Introduction

Early diagnosis and treatment can considerably improve the prognosis of serious medical conditions. One of the non-invasive methods of detecting possible early disease, and the need for further investigation, is the measurement of markers in exhaled air by mass spectrometry. However, the equipment for this technique is large, expensive and fixed. A smaller, less expensive portable quadrupole mass spectrometer system has been developed.

Diagnosis of Helicobacter pylori by carbon-13 urea breath test using a portable mass spectrometer

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Abstract

Context: In the non-invasive detection of markers of disease, mass spectrometry is able to detect small quantities of volatile markers in exhaled air. However, the problem of size, expense and immobility of conventional mass spectrometry equipment has restricted its use. Now, a smaller, less expensive, portable quadrupole mass spectrometer system has been developed.

Helicobacter pylori has been implicated in the development of chronic gastritis, gastric and duodenal ulcers and gastric cancer.

Objectives: To compare the results obtained from the presence of H. pylori by a carbon-13 urea test using a portable quadrupole mass spectrometer system with those from a fixed mass spectrometer in a hospital-based clinical trial.

Methods: Following ethical approval, 45 patients attending a gastroenterology clinic at the Royal Liverpool University Hospital exhaled a breath sample into a Tedlar gas sampling bag. They then drank an orange juice containing urea radiolabelled with carbon and 30 min later gave a second breath sample. The carbon-13 content of both samples was measured using both quadrupole mass spectrometer systems. If the post-drink level exceeded the pre-drink level by 3% or more, a positive diagnosis for the presence of H. pylori was made.

Results: The findings were compared to the results using conventional isotope ratio mass spectrometry using a laboratory-based magnetic sector instrument off-site. The results showed agreement in 39 of the 45 patients.

Conclusions: This study suggests that a portable quadrupole mass spectrometer is a potential alternative to the conventional centralised testing equipment. Future development of the portable quadrupole mass spectrometer to reduce further its size and cost is indicated, together with further work to validate this new equipment and to enhance its use in mass spectrometry diagnosis of other medical conditions.

Keywords

Helicobacter pylori, portable quadrupole mass spectrometer, urea breath test

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A clinically important early detection is the *Helicobacter pylori* infection of the stomach. *H. pylori* has an important role in the development of active chronic gastritis and gastric and duodenal ulcers1–4 and of gastric cancer.5 The World Health Organization's International Agency for Research on Cancer classifies the bacterium as a grade 1 carcinogen.6 *H. pylori* secretes the enzyme urease, converting urea into ammonia and bicarbonate. Of the various available tests for the *H. pylori* infection, carbon-13 and carbon-14 urea breath tests are well established and have the advantages of being rapid, non-invasive and having high sensitivity and specificity.7–10

Therefore, the aim of this study was to compare the results obtained for the presence or absence of *H. pylori* by a carbon-13 urea breath test using the portable QMS system with those from a fixed mass spectrometer in a hospital-based clinical trial.

**Patients and method**

**Patients**

Ethical approval for the study was obtained from the Royal Liverpool and Broadgreen University Hospitals Trust Ethics Committee. In all, 45 patients aged 30–60 years attending the Gastroenterology Department of the Royal Liverpool University Hospital, with upper gastrointestinal symptoms and suspected of having *H. pylori* colonisation of the stomach, gave written informed consent to participate in the study.

The clinical procedure for each patient was that an initial breath sample was taken as a baseline. The patient then drank 100 mL of unsweetened orange juice containing 75 mg of C13 urea. Thirty minutes later, a second breath sample was taken. The breath samples were gathered in Tedlar gas sample bags, size 25 × 22.5 cm2.11

**Analysis of breath samples**

A portable QMS system (Figure 1) with a triple filter, closed ion source and heated capillary inlet (MK2 Cirrus) was used to measure carbon-13 levels in the breath samples in the Tedlar bags.11–13 The bag was connected to the open value of the QMS and sampled for 10 s to ensure uniform flow. The QMS sampling programme utilised six consecutive scans.

The diagnosis made using the portable QMS was compared with the diagnosis made in the usual manner by sending patient breath samples to a central off-site isotope ratio mass spectrometry (IRMS) facility.

