Clinical Utility of Histological Examination of Gastric Ulcer Margin to Diagnose Helicobacter Pylori Infection

Mu-Shien Lee, MD; Chi-Ju Yeh1, MD; Hsing-Yu Chen1, MD; Yung-Kuan Tsou, MD; Cheng-Hui Lin, MD; Jau-Min Lien, MD

Background: To investigate the effectiveness of histological examination of ulcer margins (HEUM) in detecting Helicobacter pylori (H. pylori) infection in patients with non-bleeding gastric ulcers (GUs).

Methods: A retrospective study included 284 patients with GU undergoing concomitant HEUM and rapid urease test (RUT) to detect H. pylori infection between January 2005 and December 2006. The slides were reviewed by an experienced pathologist (revised HEUM) in the 52 patients with inconsistent results on the initial HEUM and RUT. H. pylori infection was defined as a positive RUT and/or revised HEUM. Detection rates of H. pylori infection for HEUM and RUT were calculated accordingly. In patients with H. pylori infection, several parameters including ulcer characteristics and pathological findings were compared between patients with negative and positive (revised) HEUM.

Results: A total of 164 (57.7%) patients had positive results of H. pylori infection. The overall detection rates of H. pylori infection on the initial HEUM, revised HEUM and RUT were 78.0% (128/164), 89.0% (146/164), and 94.5% (155/164), respectively. For antrum ulcers, the respective detection rates were 81.0% (85/105), 92.4% (97/105), and 93.3% (98/105), for angulus ulcers, 78.6% (22/28), 85.7% (24/28), and 100% (28/28), and for proximal stomach ulcers, 61.9% (13/21), 81.0% (17/21), and 90.4% (19/21). In patients with H. pylori infection, gastric malignancy was more frequently observed in patients with false negative than true positive HEUMs.

Conclusions: HEUM might be not sensitive enough for diagnosing H. pylori in patients with GU. It was especially insensitive when the ulcers were in the proximal stomach, the ulcers were malignant, or the slides were interpreted by pathologists in a rotating manner.

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Key words: histology of gastric ulcer biopsy, helicobacter pylori infection, rapid urease test

Helicobacter pylori (H. pylori) infection is a major etiological cause of peptic ulcers.1,2 The eradication of this pathogen reduces ulcer recurrence and the methods for doing this are cost effective.3,4 Therefore, the development of accurate tests to detect this organism is important. Current guidelines...
suggest that *H. pylori* can be diagnosed by several invasive and noninvasive methods. Invasive methods are biopsy-based tests which require endoscopy, including a rapid urease test (RUT), histology, culture, and polymerase chain reaction. When endoscopy is indicated, a rapid RUT is generally the test of choice because of its simplicity, low cost, and relatively rapid results. In patients with gastric ulcers (GUs), however, multiple biopsy specimens must be obtained from the ulcer margin to distinguish benign from malignant ulcers. *H. pylori* status has been recommended to be included in the diagnostic phrase if they are detected in any of the biopsy specimens. Therefore, this histological examination of ulcer margins (HEUM) will also reveal *H. pylori* infection in patients with GU, but there are few studies that have evaluated the accuracy of this test. Despite a lack of evidence in the literature, using HEUM to diagnose *H. pylori* infection in GU patients has been a common practice in Taiwan because of health insurance reimbursement restrictions. Currently, the only test for *H. pylori* infection which is paid for by insurance is the urea breath test performed after anti-*H. pylori* therapy for patients with peptic ulcers documented on endoscopy. The mean number of specimens was 4.2 (range from 2-9). The biopsy specimens were histologically examined by hematoxylin and eosin (H & E) staining in all cases. These H & E-stained slides were interpreted by faculty members of the Department of Pathology in a rotating manner. The primary goal of the ulcer biopsy was to exclude gastric malignancy, but *H. pylori* status was also included in the pathologic reports (initial HEUM) in all cases. In 7 patients, histopathology showed the GUs were malignant.

When the results of the initial HEUM and RUT were inconsistent, an experienced pathologist (Yeh CJ) who was blinded to the results of the RUT and initial HEUM reviewed all H & E-stained slides to diagnose *H. pylori* infection (revised HEUM).

**Definition of *H. pylori* infection and statistical analysis**

For the purpose of analysis, patients with *H. pylori* infection were defined as follows: (1) The

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**METHODS**

**Patients**

In a 2-year period between 2005 and 2006, 1615 patients had endoscopically diagnosed GUs at Chang Gung Memorial Hospital (Division of Digestive Therapeutic Endoscopy; Linkou Center, Taipei and Taoyuan Branches). Among them, 284 patients undergoing concomitant GU biopsy and RUT were enrolled in this study. Follow-up endoscopic procedures to document ulcer healing were not included in the study. Patients with the following criteria were also excluded: age younger than 20 years, gastrectomy prior to endoscopy, prominent ulcerative mass on endoscopy, active ulcer bleeding, and intake of antibiotics or proton pump inhibitors within 2 weeks before endoscopy. Patient data and endoscopic findings, as well as pathologic reports were collected and analyzed retrospectively.

