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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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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. Now, a smaller, less expensive portable quadrupole mass spectrometer (QMS) system has been developed which could be utilised at more health service locations. Before this portable QMS can be used with confidence, it is necessary to measure its performance in a clinical setting against a currently used mass spectrometer technique.

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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 ulcers[1–4] 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 $25 \times 22.5\,cm^2$.[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

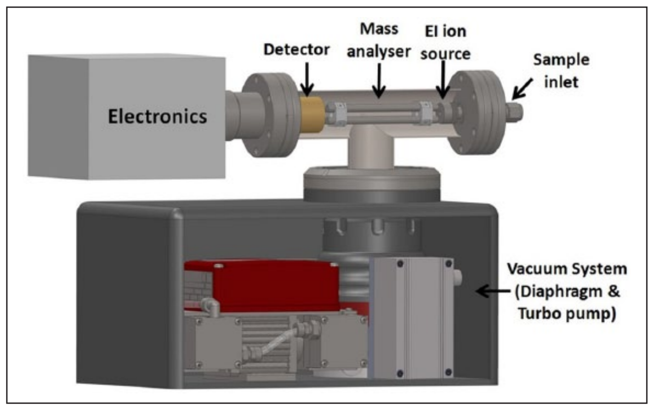

Figure 1. Schematic diagram of the portable QMS and vacuum system.

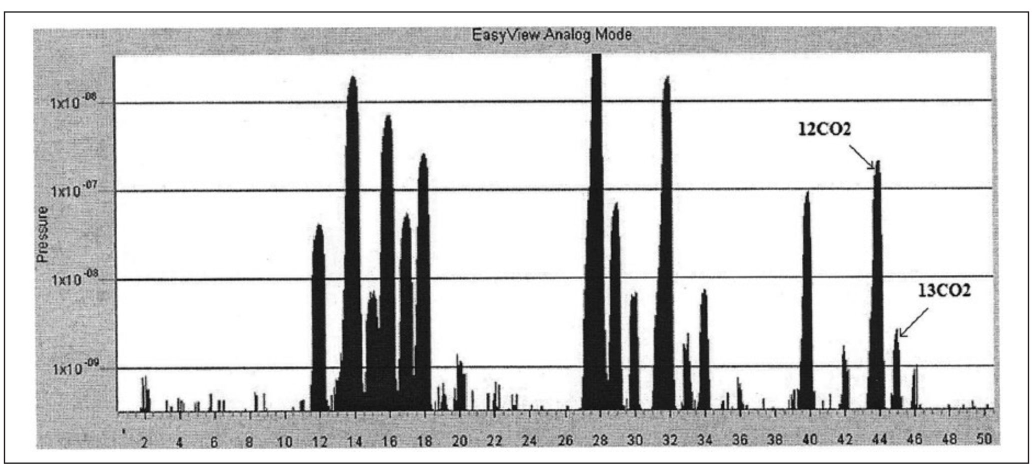

Figure 2. QMS output from patient breath with a log scale showing spectral peaks at m/z 44 and 45 corresponding to $^{12}CO_2$ and $^{13}CO_2$.

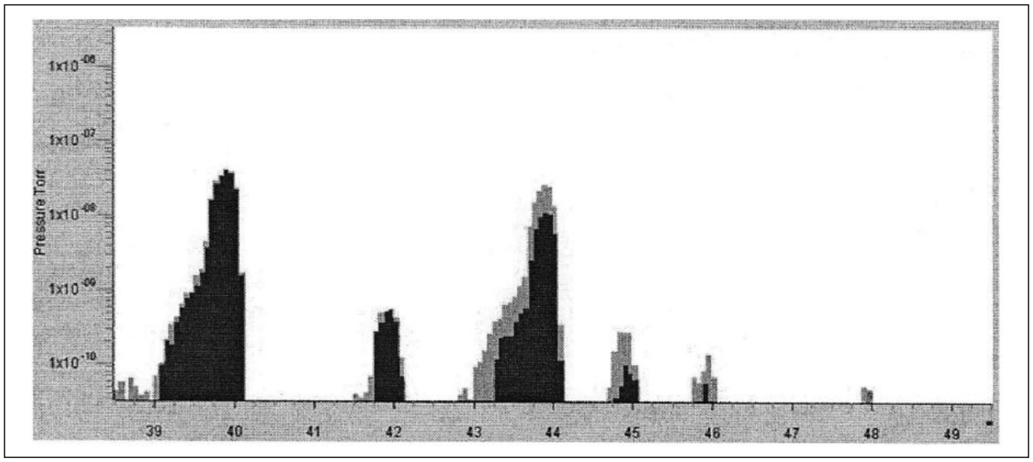

Figure 3. Patient 4. The pre-drink and post-drink breath samples superimposed; lighter shading is post-drink sample. The difference between the samples suggests a positive diagnosis.

