infection require endoscopy and sampling of the gastric mucosa. Invasive tests such as : the urease test, culture, histology, polymerase chain reaction and molecular typing are available. The initial gold standard for the diagnosis of *H. pylori* infection has been histological examination, culture, or both. However, since the report of Graham (4), the number of papers on the accuracy of the UBT as a clinical diagnostic test and as a research tool are increasing. Cutler *et al.* (6) determined and compared the accuracy of invasive and non-invasive tests. Sensitivity and specificity of *C-UBT was respectively 90.2 and 95.8%. The test showed also to be as accurate in predicting *H. pylori* status in untreated patients as the invasive CLO-test and Warthin-Starry stain. Another recent study validated the test in clinical practice both before and after treatment (7). Comparison of diagnostic performance against histology showed an accuracy of respectively 94.8 and 95.2%. Home-based testing resulted in a decrease of specificity and sensitivity of 5% (8). Still both remained above 90%. Also in children, the ^{13}C-UBT is highly accurate for the detection of *H. pylori* infection (9).

Urea can be labelled with the radioactive isotope (^{14}C) or with the stable isotope (^{13}C). The ^{14}C-UBT can be done in any hospital that has a nuclear medicine department. It requires liquid scintillation counting for analysis, a technique that is generally available. The cost of the test is low and ^{14}C is a not naturally occurring isotope. To reduce dose and to overcome the problem of false positive results due to mouth contamination, a microdose version of the test was recently proposed (10). Thirty seven kBq is given orally in capsule. This modified version is highly reliable and reproducible for detection of *H. pylori* infection. A major drawback of the test, however, is its radioactivity. ^{14}C is an isotope with a very long half-life. Although, the dose in a typical test is only 185 kBq and exposure is roughly equivalent to one day's background radiation, repetition of the test is rather debatable. It is also considered unethical to administer radioactive substrates to children and pregnant women. Moreover, there is the problem of long-term storage of radioactive waste and the fact that the test is only possible in hospital.

The major advantage of the use of stable isotopes over radioactive tests is safety. The ^{13}C-urea breath test can be used repeatedly, at home and also in children and pregnant women. ^{13}C is a stable, non radioactive isotope which occurs naturally. Due to this background level of ^{13}C, changes in ^{13}C enrichment of the breath CO_2 should be large enough to be detected by appropriate instrumentation. Isotope-ratio-mass spectrometer (IRMS) is the standard, but expensive equipment to detect minimal differences in ^{13}C enrichment of CO_2 (11). This high precision is not necessarily required for the urea breath test, but is indispensable for the analysis of other ^{13}C breath tests when multiple breath samples have to be analysed.

To reduce the costs in test execution, centralisation of analysis has to be considered. It is know that storage has no effect on the results (7) and test tubes can be sent by mail to the central laboratory. An alternative is the use of the simple optical method called isotope-selective non-dispersive infrared spectrometry (NDIRS) to measure $^{13}CO_2/^{12}CO_2$ ratios. It gives on-line results and is easier and cheaper than IRMS (12). Recently, a Laser Assisted Ratio Analyser (LARA) was evaluated and showed a good accuracy (13).

In clinical practice and for research, the ^{13}C-urea breath test is recommended. If not available, the microdose version of the ^{14}C-urea breath test seems to be a good alternative.

A huge amount of modifications on methodology were published since the original paper of Graham (4). Everybody use his own protocol and comparison of results is rather difficult. At the 10th anniversary, one can question if something fundamental has changed and which problems we are dealing with today.

Initially a dose of 5 mg ^{13}C labelled urea per kg body weight was used to perform the *C-UBT (4). Stable isotopes are expensive, therefore one should use the lowest adequate dose. Eggers *et al.* determined the lower range of substrate to approximately 1 mg/kg, and they recommended 75 mg (14). It has to be stressed that different doses can have an influence on the optimum cut-off value of the test (7). Reassessment is necessary when changing doses and test conditions.

If labelled urea is given in non-nutrient liquids, it comes into contact with the stomach for only a short time. This rapid emptying might cause false-negative results. A test meal is given to delay gastric emptying and to increase the contact time between ^{13}C-urea and the gastric mucosa. Several test meals such as Nutridrink®, Ensure®, full cream milk, 10% glucose polymer solution, 0.1 N citric acid solution, ... are currently used. It has been shown that the composition of the test meal is of no influence. Important is that gastric emptying is delayed in a reasonable way.

False positive results are especially likely in patients with achlorhydria and those on long-term Omeprazole therapy (15). Probably due to colonisation of the stomach by urease-containing bacteria normally not present in the stomach. Also urease-producing bacteria present in the oropharynx may yield false-positive results. This can be markedly reduced or eliminated by prestudy cleansing with toothpaste (16) or citric acid (14). In practice, contamination of the buccal cavity is of minor importance if the test substrate is administered in the middle of the test meal. It covers the surface of the cavity by a small liquid protective layer, and the urea is completely rinsed out of the mouth by the second half of the meal. Also the shape of the excretion curve (% dose/hour) is very informa-

tive. A very quick peak (< 15 min) indicates oral urease activity. Another possibility to overcome this problem of urea hydrolysis in the oropharynx and to eliminate the need of a test meal, is a modified breath test which has recently been proposed (17). ^{14}C-urea is supplied in a gelatine capsule without test meal and it releases his content in the stomach. Diagnostic reliability of the test, based on a single breath sample 10 min after capsule intake, was 99.8%.

