Adjustment of Cut-off Values in ELISA for Detection of *Helicobacter pylori* Infection

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ABSTRACT

**Aim:** to detect *Helicobacter pylori* infection in Indonesian patients.

**Methods:** serum samples were collected from patients referred for upper endoscopy. Gastric biopsies were taken for polymerase chain reaction (PCR) examination. The gold standard for diagnosing *H. pylori* infection was 294 bp amplification target of PCR examination with primer ureC. The serum samples were tested for *H. pylori* antibodies using Biohit Anti-*H. pylori* ELISA according to the manufacturer instructions. By using analysis of receiver operating characteristic (ROC), an optimal cut-off point of serum IgG *H. pylori* and area under curve (AUC) ROC were determined. The accuracy of test was calculated according to a new cut-off value. Significance level was determined by p value < 0.05.

**Results:** a total of 81 patients were recruited, 31 (41.9%) subjects were *H. pylori*-positive. We found a new optimal cut-off point on serum IgG *H. pylori* at 15.2 IU/mL with AUC ROC of 0.84. By using the new cut-off value, we conclude that the Biohit Anti-*H. pylori* ELISA has a good sensitivity and specificity, i.e. 94.1% (95% CI 59.9 - 89.6) and 97.9% (95% CI 92.3 - 100.0) respectively.

**Conclusion:** by adjusting new cut-off values for Indonesian patients, we are able to improve the performance of Biohit Anti-*H. pylori* ELISA. This study illustrates the importance of local validation.

**Key words:** *Helicobacter pylori*, polymerase chain reaction, ureC primer, receiver operator curves, IgG.

INTRODUCTION

*H. pylori* is the most commonly found pathogen bacteria in the gastrointestinal tract and its existence is associated with chronic gastritis, gastroduodenal ulcer, gastric adenocarcinoma and MALT (Mucosa-Associated Lymphatic Tissue Lymphomas). Moreover, the World Health Organization has included *H. pylori* as a class I carcinogen due to its strong correlation with gastric cancer. For preventive purpose, a simple and non-invasive tests for detecting *H. pylori* infection is required.

*H. pylori* antibodies found in human serum are indicators for *H. pylori* infection. Positive *H. pylori* IgG results for diagnosis of *H. pylori* infection has been proven in reports of various countries with high sensitivity and specificity. However, the results are different among countries, especially between developed and developing countries. Such differences may arise due to different optimal cut-off value of IgG level compared to the cut off determined by the manufacturer of antibody serologic test kit for *H. pylori*. The optimal cut-off value which has been determined by the manufacturer may not always be used for various different population or sub-groups.

To obtain an optimal cut-off value of a test, we can use receiver operating characteristic (ROC) analysis in local population.

The aim of the study was to determine the optimal cut-off value for Biohit Anti-*H. pylori* ELISA serologic test at Margono Hospital in Purwokerto, Central Java.

METHODS

Our study used consecutive sampling method, i.e. all patients with dyspepsia who were hospitalized at the Department of Internal Medicine, Faculty of Medicine and Health, University of General Soedirman/Margono...
Hospital, Purwokerto and who had fulfilled the inclusion and exclusion criteria.

The inclusion criteria were patients aged over 18 years, who have signed informed consent and had complete data, particularly \textit{H. pylori} PCR result and IgG \textit{H. pylori} level. The exclusion criteria were patients with alarm signs such as having family history of gastrointestinal cancer, loss of weight due to unknown etiologies, progressive dysphagia, odinophagia, deficiency anemia of unknown origin, persistent vomiting, lymphadenopathy, hyperbilirubinemia, gastroesophageal reflux, history of gastrointestinal surgery, pregnancy and medication use (proton pump inhibitor, H2-receptor antagonist, non-steroid anti-inflammatory drugs and antibiotics) within 4 weeks prior to the study.

The gold standard for \textit{H. pylori} detection was performed by polymerase chain reaction (PCR) method. The examined materials were obtained from gastric specimen through endoscopic biopsy. The reagent kit was PCR Core System Promega #M7660, Promega Corp. Madison WI, USA). UreC/gImM primer was utilized with following sequence:

- \text{ureC}1 5’-GGATAAGCTTTAGGGTGTTAGGGG-3’
- \text{ureC}2 5’-GCTTGCTTTCTAACACTAACGCGC-3’

(Invitrogen, Tokyo Japan)

The criteria of positive \textit{H. pylori} infection was determined if the amplification target of 294 bp was found. (Figure 1).

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The ELISA test was performed by using Helicobacter pylori IgG ELISA KIT (Cat. No. 601040.01) according to the manufacturer instructions. The IgG cut-off point determined by the manufacturer was 0-30 IU/mL.

After collecting data, we performed data cleaning, coding, tabulating and data entry. On descriptive analysis, the categorical variables were expressed as distribution of frequency and proportion (n and %); while the continuous data were expressed as mean value and standard deviation as well as other necessary statistical data.

In order to determine the optimal cut-off value of IgG \textit{H.pylori} level, ROC was used as well as the determination of area under curve (AUC). Based on the cut-off value, then the sensitivity, specificity, likelihood ratio and predictive value could be concluded.

The significance level of statistic test was determined by \( p \) value < 0.05. Statistical analysis was performed by using the MedCalc program version 11.3.0.0.

**RESULTS**

During the period of January to December 2005 at the Department of Internal Medicine, in Margono Hospital, Purwokerto, there were 16,258 hospitalized patients. 104 patients underwent endoscopic gastric biopsy for PCR ureC examination and the patients serum was examined for IgG \textit{H.pylori} serologic test. The prevalence of \textit{H. pylori} as expressed by positive PCR ureC was 38 subjects (36.5%).

