Clinical aspects in Helicobacter pylori infections

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CHAPTER 6

Post-eradication evaluation of \textit{H. pylori} status:
the $^{13}$C urea Laser Assisted Ratio Analyzer
(LARA$^{TM}$) breath test.
Post-eradication evaluation of \textit{H. pylori} status: the $^{13}$C-urea Laser Assisted Ratio Analyzer (LARA™) breath test

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Submitted

Summary

**Background:** urea breath testing has been shown to be a reliable non-invasive test for diagnosing \textit{H. pylori} infection. Drawbacks of the urea breath tests have been the requirement of expensive and complex instrumentation. A novel, accurate and economical LARA™ system, based on laser optogalvanic spectroscopy, was developed to measure $^{13}$C/$^{12}$C ratios in breath. Relatively few data are available on the reliability of breath testing in the post-eradication setting.

**Aim:** To establish the sensitivity and specificity of the $^{13}$C LARA™ urea breath test in the evaluation of \textit{H. pylori} eradication.

**Methods:** \textit{H. pylori} positive dyspeptic patients received various eradication regimens. Five to seven weeks after cessation of eradication therapy a second endoscopy with 8 biopsies from antrum and corpus for histology, culture and rapid urease test was performed, as well as the LARA™ breath test. Breath samples were taken at 20, 30, 40 and 60 minutes after urea ingestion. The cut off value was set at 6.1 ± 0.6 delta units. In the reference test, \textit{H. pylori} was considered eradicated when culture, histology and CLO were all negative in all biopsies.

**Results:** 181 patients were included. Sensitivity and specificity of the LARA™ urea breath test were 91 and 96%, respectively at 5-10 weeks after eradication therapy, and 100 and 98% at 3-6 months after eradication therapy.

**Conclusion:** The $^{13}$C LARA™ urea breath test is reliable for post-therapy evaluation of \textit{H. pylori} status. This non-invasive method may well become the test of choice for \textit{H. pylori} post-therapy monitoring because of its simplicity, non-invasiveness and reduced cost.

Introduction

The gold standard to detect the presence or absence of \textit{H. pylori} is made by endoscopy and multiple gastric biopsies for histological examination, culture and rapid urease tests; this however carries the disadvantage of being expensive, invasive and time consuming. Detection of \textit{H. pylori} by ELISA serology reflects not only current but also previous exposure to \textit{H. pylori}. In addition
because antibody titers can take up to six months to fall after successful treatment, ELISA tests cannot be used to assess the efficacy of treatment regimens, when used early after therapy. The 13/14-Carbon (C) urea breath test is a non invasive test that has been reported to provide high sensitivity and specificity to detect the presence or absence of an *H. pylori* infection in adults as well as in children.\(^1\) However, only a few data are available regarding the value of breath testing after antibiotic therapy.

The principle of the urea breath test is based on the identification of urease activity utilizing \(^{13}\)C- or \(^{14}\)C-labeled urea. The urea is metabolized by urease, produced by *H. pylori*, yielding labeled CO2, which is absorbed across the gastric mucosa and expired in the breath where it can be measured. The advantage of breath testing is clearly non-invasiveness, cost effectiveness and simplicity. Furthermore the absence of a sampling error favors the use of breath tests.

Initially the \(^{14}\)C-labeled urea breath tests was validated and appeared both sensitive and specific in detecting *H. pylori* infection in humans.\(^6\)\(^7\) The drawbacks of \(^{14}\)C-labeled urea breath tests are problems with radioactive material and the inherent precautions that have to be taken. Later a \(^{13}\)C-urea breath test method became available that did not have these drawbacks. \(^{13}\)C is a stable, non-radioactive, naturally occurring isotope of carbon that presents no risks to human subjects. Special purpose mass spectrometers, called isotope ratio mass spectrometers (IRMS) are now used to obtain sufficient precision and accuracy to measure stable isotopes. Mass spectrometry is not regularly used in the clinic because of the high cost of such devices and the need for trained operators. For this reason the NIH consensus conference of 1994 concluded that there was a significant need for an easy to use, reliable and low cost \(^{13}\)C-urea breath test for clinical use.\(^8\)

Infrared detection methods have also been developed to measure stable isotopes; however these methods may be less accurate as mass spectroscopy.\(^9\)\(^10\)

The Laser Assisted Ratio Analyzer (LARA™) System consists of a stable isotope analyzer, which measures the ratio of 13-carbon to 12-carbon via a novel technology, based on the use of laser optogalvanic effect spectroscopy. With this technique \(^{13}\)C/\(^{12}\)C-isotope ratio's can be determined with a very high precision.\(^11\)\(^12\) The LARA™ system is a fully automated device that contains 2 carbon dioxide lasers. It is relatively cheap, safe, easy to handle and can be used in any hospital without the need for a specialized technician.