**Gastric ulcers**

A GU was defined by endoscopic findings as a mucosal ulceration in the stomach regardless of its size. GUs were classified by location in the antrum, angulus, or proximal stomach (including the body, fundus, and cardia). Patients with ulcers at more than 2 locations (n = 39) were classified according to the biopsied ulcer. The largest or morphologically abnormal ulcer was usually chosen as the biopsy site. In patients with more than one ulcer (n = 141), the ulcer size was calculated according to the biopsied ulcer.
RUT and revised HEUM were both positive (n = 137); (2) Both the initial and revised HEUM were positive and the RUT was negative (n = 9); (3) The RUT was positive and both the initial and revised HEUM were negative (n = 18) (Table 1). Accordingly, there were 164 (57.7%) patients with H. pylori infection in this study.

Quantitative data were expressed as mean ± standard deviation. Differences were compared using the two-sample t-test for continuous variables and the χ²-test or Fisher’s exact test for categorical variables. The analyses were performed with the statistical software SPSS 18.0 version for Windows. A p value of < 0.05 was considered statistically significant.

Ethics
The study protocol was approved by the ethics committee at Chang Gung Memorial Hospital (IRB No: 98-2704B).

RESULTS

A total of 284 patients with GUs were analyzed. The mean age of the patients was 57.6 ± 15.6 years (range, 27–91 years); 57.4% of them were men. The size of the GUs was 8.5 ± 6.1 mm (range, 2–30 mm).

Initial HEUM and RUT
The results of the initial HEUM and RUT (Table 1) were consistent in 232 (81.7%) patients: both tests were positive in 119 patients and both were negative in 113 patients. In the remaining 52 (18.3%) patients, the HEUM was positive and RUT was negative in 16 patients, and the HEUM was negative and RUT was positive in 36 patients.

Revised HEUM and RUT
Results of the revised HEUM and RUT are shown in Table 1. After pathological review of the 52 patients with initially inconsistent results between HEUM and RUT, 9 (17.3%) cases remained positive and 18 (34.6%) remained negative for H. pylori infection. In the remaining 25 (48.1%) cases, the results turned out to be consistent with those of the RUT (Table 1) in 7 of the 16 patients with initially positive HEUMs and 18 of the 36 patients with initially negative HEUMs. Accordingly, the revised HEUM and RUT were consistent in 257 (90.5%) patients: both were positive in 137 patients and both were negative in 120 patients. The HEUM remained positive and the RUT was negative in 9 patients. The HEUM remained negative and the RUT was positive in 18 patients.

Detection rates of HEUM and RUT
Detection rates of H. pylori infection for each test are shown in Table 2. According to the definition of the study, 164 (57.7%) of the 284 patients were positive for H. pylori infection. The detection rates of H. pylori infection for the initial HEUM, revised HEUM, and RUT were 78.0% (128/164), 89.0% (146/164), and 94.5% (155/164), respectively.

Table 1. Histological Examination of Ulcer Margin and Rapid Urease Test for Detection of H. Pylori Infection in Patients with Gastric Ulcers

<table>
<thead>
<tr>
<th>Test results</th>
<th>Initial HEUM (n = 284)</th>
<th>Revised HEUM (n = 284)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEUM (+) RUT (+)</td>
<td>119</td>
<td>137</td>
</tr>
<tr>
<td>HEUM (+) RUT (–)</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>HEUM (–) RUT (+)</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td>HEUM (–) RUT (–)</td>
<td>113</td>
<td>120</td>
</tr>
</tbody>
</table>

Abbreviations: HEUM: histological examination of ulcer margin; RUT: rapid urease test.

Table 2. Detection Rates of H. Pylori Infection at Different Locations

<table>
<thead>
<tr>
<th>Overall (n = 164)</th>
<th>Antrum ulcers (n = 105)</th>
<th>Angulus ulcers (n = 28)</th>
<th>Proximal stomach ulcers (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEUM Initial</td>
<td>RUT</td>
<td>HEUM Initial</td>
<td>RUT</td>
</tr>
<tr>
<td>Detection Rate</td>
<td>(n)</td>
<td>Detection Rate</td>
<td>(n)</td>
</tr>
<tr>
<td>78.0%</td>
<td>(128)</td>
<td>81.0%</td>
<td>(85)</td>
</tr>
<tr>
<td>89.0%</td>
<td>(146)</td>
<td>92.4%</td>
<td>(97)</td>
</tr>
<tr>
<td>94.5%</td>
<td>(155)</td>
<td>93.3%</td>
<td>(98)</td>
</tr>
</tbody>
</table>

Abbreviations: HEUM: histological examination of ulcer margin; RUT: rapid urease test.
Detection rates of HEUM and RUT according to ulcer location

The results according to ulcer location are shown in Table 2. A total of 16 patients were excluded for analysis because the ulcer location was unclear in the chart records (n = 2) or biopsy samples were obtained from 2 or more ulcer locations and were stored in a container for pathological examination (n = 14). As a result, 268 patients were included in this analysis. One hundred and ninety-seven (73.5%) patients had antrum ulcers, 35 (13.1%) had angulus ulcers, and 36 (13.4%) had proximal stomach ulcers. For antrum ulcers, 105 (53.3%) patients had H. pylori infection. The detection rates for the initial HEUM, revised HEUM, and RUT were 81.0% (85/105), 92.4% (97/105), and 93.3% (98/105), respectively. For angulus ulcers, 28 (80.0%) patients had H. pylori infection. The detection rates for the initial HEUM, revised HEUM, and RUT were 78.6% (22/28), 85.7% (24/28), and 100% (28/28), respectively. For proximal stomach ulcers, 21 (58.3%) patients had H. pylori infection. The detection rates for the initial HEUM, revised HEUM, and RUT were 61.9% (13/21), 81.0% (17/21), and 90.4% (19/21), respectively.

