An Evaluation of Whole Blood Testing for *Helicobacter pylori* infection in Chinese

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Abbreviations used in this paper: *Helicobacter pylori*, *H. pylori*; ^{13}C-urea breath test, ^{13}C-UBT;

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ABSTRACT

BACKGROUND : Near patient tests for *Helicobacter pylori* (*H. pylori*) were developed to assist in the management of dyspepsia patients in general practice. Most studies were performed in western population.

AIM : To evaluate the rapid whole blood test (Flexpack HP) for *H. pylori* in Chinese population.

METHODS : Consecutive dyspeptic patients referred for upper endoscopy were recruited. During upper endoscopy, biopsies were taken from the antrum and corpus for CLO test and histological examination. After endoscopy, the whole blood test (FlexPack HP) was performed according to the manufacturer's instruction. Patients then received a ^{13}C-urea breath test (^{13}C-UBT). Results of the whole blood test were compared with the gold standard (CLO test, histology and ^{13}C-UBT).

RESULTS: 294 consecutive patients gave a valid Flexpack HP result for interpretation. Mean age of patients was 47.7 (range 15-85) years. Analysis showed a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 58.2%, 92.2%, 90.6%, 63.2% and 73.1% respectively. There was no influence by the age of the patient and the familiarity of the operators.

CONCLUSION: The FlexPack HP whole blood test showed good specificity but lacked sensitivity. It is not sensitive enough to be used in general practice setting for the test and treat approach in Chinese population.

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection plays an important role in the pathogenesis of peptic ulcer disease and adenocarcinoma of stomach.[1] The development of effective antimicrobial therapy for *H. pylori* was proven to be an effective treatment of peptic ulcer disease. The eradication of *H. pylori* significantly reduced the relapse of peptic ulcers.[2-9] Endoscopy is the most reliable method for the diagnosis of peptic ulcer disease and gastric cancer. However, majority of patients presenting with dyspepsia has normal endoscopic findings. Thus empirical treatment of *H. pylori* infection in dyspeptic patients remains an attractive approach in general practice. It may increase the cost-effectiveness of the management of patients with dyspepsia. Rapid whole blood tests for *H. pylori* infection were developed to assist in the management of patients with dyspepsia in general practice. The results of these tests are usually available in less than ten minutes. Test and treat approach using these kits has been estimated to reduce the demand for endoscopy by 25 to 37%.[10,11,12] Most of the validation studies performed so far were in non-Chinese population. Thus we carried out a prospective study to validate the use of rapid whole blood test in Chinese population.

METHODS

Patient Population

Three hundred and seventh Chinese patients referred to the endoscopy unit of Department of Medicine, Queen Mary hospital for investigation of dyspepsia were recruited. Dyspepsia was defined as persistent or recurrent upper abdominal pain or discomfort over the proceeding 3 month period. Informed written consent was obtained from all patients participated in the trial. Exclusion criteria included patients with a past history of *H. pylori* eradication therapy; previous gastric surgery; patients taking antibiotics, H_2 receptor blockers, bismuth or proton pump inhibitors in the preceding 4 weeks and blood transfusion within 6 months before endoscopy.

Gastric biopsies

During upper endoscopy, 3 antral biopsies and 2 corpus biopsies were taken. One antral biopsy was used for rapid urease test (CLO test) and the rest were sent for histological examination of *Helicobacter pylori* status by hematoxylin and eosin stains. Specimens were read by experienced pathologists who were blinded to all clinical information, including the CLO test results.

Flexpack HP whole blood test

After endoscopy, the whole blood test was performed using the Flexpack HP kit (Abbott Diagnostic, Illinois, USA, kind gift from Abbott Laboratories Ltd., Hong Kong) according to the manufacturer's instruction. Flexpack HP kits were stored at 4^0C before use. Individual

packs were brought to room temperature shortly before use before removal from the sealed pouch. The results were read at 4 minutes by two staffs throughout the study. The validity of the test results required agreement between the two staffs. Discrepant results were reported as invalid. The test measured human IgG antibodies to *H. pylori* in whole blood using the principle of reverse-flow immunochromatography. Briefly, a sample of whole blood is collected from a fingerstick using the Flexpack HP Capillary Tubes included in the kit. The sample is applied to the bottom of the sample Pad, which retained the cross-linked blood cells and allows the plasma to flow through to the chromatographic Test Strips. The plasma and the tracer dye then migrate together through a band of immobilized *H. pylori* antigen (test line). This allows specific antibodies to *H. pylori*, if present, to bind to the antigen. The test card is closed once the plasma and the dye reach the limit line. This initiates the reverse-flow chromatography step. The conjugate then pass through the test line and the control line (immobilized conjugate-specific antibody). The appearance of 2 distinct visible lines (1 control and 1 test line) indicated a positive test. The appearance of a single line over the control line indicates a negative result. The absence of a control line indicates an invalid result.