There were 81 eligible subjects with subject characteristics, presented on Table 1.

The youngest age of subjects was 45 years and the oldest was 75 years; while the mean age was 56.8 ± 9.1 years. The proportion of male and female subjects was nearly equal, i.e. 40:41. The status of \textit{H. pylori} with positive PCR ureC was found in 34 subjects (41.9%) and negative ureC was found in 47 subjects (58.1%).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45-75 years</td>
</tr>
<tr>
<td></td>
<td>Mean/standard deviation</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40 subjects (49.9%)</td>
</tr>
<tr>
<td>Female</td>
<td>41 subjects (50.1%)</td>
</tr>
<tr>
<td>\textit{H.pylori} status</td>
<td></td>
</tr>
<tr>
<td>Positive UreC</td>
<td>34 subjects (41.9%)</td>
</tr>
<tr>
<td>Negative UreC</td>
<td>47 subjects (58.1%)</td>
</tr>
<tr>
<td>IgG \textit{H.pylori} level (IU/ml)</td>
<td>3.30-52.00</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>Mean/standard deviation</td>
</tr>
</tbody>
</table>

The range of IgG \textit{H. pylori} level was 3.30 – 52.00 IU/ml with mean value of 11.77 IU/mL and standard deviation of 8.27 IU/mL. The mean IgG level in \textit{H.pylori} positive ureC was 17.3 ± 6.7 IU/mL; while in \textit{H. pylori} negative ureC was 8.8 ± 2.5 IU/mL. The unpaired t-test demonstrated that there was statistically significant difference between positive and negative \textit{H. pylori} ureC regarding the IgG \textit{H. pylori}.
level (p = 0.00).

By using ROC, we found optimal cut-off value for IgG H. pylori more than 15.2 IU/m with AUCROC = 0.84 (Figure 2).

For the purpose of accuracy calculation including sensitivity, specificity, likelihood ratio and predictive value, a table of 2 X 2 was used as presented on Table 2.

<table>
<thead>
<tr>
<th>IgG H.pylori level</th>
<th>H.pylori ureC&lt;+&gt;</th>
<th>H.pylori ureC&lt;-&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 15.2 IU/mL</td>
<td>TP(32)</td>
<td>FP(1)</td>
</tr>
<tr>
<td>&lt; 15.2 IU/mL</td>
<td>FN(2)</td>
<td>TN(46)</td>
</tr>
</tbody>
</table>

The optimal point value of IgG H.pylori level was 15.2 IU/mL, the accuracy of such examination included sensitivity (Se) = TP / (TP+FN) = 32/(32+2) = 94.1%; specificity (Sp) = TN / (TN+FP) = 46/(46+1) = 97.9%; likelihood ratio positive (LR+) = Se/(1-Sp) = 0.94/ (1-0.98)=47; likelihood ratio negative(LR-) = (1-Se)/ Sp=(1-0.94)/0.98=0.06; positive predictive value(PPV) = TP/(TP+FP) =32/(32+1) = 96.9%; negative predictive value (NPV) = TN/(TN+FN) = 46/(46+2) = 95.8%.

DISCUSSION

The prevalence of H.pylori infection worldwide varies either among countries or among populations in a country. In developing countries, the prevalence of H.pylori infection is high, approximately about 80-90%; while in developed countries, the prevalence is less than 40%. Such different prevalence is caused by age of acquisition at initial H. pylori infection and different method of examination for H. pylori detection. A study in Jakarta reported the prevalence of H. pylori infection in dyspepsia patients by using urea breath test as high as 19%. By using the PCR ureC method, our study reported 36.5% prevalence of H. pylori; while other investigators reported prevalence ranges from 33-70.1%.

UreC primer is the best primer for detecting H. pylori since its amplification is only found in H. pylori and absent for other positive urease bacteria; therefore, no false positive or false negative result will be found. Hence, it can be used as gold standard for detecting the presence of H.pylori infection.

Optimal cut-off point for H. pylori IgG level was 15.2 IU/mL, which can be utilized accurately to substitute the gold standard examination for detecting H.pylori infection due to various reasons. The first reason is proven by using the ROC model to calculate the AUCROC. The AUCROC value between 0.8-0.9 can be considered as very good. In our study, the AUCROC was 0.84.

Other reasons are the observation on accuracy results including sensitivity, specificity, likelihood ratio and predictive value. For diagnostic purpose, ideally there should be a test with high sensitivity and specificity, reaching up to 100% for each. However, such test has not been present yet. Our study reported nearly 100% sensitivity and specificity, i.e. 94.1% and 97.9%. Likelihood ratio (LR) is the likelihood that a given test result would be expected in a patient with target disorder compared to the likelihood that same result would be expected in a patient without the target disorder. LR + is the likelihood of positive results with target disorder; while LR – is the likelihood of positive results in subjects without target disorder. LR+ value greater than 1 indicates test result correlated to subjects with disease; while LR – value less than 1 was associated with subjects without disease. Our study result showed LR + of 47 and LR– of 0.06.

Predictive value is used to recognize the test that results in correct diagnosis. The optimal cut-off value is perfect when PPV = 1 and NPV =1.2 In our study, the predictive value was almost perfect with PPV = 96.9% and NPV = 95.8%.

Similar studies have also been conducted by Szetos ML et al and Xia HHX et al to determine optimal cut-off value of several commercial serological kits for detection of H. pylori in Chinese population.

CONCLUSION

It can be concluded that by conducting adjustment for cut-off point in Indonesian patients at the Margono Hospital, Purwokerto, we can improve the performance of Biohit Anti H.pylori ELISA test.
REFERENCES