Pre-eradication, the LARA™ urea breath test yielded a sensitivity and specificity of over 95% in both a European and a North American multicenter trial.\(^14\)\(^15\) The aim of this study was to establish the sensitivity and specificity of the LARA™ urea breath test when used to monitor the efficacy of *H. pylori* eradication therapy.

**Patients and methods**

**Patient selection**

Dyspeptic patients who were referred for upper gastro-intestinal endoscopy were considered for the study. *H. pylori* positive patients could be included when treatment of the *H. pylori* infection was thought to be indicated by the attending physician; when patients were over 18 years of age
and able to understand and comply with the protocol. All patients signed a written informed consent. Females with childbearing potential needed to have a negative pregnancy test and had to use a reliable birth control method. Exclusion criteria were: the use of bismuth compounds, antibiotics or proton pump inhibitors during four weeks prior to the start of the study; gastric surgery resulting in the removal of the antrum; active bleeding ulcers; medical necessity for chronic treatment with a proton pump inhibitor; pregnant or lactating women, women of childbearing potential not using a reliable birth control method; serious medical illness precluding enrollment in the study; alcohol or drug abuse and treatment with any investigational drug other than trial for *H. pylori* within the last four weeks.

**Methods**

A first LARA™ breath test was performed at study entry, before eradication therapy was given. Thereafter patients received various triple eradication regimens. All patients underwent a second endoscopy followed by a second LARA™ breath test, 5 to 10 weeks after the end of the eradication regimen. A third LARA™ breath test was performed after 3 to 6 months after eradication therapy. At each endoscopy eight biopsies were obtained, two for histology, one for culture and one for CLO test from both antrum and corpus. Pre-eradication, patients were considered to have an *H. pylori* infection when culture was positive or when at least histology and CLO-test were positive. Post-eradication *H pylori* was considered absent only when all three biopsy-based tests (culture and histology and CLO-test) were negative in both antrum and corpus biopsies (=reference test).

Proton pump inhibitors, antibiotics and bismuth-preparations were not allowed during at least 28 days before the endoscopy and breath tests were performed. H2-blockers were allowed during the study. The CLO-test as placed in an incubator at 37 °C and read after 24 hours. The four biopsies obtained for histopathological assessment of *H. pylori* were placed in two separate vials containing buffered, neutral 3.7% formaldehyde solution (10% formalin). Specimens were processed using the paraffin embedding technique, and stained with the modified Giemsa method. Complementary staining, e.g. immunohistochemical staining and in situ hybridization was performed when considered necessary.

The LARA™ ¹³C-urea breath test was performed a minimum of one-hour following endoscopy, using the protocol developed by the manufacturer. After baseline breath samples were taken, patients consumed a nutrient dense meal (Ensure), to delay gastric emptying. Then the patient ingested 100 mg of ¹³C-labeled urea, dissolved in 50 ml of sterile purified water. Breath samples were taken at 20, 30, 40 and 60 minutes after urea-ingestion. The ¹³C/¹²C-ratio (measured in ‘delta units’) in the exhaled breath was measured by the LARA™ system.

We used a cut off value for the ¹³C/¹²C-ratio of 6.1 with a grey zone of ± 0.6, as was derived from Receiver Operator Characteristics analysis (ROC) to give optimal results. Patients with delta values above 6.7 were regarded as having a positive LARA™ test, indicating an *H. pylori* infection. Patients with delta values below 5.5 were regarded as having a negative breath test,
Table 1 *Number of analyzable patients.*

<table>
<thead>
<tr>
<th>Total inclusion</th>
<th>2nd LARA™ breath test</th>
<th>3rd LARA™ breath test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not analyzable because of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wrongly included</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>received no eradication therapy</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>prohibited concomitant medication</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>lost to follow-up</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>Non analyzable breath test</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Grey zone value</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total analyzable patients</td>
<td>159</td>
<td>127</td>
</tr>
</tbody>
</table>

indicating absence of *H. pylori*. Results between 5.5 and 6.7 delta units were considered indeterminate or ‘grey-zone’-values and were not further analyzed.

**Statistical analysis**

The sensitivity, specificity, positive predictive value and the negative predictive value were calculated for the second and third LARA™ breath test as compared to the gold standard reference test.