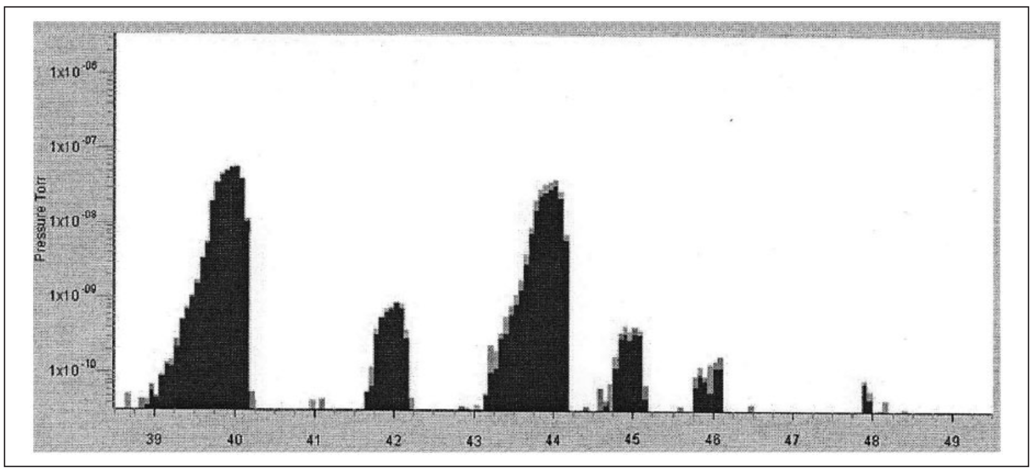

Figure 4. The pre-drink and post-drink breath samples superimposed; the lighter shading is the post-drink sample. The lack of difference between samples suggests a negative diagnosis.

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 *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 *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 *H. pylori*. If the infection becomes chronic, debilitating illness from gastric and duodenal ulceration and gastric cancer can follow,[5] 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

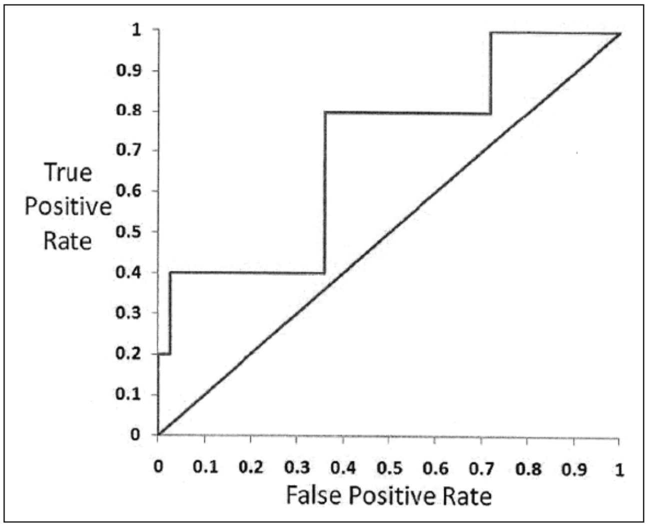

Figure 5. An ROC curve with the curves constructed by plotting the true-positive rate (sensitivity) against the false-positive rate (specificity). A positive diagnosis was 3% above the baseline of the initial reading.

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	Negative	Positive
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

biopsy-based tests.[14,15] *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.[16,17] 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 ($^{13}C/^{12}C$), hydrogen ($^{2}H/^{1}H$), nitrogen ($^{15}N/^{14}N$) or oxygen ($^{18}O/^{16}O$). 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.[11] 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.[18] 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

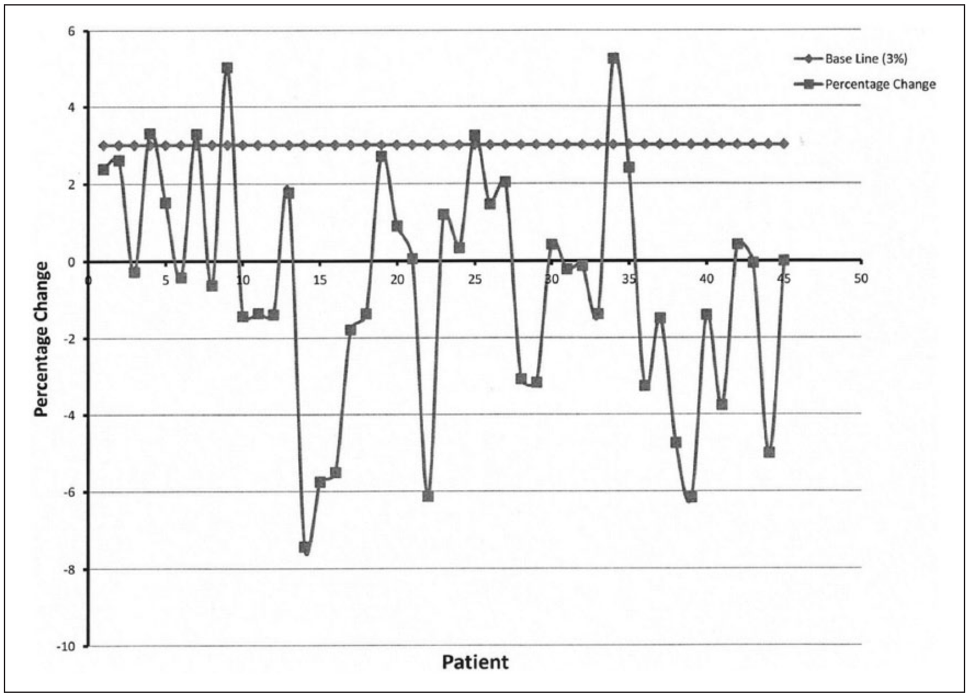

Figure 6. A scatter plot of the result for each patient using the portable QMS. The cut-off for a positive diagnosis was 3% above baseline.

samples do not provide 100% sensitivity and specificity of diagnosis for Savarino et al.[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 sample size was limited by the number of suitable patients attending this clinic and consenting to participate. However, with each patient acting as their own control, this sample was sufficient 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%.[18] The spread of the results in the scatter plot of our results (Figure 6) was comparable to that in the study of Hegedus et al.[19] These investigations also used cut-off levels on the measured values in order to simplify the test and interpretation process.[18]

Using the portable QMS, the test was straightforward to perform and gave a rapid result. The one off cost of this portable 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 availability. With further instrument development, for example, the use of hyperbolic electrodes, an improved, miniaturised portable 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 commercial 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 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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