Diagnostic accuracy of serological kits for *Helicobacter pylori* infection with the same assay system but different antigens in a Japanese patient population

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*Helicobacter pylori* infection is thought to be a causal risk factor for gastric carcinoma (Nomura et al., 1991; Shimizu et al., 1999). Many ELISA kits for detection of *H. pylori* antibodies are available and have reportedly provided highly reliable results in Western countries (Evans et al., 1989; Crabtree et al., 1991; Jensen et al., 1993; Schembri et al., 1993; Marchildon et al., 1996; Wilcox et al., 1996; Meijer et al., 1997; Leung et al., 1999; Raymond et al., 1999). However, diagnostic accuracy of kits made in Western countries has been reported to be lower when used among Oriental populations. Diagnostic accuracy of two serological kits [HM-CAP and HM-CAP with antigens extracted from clinically isolated Japanese *H. pylori* strains (J-HM-CAP)] was investigated in 440 samples from a Japanese patient population by using the 13C-urea breath test as gold standard. According to the original optimal cut-off value, HM-CAP provided 87.5% sensitivity and 84.8% specificity with 86.8% accuracy and J-HM-CAP provided 95.5% sensitivity and 81.9% specificity with 92.3% accuracy. This study suggests that antigens from HM-CAP are satisfactory for examining a Japanese patient population, but that using local antigens improves accuracy.

Introduction

*Helicobacter pylori* infection is thought to be a causal risk factor for gastric carcinoma (Nomura et al., 1991; Shimizu et al., 1999). Many ELISA kits for detection of *H. pylori* antibodies are available and have reportedly provided highly reliable results in Western countries (Evans et al., 1989; Crabtree et al., 1991; Jensen et al., 1993; Schembri et al., 1993; Marchildon et al., 1996; Wilcox et al., 1996; Meijer et al., 1997; Leung et al., 1999; Raymond et al., 1999). However, diagnostic accuracy of kits made in Western countries has been reported to be lower in Chinese patients (Leung et al., 1999). We also found that imported serological kits yielded many intermediate results for Japanese patients; therefore, their effectiveness seems somewhat limited in a Japanese patient population (Miwa et al., 2000).

To gain a better understanding of the variation in performance of these kits in a non-Western patient population, we compared the diagnostic accuracy of two serological kits [HM-CAP and HM-CAP with antigen extracted from clinically isolated Japanese *H. pylori* strains (J-HM-CAP)] in our Japanese patient population.

Methods

**Patients.** We measured *H. pylori* IgG antibody in 440 stock serum samples frozen at −80 °C, which were obtained from patients admitted to Juntendo University Hospital for dyspeptic symptoms. These conserved serum samples were included in a previous study (Miwa et al., 2000).

**Urea breath test.** In these 440 patients, the 13C-urea breath test and blood-draw for serological antibodies to *H. pylori* were performed. The sample included 305 males and 135 females, with a mean age of 47.2 ± 14.0 (range, 19–85) years. They were confirmed not to have used a proton-pump inhibitor or antimicrobials that might affect the 13C-urea breath-test value. In addition, they had not undergone any previous *H. pylori* eradication therapy. All patients provided informed consent before the 13C-urea breath-test procedure. The blood draw and 13C-urea breath test were performed before treatment drugs were prescribed to eradicate the bacteria; both procedures were carried out within 1 month. We used a modified 13C-urea breath-test method that involved the following procedures: overnight fasting, 100 mg dosage of 13C-urea, maintaining patients in a sitting position, tooth-brushing and mouth-washing before and immediately after undergoing a 20 min

**Abbreviations:** EV, ELISA value; ROC, receiver operator characteristic.
point breath sampling and a 5‰ cut-off value. This modified breath test provided 96.7% specificity and 96.5% sensitivity (Miwa et al., 1997, 1998).

ELISA assay. In this study, we evaluated and compared the diagnostic accuracy of imported and domestic ELISA kits for detection of IgG antibodies to *H. pylori*: HM-CAP (Enteric Products, Westbury, NY, USA) and J-HM-CAP (Kyowa Medex, Japan). J-HM-CAP uses the same assay system as HM-CAP, except for the antigens: those used in HM-CAP are derived from American *H. pylori* strains, but J-HM-CAP uses mixed antigens derived from four Japanese strains. Two strains were from gastric ulcer patients, one from a gastric carcinoma patient and one from a non-ulcer dyspepsia patient (Marchildon et al., 2003). The assay was performed according to the manufacturer’s instructions. According to the recommended cut-off value [2.3 ELISA value (EV)] of HM-CAP, results were determined to be positive, negative or intermediate. Results also determined to be positive or negative according to the appropriate cut-off value estimated from the receiver operator characteristic (ROC) curve for each kit. All samples were assayed simultaneously in a blind fashion in terms of accompanying clinical information on patients.