All patients then received a ^{13}C-urea breath test following standard protocol measured by an isotope ratio mass spectrometer in the Simon K.Y. Lee Digestive Disease Center, Queen Mary Hospital. The definition of *H. pylori* infection in this study required at least two of the

three tests (CLO test, histology and ^{13}C-urea breath test) were positive. The absence of *H. pylori* infection required all three tests to be negative. This definition was used as the "gold standard" in this study.

Statistical analysis

The statistics used included chi-square test and Fisher's exact test when appropriate. A *P* value of 0.05 or less is considered statistically significant. The sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy were calculated. Ninety-five percent confidence intervals were calculated for proportions.

In order to evaluate the effect of operator familiarity on the accuracy of the test, we arbitrary divide the study population into two half according to the entry time into the study. The sensitivity and specificity of each half was calculated and compared with each other.

RESULTS

Thirteen cases were excluded from analysis because they could not be classified according to our gold standard for the diagnosis of *H. pylori* infection, i.e. only 1 positive test out of the three tests. Out of the 304 cases, 294 patients gave a valid result for interpretation. The mean age of 294 patients was 48 (range 15 to 85) years. There were 99 males and 195 females. Most of the patients were ethnic Chinese (98 percent). Four patients were Filipinos. 165 patients (56 percent) were diagnosed as *H. pylori* positive by our criteria of gold standard. Of

these 165 *H. pylori* positive patients, 18 (10.9 percent) patients had gastric ulcer, 24 patients (14.5 percent) had duodenal ulcer and 9 patients (5.4 percent) had gastro-duodenal erosions. Eight patients with *H. pylori* positive gastric ulcer and nine patients with *H. pylori* positive duodenal ulcer had a negative test by Flexpack HP.

One hundred and twenty-nine patients were *H. pylori* negative by our gold standard. Of these 129 patients, 3 patients had gastric ulcer (2.3 percent), 2 patients had duodenal ulcer (1.6 percent), and 1 patients had gastro-duodenal erosions (0.8 percent). *H. pylori* status correlate strongly with the presence of duodenal ulcer (P=0.001), gastric ulcer (P<0.001) and gastro-duodenal erosions (P=0.01).

The sensitivity of the Flexpack HP (whole blood) test using the ^{13}C UBT, CLO and histology (2 out of 3) as gold standard was 58.2 percent, specificity of 92.2 percent, positive predictive value of 90.6 percent, negative predictive value of 63.3 percent and overall accuracy of 73.1 percent(Table 1). The sensitivity and specificity of the test for patients under 45 years old were 55.4 percent and 95.3 percent respectively. For patients over 45 years old, the sensitivity and specificity were 60.4 percent and 89.2 percent respectively(Table 1). The prevalence of *H. pylori* infection was similar between patients under 45 years of age (53.6 percent) and patients over 45 years of age (58.3 percent) (P = NS). The prevalence of *H. pylori* infection was also similar between male (58.6 percent) and female patients (54.9 percent) (P = NS).

When the study population was divided into two groups according to their chronological order of entry into the study, there was no statistical difference between the two groups in terms of sensitivity, specificity, positive and negative predictive values, and accuracy (Table 2). The sex and age distributions were similar between the two groups. In fact, the sensitivity appeared higher in the first half (64.9%) when compared to the second half of the study (52.2%). Thus operator familiarity did not significantly affect the accuracy of the test.

DISCUSSION

The Flexpack HP for IgG antibodies to *H. pylori* in whole blood is advocated as a rapid, visually read, qualitative immunochromatographic method for the diagnosis of *H. pylori* infection in primary practice and facilitates referral to specialists or gastroenterologists. The accuracy of the test remained an important issue in this regard. The sensitivity and specificity of the Flexpack HP (whole blood) test obtained in this study was 58.2% and 92.2% and with a positive and negative predictive value of 90.6% and 63.2%. The sensitivity and specificity of the test were similar between patient below 45 years of age (55.4%, 95.3%) and patients over 45 years of age (60.4%, 89.2%).