**Analysis of results**

The measurements obtained were averaged and recorded. The percentage change in C13 content was obtained from mass spectra using a MATLAB programme. Figure 2 shows the output with a log scale and spectral at m/z 44 and 45. The pre-drink and post-drink measurements were used to determine the presence or absence of *H. pylori*.

Figure 3 shows the readings for one patient with pre-drink and post-drink findings superimposed to illustrate the differences between the two sets of readings. As the post-drink...
reading was more than 3% greater than the pre-drink reading, a positive diagnosis was made.

Figure 4 shows the readings for another patient where the ratio of the peak heights for m/z = 44 and m/z 45 remained unchanged after the urea-containing drink. This led to a negative diagnosis, that is, the absence of \textit{H. pylori}.

A receiver operating characteristic (ROC) plot (Figure 5) was constructed for the portable QMS results compared with the conventional findings.

**Results**

The test methodology was found to be straightforward to use and gave a rapid result. The portable QMS equipment and the Tedlar bags were used without any problems. The results of the 45 patients are summarised in Table 1. For 39 of the 45 patients, the results using the portable QMS were the same as the conventional IRMS results; there were three false positives (patients 7, 9 and 25) and three false negatives (patients 17, 21 and 42) giving an overall agreement of 87%. In the ROC plot, the curves were constructed by computing the sensitivity and specificity of the clinical findings in predicting the presence or absence of \textit{H. pylori}. The area under the curve is 0.71, grading the accuracy of the carbon-13 urea breath test as ‘fair’.

**Discussion**

The value of early diagnosis to enhance treatment outcomes is well demonstrated in the case of infection with \textit{H. pylori}. If the infection becomes chronic, debilitating illness from gastric and duodenal ulceration and gastric cancer can follow, yet curative treatment at the appropriate stage may require only outpatient medication.

The carbon-13 urea breath test is rapid and non-invasive, while having good sensitivity and specificity which have been shown to range from 90% to 100% in comparison with
Figure 5. An ROC curve with the curves constructed by plotting the true-positive rate (sensitivity) against the false-positive rate (specificity). A positive diagnosis was 3% above the baseline of the initial reading.

biopsy-based tests. H. pylori produces a significant quantity of the enzyme urease in the gastric mucosa of the infected individual. The ingested carbon-13 labelled urea is hydrolysed by the urease. Ammonia and carbon dioxide are produced, diffused into the blood and are excreted in the breath via the lungs. IRMS may be carried out using a variety of methods; one of the most popular uses gas chromatography combustion (GC-C) MS to ascertain the relative ratio of light stable isotopes of carbon (13C/12C), hydrogen (2H/1H), nitrogen (15N/14N) or oxygen (18O/16O). The time taken to send the breath samples away, process them and return the results to the hospital clinician can be upwards of several days.

Several methods for capturing and transporting the breath samples have been used, including canisters, absorbing agents and Teflon or Tedlar bags. The sample containers need to be easy to handle and able to store the sample securely for a period of time. Canisters are not appropriate for breath measurement as they need to be evacuated before sampling. Absorbing agents like Tenax are compound specific. Teflon bags are relatively expensive and fragile, while Tedlar bags as used in this study are tough, durable and chemically inert to a wide range of compounds. They were found to be easy to use.

The threshold increase in the post-drink to pre-drink samples of 3% was chosen to allow for sampling variation (Figure 6). The results obtained agree reasonably well with the conventional QMS results (87% agreement), indicating that using a portable QMS is promising as a potential alternative to the expensive centralised testing facility. Even using different fixed IRMS machines, there are some differences in the results obtained. For example, in testing a large sample using two different conventional IRMS machines, Savarino et al. found that there was close but not complete correlation (r=0.98) and sensitivity and specificity of 97%–100%. The ROC plot of the results provided a graphical indication of the performance of the test in differentiating normal and diseased state.