Statistics. For statistical analysis, $\chi^2$ testing and a 95% confidence interval were used, with a $P$-value of $<0.05$ being regarded as statistically significant.

Results and Discussion

Three hundred and thirty-five of 440 patients were diagnosed as *H. pylori*-infected, and the remaining 105 patients as infection-negative, by the $^{13}$C-urea breath test. By using these results as gold standard, we evaluated the diagnostic accuracy of the two serological kits.

First of all, we used the recommended cut-off value (2.3 EV) of the imported serological kit to analyse serology results. We determined results as positive, negative or intermediate according to the recommended cut-off value and calculated the diagnostic accuracy of serology after excluding patients with intermediate values (Table 1). Sensitivity of J-HM-CAP (97.0%) was higher than that of HM-CAP (94.0%). Specificities of J-HM-CAP and HM-CAP were 76.6% and 82.4%, respectively. The intermediate result of J-HM-CAP was 3.0%.

**Table 1.** Comparison of serological and breath-test results by using the recommended HM-CAP cut-off value

<table>
<thead>
<tr>
<th>Serology results</th>
<th>Urea breath test</th>
<th>Diagnostic accuracy of serology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>J-HM-CAP Positive (2.3+)</td>
<td>323</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Intermediate (1.8–2.2)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig. 1. ROC curve for J-HM-CAP. Italicized numbers represent cut-off values. Table indicates sensitivity and specificity with cut-off value at 2.0, 2.3, 2.5, 2.7, 3.0 and 3.5 EV.

Fig. 2. ROC curve for HM-CAP. Italicized numbers represent cut-off values.
Table 2. Comparison of serological and breath-test results by using the optimal cut-off value estimated from each ROC curve

Data are expressed with 95 % confidence interval. Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

<table>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>J-HM-CAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (2·7+)</td>
<td>320</td>
<td>19</td>
</tr>
<tr>
<td>Negative (0–2·6)</td>
<td>15</td>
<td>86</td>
</tr>
<tr>
<td>Positive (2·5+)</td>
<td>293</td>
<td>16</td>
</tr>
<tr>
<td>Negative (0–2·4)</td>
<td>42</td>
<td>89</td>
</tr>
<tr>
<td>HM-CAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (2·5+)</td>
<td>103</td>
<td>96</td>
</tr>
<tr>
<td>Negative (0–2·5)</td>
<td>41</td>
<td>99</td>
</tr>
</tbody>
</table>

*P < 0·01 versus HM-CAP.

(13/440), which was better than that of HM-CAP [4·3 % (19/440)]. With this cut-off value, there were no significant differences in diagnostic accuracy between the kits.

We then plotted original ROC curves for J-HM-CAP and HM-CAP to establish the appropriate cut-off value of each kit for our patient population (Figs 1 and 2). The ROC curve for J-HM-CAP is shown in Fig. 1; the graph depicts the optimal cut-off value of J-HM-CAP in our patient population to be 2·7 EV, showing that the specificity of this kit had improved from 76·6 to 81·9 % with little decline in sensitivity (Table 2). The optimal cut-off value of HM-CAP in our patient population was 2·5 EV (Fig. 2). Under each optimal cut-off value, the sensitivity, negative predictive value and accuracy of J-HM-CAP were significantly different (P < 0·01) from those of HM-CAP (Table 2).

Numerous reports have indicated differing performance for these serological kits in various populations. For example, sensitivity and specificity of HM-CAP in the USA have been reported to be respectively 98·7 and 100 % (Evans et al., 1989) and 98·4 and 96·4 % (Marchildon et al., 1996), but in Denmark, they have been reported to be 81 and 71 % (Jensen et al., 1993) and in China, 72·7 and 68·4 % (Leung et al., 1999). Our study also showed the sensitivity and specificity values of HM-CAP in Japan to be lower than those in the USA.

There may be several reasons for these differences, such as the presence of strain heterogeneity in different geographic regions (Ohtsuka et al., 1997), geographic variation in cross-reactivity to other intestinal pathogens (Graham et al., 1996) and various immunological responses to H. pylori antigens in different patient populations (Khanna et al., 1998).

Marchildon et al. (2003) also tested 13C-urea breath test-characterized serum samples from Japanese patients by using both J-HM-CAP and HM-CAP kits. Compared with their results, specificity and accuracy of both J-HM-CAP and HM-CAP were lower in our study. This may be because the diseases in their subjects were different or because we adopted a higher 13C-urea breath test cut-off value.

In conclusion, we found that J-HM-CAP was more suitable for use in a Japanese patient population and that although HM-CAP proved to be satisfactory for use in this population, changing its cut-off value or using local antigens served to improve its accuracy. Our study suggests that using local antigens may improve the diagnostic accuracy of serological kits for H. pylori infection.

Acknowledgements

We would like to thank Ms Oikawa and Ms Endo for technical assistance with this study.

References


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