Existing studies show variation in the accuracy of this test, with reported sensitivities ranging from 84 to 95 percent and specificities ranging from 74 to 97 percent [13-23]. The study population in this study was ethnic Chinese. Our results were quite different from that obtained from another study in Chinese population which reported a sensitivity and

specificity of 81.8 percent and 83.6 percent respectively [22]. The marked difference between our results and the other Chinese study was uncertain. Our study required agreement between two staffs to establish a positive or a negative result. Discrepant results were reported as invalid. The study reported by Leung *et al*. used one single observer throughout the study[22]. This agreement system used in our study might lower the positivity rate. The presence of a faint line in the expected positive position was difficult to interpret and affected the overall validity of the test kits. The reporting of borderline cases may be very difficult and subject to individual variation. It had also been reported that the lower sensitivity of the whole blood test may be partially attributed to challenges associated with collection of blood from a finger stick, not the performance of the test as suggested by Sadowski *et al*.[20] Furthermore, methodological dissimilarities exist between different studies. It may account for difference in the results obtained [13-23].

The low sensitivity of the test in this study indicated that the test was insensitive in Chinese population. It has been shown that the overall heterogeneity of *H. pylori* was greater than that of most bacteria. [24-26] The use of DNA fingerprinting and restriction fragment length polymorphism has demonstrated significant genetic variations among different isolates. A previous report has shown substantial differences between the *cagA* sequences of *H. pylori* strains from China and the Netherlands.[27] Furthermore, the study of geographic distribution of *vacA* alleic types by polymerase chain reaction and reverse hybridization assay also showed different geographical prevalence of different *H. pylori* genotypes.[28]

Thus antigenic difference between strains from Asian and western population remains a distinct possibility to account for the lower sensitivity of the test in Asian population. Our findings are corroborated with the study reported by Leung *et al*. [22] and the other study from Leicester, United Kingdom which has a strong South Asian representation.[29]. On the other hand, it is unknown which are the major *H. pylori* antigens present in the Flexpack HP test. And whether CagA is an important part of it. Some other studies have shown antigenic conservation among strains regardless of their geographical origin.

We observed a higher sensitivity of the test in the first half of the study compared with the second. Although this did not reach statistical significance, there appeared to be some difference in interpretation with time. One of the possibilities would be the longer shelf time for storage of the test kits. The kits were obtained altogether at the beginning of the study and stored in our laboratory before use.

The value of the whole blood test for *H. pylori* lies on its ability to influence the treatment plan and the referral rate in general practice. If endoscopy had been taken only for patients with positive tests, nine of the 24 duodenal ulcers and eight of the 18 gastric ulcers would have been missed. *H. pylori* status also had a strong association with gastric cancer particular in the Asian population in which the incidence of gastric cancer is high.[30] The low sensitivity of the whole blood test to detect *H. pylori* infection may strongly influence the outcome of these patients. Our results suggested that the Flexpack HP may have an unacceptable sensitivity rate when used in the general population with dyspepsia.

In conclusion, the FlexPack HP whole blood test showed good specificity but lacked sensitivity in Chinese population. It does not provide the required accuracy to be used in general practice setting.

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Table 1. Sensitivity, specificity, positive and negative predictive values, and accuracy with 95 percent confidence interval of Flexpack HP whole blood test using C^{13} urea breath test, CLO and histology (2 out of 3) as golden standard

	Overall	Age < 45	Age ≥ 45
Sensitivity (%)	58.2 (50-66)*	55.4 (43-67)	60.4 (50-71)
Specificity (%)	92.2 (86-96)	95.3 (87-99)	89.2 (79-96)
Positive predictive value (%)	90.6 (83-95)	93.2 (81-99)	88.7 (78-95)
Negative predictive value (%)	63.2 (56-70)	64.9 (54-74)	61.7 (51-72)
Accuracy (%)	73.1 (68-78)	73.9 (66-81)	72.4 (65-79)

* 95 percent confidence intervals shown in brackets.

Table 2. Sensitivity, specificity, positive and negative predictive values, and accuracy with 95 percent confidence interval of Flexpack HP whole blood test when the study population was divided into two halves according to their chronological entry into the study

	First half	Second half
Sex (M/F)	51/100	50/103
Mean Age	47.9	47.7
Sensitivity (%)	64.9 (53-75)*	52.3 (41-63)
Specificity (%)	88.1 (78-95)	96.8 (89-100)
Positive predictive value (%)	86.2 (75-94)	95.8 (86-99)
Negative predictive value (%)	68.6 (58-78)	58.8 (49-68)
Accuracy (%)	75.7 (68-82)	70.7 (63-78)

* 95 percent confidence intervals shown in brackets.