**Results**

**Patients**

A total of 181 patients with peptic ulcer disease or functional dyspepsia were included in the study; 127 in the Academic Medical Center in Amsterdam, The Netherlands and 54 in the San Orsola Hospital in Bologna, Italy. Ninety-four men and 87 women were included, with a mean age of 47 years (range 18–85). The second LARA™ breath test, performed 5 to 10 weeks after the eradication therapy, was compared to the reference test in 159 patients (Table 1). One patient was wrongly included because he/she had a positive CLO test at baseline, but pathology and culture later appeared negative; two patients refused the eradication regimen; one patient used a proton pump inhibitor during the study period; two patients moved abroad; eleven patients refused a second endoscopy and/or breath test; one patient abusively had only baseline breath samples taken, another patient had a not valid breath test result due to instrument protocol deviations; breath test results of one other patient were unable to be processed because of inadequate baseline samples and in 3 patients a second LARA™ breath test result was in the ‘grey zone’.

A total of 3 indeterminate results were noted for the second breath test; no values in the grey zone area were found for the third breath test.

The third LARA™ breath test at 3 to 6 months after eradication therapy could be compared to the reference test in 127 patients.

No adverse events have occurred after any of the LARA™ breath tests.
In 12 patients a discrepancy was seen between the reference test and the second and/or third LARA™ breath test (TABLE 2). In all other analyzable patients the breath tests yielded the same result as the reference test.

Pre-eradication the sensitivity of the first LARA™ breath test was 95% (data not shown). The specificity of the first LARA™ breath test could not be determined because we only included patients with an H. pylori infection.

The second LARA™ breath test, performed 5 to 10 weeks after eradication therapy, compared to the gold standard showed a sensitivity of 91% and a specificity of 96% (TABLE 3).

The third LARA™ breath test, performed 3 to 6 months after eradication therapy, compared to the reference test showed a sensitivity 100% of and a specificity of 98% (TABLE 4).

When using only the 30 and 60 minute LARA™ delta values, similar results were obtained, as can be seen from the raw data in Table 2.

Comparing the second to the third LARA™ breath test in all patients showed 4 discordant results (see TABLE 2). In one patient the third LARA™ was regarded false positive, because the post eradication reference test as well as the second LARA™ breath test were all negative. Three patients were regarded to have a false positive second breath test, with negative reference test and negative third LARA™ breath test.

Sensitivity and specificity of the LARA™ urea breath test
Table 3. Second LARA™ breath test, 5-10 weeks after eradication therapy, compared to gold standard.

<table>
<thead>
<tr>
<th>Reference test (Histology + culture + CLO)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LARA™ positive</td>
<td>21</td>
</tr>
<tr>
<td>LARA™ negative</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference test (Histology + culture + CLO)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third LARA™ positive</td>
<td>17</td>
</tr>
<tr>
<td>Third LARA™ negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
</tr>
</tbody>
</table>

Sensitivity: 91% (95% CI: 72-99)
Specificity: 96% (95% CI: 91-98)
Positive predictive value: 78%
Negative predictive value: 98%

**Post-eradication H. pylori was considered absent only when all culture and histology and CLO-test were negative in both antrum and corpus biopsies.**

Table 4. Third LARA™ breath test, 3-6 months after eradication therapy, compared to gold standard.

<table>
<thead>
<tr>
<th>Reference test (Histology + culture + CLO)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LARA™ positive</td>
<td>17</td>
</tr>
<tr>
<td>LARA™ negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
</tr>
</tbody>
</table>

Sensitivity: 100% (95% CI: 81-100)
Specificity: 98% (95% CI: 94-100)
Positive predictive value: 89%
Negative predictive value: 100%

**Post-eradication H. pylori was considered absent only when all culture and histology and CLO-test were negative in both antrum and corpus biopsies.**

Discussion

This study shows a high sensitivity and specificity of the LARA™ 13C-urea breath test to test for the success of eradication therapy. Five to ten weeks after eradication therapy the sensitivity and specificity of the LARA™ system were 91 and 96%, respectively. When the reference test was compared to the third LARA™ breath test, performed 3-6 months after eradication therapy, the results were even better with a sensitivity and specificity of 100% and 98%, respectively.

Although breath testing has shown high sensitivity and specificity in untreated patients, only a few groups have studied the usefulness of breath testing after anti-Helicobacter therapy. Published data on the reliability of 13C- and 14C-urea breath tests, performed post-eradication therapy are listed in Table 5. These data show a mean sensitivity and specificity of the breath testing after eradication therapy of 94 and 89%, respectively.