The expression of the results of the ^{13}C-urea breath test varies widely. Mostly delta per mille over baseline (DOB) is used. It represents the difference between the δ-value at any sampling tune and the baseline δ-value at time 0. Commonly a test is regarded positive if the highest excess $\delta^{13}CO_2/^{12}CO_2$ was above 5 (8) or 6 (18) ‰. However, an excretion curve has to be constructed in order to identify the peak. The data of Thijs *et al.* showed that when a single sample technique is used, the sample should be taken at least 50 min after dosing to maximise sensitivity (8). At our laboratory, the duration of the ^{13}C-urea breath is 60 min and samples are taken every 15 min. The test dose is 75 mg ^{13}C-urea in adults and it is administered with 200 ml of a liquid meal (Nutridrink®, Nutricia). It has been shown that DOB-values higher than 3.5 at 30 min after substrate intake can be considered as cut off value. Nevertheless we still continue to take samples over a one hour period to have a control on mouth contamination, to be sure of the 30 min-value, and to be independent of the dose administered (which is of importance in the children's hospital). The analytical data are also expressed as percentages of $^{13}CO_2$ recovery per hour of the administered dose at 15, 30, 45 and 60 minutes. By trapezoidal rule, the percentages of $^{13}CO_2$ cumulative values (% CD) are calculated for the different time points. This way of expression takes into account the basal CO_2 output, is independent of dose and has a more physiological meaning.

In 1991, Logan *et al.* (19) proposed a standardised European protocol to perform urea breath testing. Today, one can state it's more realistic to validate each methodology, than to create a uniform UBT protocol.

Clinical indications

The urea breath test can be considered as the "gold standard" to evaluate *H. pylori* eradication therapy. Klein *et al.* (7) reported an accuracy of 95% post-eradication treatment. However, reliable assessment can only be done after 4 to 6 weeks after eradication (20). Recently, two studies (21,22) focused on the effect of gastric acid suppressive drugs on accuracy of UBT. If the test was performed during therapy with Lanzoprazole, Omeprazole or high dose Ranitidine, respectively 61%, 38.5% and 18% of false negative results were obtained. The medication effect resolved within 5 days of drug cessation and is probably induced by the suppressive effect of these agents on gastric acid secretion.

Control of eradication is necessary in patients with a complicated peptic ulcer disease, a gastric ulcer or after treatment for a low grade Malt Lymphoma. In patients who were adequately treated for an uncomplicated peptic ulcer, it is generally accepted that confirmation of their eradication isn't obligatory if they are asymptomatic post-treatment. This attitude is mainly based on the high incidence of *H. pylori* infection (95%) and the good therapeutic success which achieves eradication rates between 80 to 90% in these patients. On the other hand, in patients with complicated ulcers, i.e. bleeding or perforation, confirmation is necessary. In these patients, adequate eradication reduces significantly the risk of rebleeding (23,24). In benign gastric ulcer disease, the relation with *H. pylori* (70-80%) isn't as strong as in duodenal ulcers. Therefore, before starting eradication a good history on non-steroidal antiinflammatory drugs must be taken and patients must be tested for *H. pylori.* In general, these patients underwent a second endoscopy after 4 to 8 weeks to confirm ulcer healing and to exclude malignancy. However, recent studies showed that a single endoscopy including careful inspection and expert pathologic interpretation of adequate, multiple biopsies, will detect greater than 98% of cancers. Malt lymphomas must be treated in specialised centres. If eradication is performed in these patients, success can be checked after 4 to 6 weeks by UBT. However, the disease as such needs strict follow up by endoscopy, echoendoscopy and histology.

Although serology is regarded "gold-standard" to perform epidemiology studies on *H.* infection, it is possible to study the prevalence of active infection in large populations (25).

Today ; the point of discussion is whether non-invasive tests can be used in primary care setting as diagnostic tool in dyspeptic patients. Last year an algorithm has been recommended by the Maastricht Consensus Report (26). If this algorithm gets validated and if there is enough scientific evidence to accept it, the UBT is the test of choice to perform primary diagnosis. However, in Belgium the test is too expensive to be performed on a large scale.

Conclusion

The *C-urea breath test is a simple, non-invasive and accurate test to demonstrate *H. pylori* infection. It would be desirable that we could come to an universal standard in test execution, dose, meal, ... In practice, however, so many laboratories already applied *C-UBT under so many different conditions that harmonisation of test execution belongs to the world of wishful thinking. Each clinical centre has right to have its own test protocol on condition that misinterpretation of test results is avoided by evaluating the test practice against other diagnostic methods. The ^{13}C-UBT can be regarded as "gold standard" to evaluate *H. pylori* in adults and children. It's also an interesting tool to perform epide-

miological studies and to have an idea of active infection in different populations. The usefulness and the diagnostic safety of the breath test as a pnmary diagnostic tool needs to be validated before unlimited use in general practice.

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