As this was a non-invasive study, and the aim was to compare the new equipment with existing equipment, biopsies were not undertaken as part of this study. Even biopsy

### Table 1. Summary results for 45 patients.

| Patient | Percentage change | Portable result | Hospital result |
|---------|-------------------|-----------------|----------------|
| 1       | 2.3805            | Negative        | Negative       |
| 2       | 2.6119            | Negative        | Negative       |
| 3       | −0.27153          | Negative        | Negative       |
| 4       | 3.3088            | Positive        | Positive       |
| 5       | 1.5157            | Negative        | Negative       |
| 6       | −0.41268          | Negative        | Negative       |
| 7       | 3.2864            | Positive        | Negative       |
| 8       | −0.6307           | Negative        | Negative       |
| 9       | 5.0282            | Positive        | Negative       |
| 10      | −1.4281           | Negative        | Negative       |
| 11      | −1.3585           | Negative        | Negative       |
| 12      | −1.392            | Negative        | Negative       |
| 13      | 1.7658            | Negative        | Negative       |
| 14      | −7.4317           | Negative        | Negative       |
| 15      | −5.7499           | Negative        | Negative       |
| 16      | −5.5067           | Negative        | Negative       |
| 17      | −1.7904           | Negative        | Positive       |
| 18      | −1.3738           | Negative        | Negative       |
| 19      | 2.7059            | Negative        | Negative       |
| 20      | 0.90435           | Negative        | Negative       |
| 21      | 0.057905          | Positive        | Negative       |
| 22      | −6.1336           | Negative        | Negative       |
| 23      | 1.2009            | Negative        | Negative       |
| 24      | 0.33432           | Negative        | Negative       |
| 25      | 3.2398            | Positive        | Negative       |
| 26      | 1.4659            | Negative        | Negative       |
| 27      | 2.0393            | Negative        | Negative       |
| 28      | −3.0747           | Negative        | Negative       |
| 29      | −3.1673           | Negative        | Negative       |
| 30      | 0.42858           | Negative        | Negative       |
| 31      | −0.22003          | Negative        | Negative       |
| 32      | −0.130014         | Negative        | Negative       |
| 33      | −1.3796           | Negative        | Negative       |
| 34      | 5.2481            | Positive        | Positive       |
| 35      | 2.4104            | Negative        | Negative       |
| 36      | −3.2602           | Negative        | Negative       |
| 37      | −1.4923           | Negative        | Negative       |
| 38      | −4.7456           | Negative        | Negative       |
| 39      | −6.1669           | Negative        | Negative       |
| 40      | −1.4061           | Negative        | Negative       |
| 41      | −3.7616           | Negative        | Negative       |
| 42      | 0.4252            | Negative        | Positive       |
| 43      | −0.046            | Negative        | Negative       |
| 44      | −5.0121           | Negative        | Negative       |
| 45      | 0                 | Negative        | Negative       |
samples do not provide 100% sensitivity and specificity of
diagnosis for Savarino et al.\textsuperscript{18} had to exclude 9 out of 124
patients from their study because of divergent results between
rapid urease testing of the biopsy specimens and the
histology.

This study was an initial clinical trial as part of an ongoing
programme of validation and improvement of a portable
instrument for breath testing. It was important to examine the
use of this novel equipment in a routine clinical setting such as
a hospital outpatient gastroenterology clinic. However, this
did impose limitations with the breath samples being tested on
site with the portable QMS but needing to be transported to the
fixed QMS and read there at a later time. Moreover, the sam-
ple size was limited by the number of suitable patients attend-
ing this clinic and consenting to participate. However, with
each patient acting as their own control, this sample was suf-
ficient for this initial clinical study. The findings of this study
can be used for the power calculations for the sample size in
the next, more extensive clinical study. Another limitation was
that the nature of the gastroenterology clinic meant that the
patients did not fast before attending. In another study, this
reduced the sensitivity of diagnosis to 86%.\textsuperscript{18} The spread of
the results in the scatter plot of our results (Figure 6) was com-
parable to that in the study of Hegedus et al.\textsuperscript{19} These investiga-
tions also used cut-off levels on the measured values in order
to simplify the test and interpretation process.\textsuperscript{18}

Using the portable QMS, the test was straightforward to
perform and gave a rapid result. The one off cost of this port-
able instrument was of the order of £15,000 which could be
reduced if multiple units were manufactured together. This is
considerably less than a conventional IRMS fixed system
(circa £150,000, depending on options), but still limits avail-
ability. With further instrument development, for example, the
use of hyperbolic electrodes, an improved, miniaturised port-
able QMS with enhanced sensitivity could be constructed and
produced at a much reduced price of circa £10,000k. The
reduced size and cost would enable it to be placed in large
general medical practices, community health clinics and com-
mercial health centres. The portable equipment (Figure 1) is
being further developed, and more extensive clinical studies
are indicated. Further work is also necessary in calibrating the
level at which a diagnosis is made with the portable QMS to
increase the agreement with the established IRMS.