In our data, two patients in whom eradication failed had a false-negative breath test 5-10 weeks after eradication therapy. Perhaps the growth and/or the urease activity of the H. pylori may not yet be at full strength after 5-10 weeks after eradication therapy and H. pylori colonization can be missed, causing a false negative breath test. In general, false negative breath tests may also occur after recent use of antibiotics, bismuth salts or proton pump inhibitors and after gastric surgery, neither of which was the case in our patients. If and to what extent H2-receptor antagonists contribute to slow down re-growth of H. pylori is unclear at the present time.

Six patients in whom eradication therapy was successful had a false-positive breath test 5-10 weeks after eradication therapy. Three of these six patients tested negative on the third breath test at 3-6 months. One of these six patients, who became symptom free after the eradication regimen without further medication, also yielded a positive third breath test. Maybe these false positive results occurred because other urease-producing bacteria are present in the stomach, such as may
Table 5 Published data on the usefulness of 13- and 14C-urea breath tests in confirming eradication of H. pylori infections

<table>
<thead>
<tr>
<th>Author</th>
<th>Breath test</th>
<th>Reference test</th>
<th>No. of studied patients</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graham</td>
<td>14C</td>
<td>Culture</td>
<td>16</td>
<td>86</td>
<td>50</td>
</tr>
<tr>
<td>Logan</td>
<td>13C</td>
<td>Culture histology</td>
<td>106</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Slomianski</td>
<td>13C</td>
<td>Rapid urease test and culture</td>
<td>118</td>
<td>97</td>
<td>71</td>
</tr>
<tr>
<td>Bazzoli</td>
<td>13C</td>
<td>Rapid urease test, culture and Histopathology</td>
<td>58</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Slepman</td>
<td>13C</td>
<td>Histopathology</td>
<td>625</td>
<td>89</td>
<td>97</td>
</tr>
<tr>
<td>Soule</td>
<td>13C</td>
<td>Rapid urease test</td>
<td>104</td>
<td>97</td>
<td>?</td>
</tr>
<tr>
<td>Lahaie</td>
<td>14C</td>
<td>Culture and/or Histopathology</td>
<td>36</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Menegatti</td>
<td>14C</td>
<td>Rapid urease test, culture and Histopathology</td>
<td>83</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Menegatti</td>
<td>13C</td>
<td>Rapid urease test, culture and Histopathology</td>
<td>83</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Van de Wouw</td>
<td>14C</td>
<td>Rapid urease test, culture and Histopathology</td>
<td>57</td>
<td>92</td>
<td>78</td>
</tr>
<tr>
<td>Ahuja</td>
<td>14C</td>
<td>Rapid urease test</td>
<td>60</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td>Hiroto Miwa</td>
<td>13C</td>
<td>Rapid urease test, culture and Histopathology</td>
<td>199</td>
<td>95</td>
<td>?</td>
</tr>
</tbody>
</table>

Occur in patients with achlorhydria or gastric atrophy, or in the presence of oral- or small bowel-urease-containing bacteria and from colonization with other Helicobacter such as H. felis. Another factor that has been suggested to be responsible for false positive results after eradication therapy is the so-called ‘delayed clearance’ of H. pylori. Delayed clearance of H. pylori indicates a decrease of delta values at 3 and 6 months to that of 1-2 months after eradication therapy. This decline in delta values after eradication therapy was found by Miwa and Slomianski. The mechanism of this delayed response is not clear, but was suggested to be caused by the presence of achlorhydria from gastric atrophy or acid suppressive medication. In our data we did not find a significant decrease in delta values from the second to the third \textsc{lara}™ breath test.

The assessment of cure after eradication therapy is often made at one month after eradication therapy. Once the breath test at 1 month shows a negative result, its reliability is very high. However, the breath test at 1-2 months after eradication therapy yields a relatively high false positive rate, as can be seen from the data from Miwa and Slomianski, as well as in our data. When a breath test is positive, especially rather soon after eradication therapy, suggesting a persisting H. pylori infection, other tests to confirm this persisting infection should be considered. Optimal results are obtained when the breath test is performed 3 to 6 months after eradication therapy.

In conclusion we found that the \textsc{lara}™ 13C-urea breath test is both sensitive and specific to test for cure of an H. pylori infection. The 13C-urea breath tests may well become the test of choice, especially in confirming cure of an H. pylori infection, because it is convenient for the patient, highly accurate, easy to use, noninvasive and relatively cheap.

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References