Conclusion
This initial study suggests that a portable QMS is a potential
alternative to the conventional centralised testing equipment.
Future development of the portable QMS to reduce its size and
cost is indicated together with more extensive trials to validate
its use and explore its application in other medical conditions.

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Declaration of conflicting interests
The portable mass spectrometer used in this trial was developed by
the Mass Spectrometry Group of the Department of Electrical
Engineering and Electronics, University of Liverpool, UK; five

Figure 6. A scatter plot of the result for each patient using the portable QMS. The cut-off for a positive diagnosis was 3% above
baseline.
members of this group are authors of this article. After this trial concluded Q-Technologies, a spin-off company of the University of Liverpool of which Prof S. Taylor, one of the authors, is the founder, director and majority shareholder, further developed the equipment and now offers it.

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**References**

1. Warren JR and Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; 321: 1273–1275.
2. Hopkins RJ, Girardi LS and Tuney EA. Relationship between *Helicobacter pylori* eradication and reduced duodenal and gastric ulcer recurrence: a review. *Gastroenterology* 1996; 110(4): 1244–1252.
3. Dowsett SA and Kowolik MJ. Oral *Helicobacter pylori*: can we stomach it? *Crit Rev Oral Biol Med* 2003; 14(3): 226–233.
4. Marshall BJ. Helicobacter pylori: past, present and future. *Keio J Med* 2003; 52(2): 80–85.
5. Marshall BJ. Helicobacter connections. *ChemMedChem* 2006; 1(8): 783–802.
6. Stratton KR, Durch JS and Lawrence RS. Vaccines for the 21st century: a tool for decision making. Washington, DC: The National Academy Press, 2000.
7. Ricci C, Holton J and Vaira D. Diagnosis of *Helicobacter pylori*: invasive and non-invasive tests. *Best Pract Res Clin Gastroenterol* 2007; 21(2): 299–313.
8. Atherton JC and Spiller RC. The urea breath test for *Helicobacter pylori*. *Gut* 1994; 35: 723–725.
9. Peeters M. Urea breath test: a diagnostic tool in the management of *Helicobacter pylori*-related gastrointestinal diseases. *Acta Gastroenterol Belg* 1998; 61(3): 332–335.
10. Logan RPH. Urea breath tests in the management of *Helicobacter pylori* infection. *Gut* 1998; 43(Suppl. 1): 47–50.
11. Steeghs MML, Cristescu SM and Harren FJM. The suitability of Tedlar bags for breath sampling in medical diagnostic research. *Physiol Meas* 2007; 28(1): 73–84.
12. Dawson PH. Principles of operation. In: Dawson PH (ed.) *Quadrupole mass spectrometry and its applications*. Amsterdam: Elsevier, 1976, pp.9–64.
13. http://www.mksinst.com/product/product.aspx?ProductID=195
14. Cutter AF, Havstad S, Ma CK, et al. Accuracy of invasive and non-invasive tests to diagnose *Helicobacter pylori* infection. *Gastroenterology* 1995; 109: 136–141.
15. Goddard AF and Logan RPH. Review article: urea breath tests for detecting *Helicobacter pylori*. *Aliment Pharmacol Ther* 1997; 11: 641–649.
16. Dore SP, Krupadas S, Borgonha S, et al. The 13c urea breath test to assess *Helicobacter pylori* infection in school children. *Natl Med J India* 1997; 10(2): 57–60.
17. Kolesnikov T and Lee A. Clinical infectious diseases – *Helicobacter pylori*. *Bailliere Tindall* 1997; 4(3): 239–244.
18. Savarino V, Mela GS, Zentillin P, et al. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for 13C-urea breath test. *Am J Gastroenterol* 1999; 94: 1203–1208.
19. Hegedus O, Ryden J, Rehnberg AS, et al. Validated accuracy of a novel urea breath test for rapid *Helicobacter pylori* detection and in office analysis. *Eur J Gastroenterol Hepatol* 2002; 14: 1–8.