Detection rates of HEUM according to number of biopsied specimens

The detection rate of H. pylori infection for the revised HEUM was 90.9% (50/55) in patients with 2 to 3 biopsy specimens, and 88.1% (96/109) in patients with 4 or more biopsy specimens. All 7 patients with malignant ulcers underwent 4 or more biopsies. When the patients with gastric malignancy were excluded from analysis, the detection rate on the revised HEUM was 91.7% (100/109) in patients with 4 or more biopsy specimens.

Factors attributed to false negative HEUM

Among the 164 patients with H. pylori infection, 18 (11%) were negative (false negative) and 146 (89%) were positive (true positive) for H. pylori infection according to the revised HEUM. Age, sex, ulcer location and size, number of biopsy specimens, and pathological findings including malignancy, intestinal metaplasia and atrophy were compared between patients with false negative and true positive HEUMs (Table 3). Only malignancy was found significantly more prevalent in patients with false negative HEUMs than in patients with true positive HEUMs.

Table 3. Comparisons between patients with false negative and true positive histological examinations of the gastric ulcer margin among the 164 patients with H. pylori infection.

<table>
<thead>
<tr>
<th>Factor</th>
<th>False negative (n = 18)</th>
<th>True positive (n = 146)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.1 ± 18.0</td>
<td>57.3 ± 14.5</td>
<td>0.45</td>
</tr>
<tr>
<td>Sex (women)</td>
<td>6 (33.3%)</td>
<td>51 (34.9%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Ulcer location* (A/G/B)</td>
<td>8/4/4</td>
<td>97/24/17</td>
<td>0.29</td>
</tr>
<tr>
<td>Ulcer size</td>
<td>11.7 ± 7.2</td>
<td>9.0 ± 6.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Number of biopsies</td>
<td>4.3 ± 1.3</td>
<td>4.2 ± 1.5</td>
<td>0.69</td>
</tr>
<tr>
<td>Malignant ulcers</td>
<td>4 (22.2%)</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>4 (22.2%)</td>
<td>27 (18.5%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Gastric atrophy</td>
<td>1 (5.6%)</td>
<td>0</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*: Two patients with false negative and 8 patients with true positive HEUMs were excluded from analysis because of unidentifiable ulcer locations.

Abbreviations: HEUM: histological examination of ulcer margin; A: antrum; G: angulus; B: proximal stomach including body, fundus, and cardia.

DISCUSSION

When endoscopy is indicated, RUT or histopathologic interpretation of H & E-stained biopsy specimens from a nonulcer part of the stomach is generally the test of choice to detect H. pylori infection. There is no study suggesting the use of HEUM. In Taiwan, however, HEUM is often used for diagnosing H. pylori infection in patients with GUs because of reimbursement restrictions from health insurance. This study showed that the revised HEUM was less sensitive (89.0%) to detect H. pylori infection than the RUT (94.5%). The detection rate was even lower (78.0%) when the slides were interpreted by pathologists in a rotating manner. Furthermore, we found that HEUM was least sensitive for patients with proximal stomach ulcers. This result may reflect the fact that the density of H. pylori is lower in the gastric body than in the antrum, and an H & E stain is less sensitive in detecting H. pylori of specimens containing only small numbers of organisms.
Special stains such as modified Giemsa, Warthin-Starry, Genta, or specific immune stains, which are reported to have higher diagnostic sensitivity than that of an H & E stain to detect H. pylori in nonulcer specimens, were not used in this study. However, the standard H & E stain is excellent to determine histological inflammation or malignancy, as well as H. pylori status, if adequate numbers of organisms are present in specimens. Since the primary goal of ulcer biopsy is to detect malignancy, the aim of this study was to demonstrate the accuracy of this routine practice (ulcer biopsy) for detecting H. pylori infection in patients with GUs. Therefore, we did not evaluate the accuracy of HEUM by special stains.

Kolts et al. reported that interpretation by an experienced pathologist is significantly better and may present an advantage over analysis by rotating pathologists in evaluating H & E-stained biopsy specimens. In the present study, the initial HEUMs were interpreted by pathologists in a routine rotating manner. After review of the slides by an experienced pathologist, 25 (48.1%) of the 52 initially inconsistent results on the HEUM and RUT turned out to be consistent. Consequently, the detection rates of H. pylori infection by HEUM increased from 78.0% to 89.0%. This result was in agreement with that of a previous report and suggested that H & E-stained biopsy specimens should be interpreted by an experienced pathologist.

Eighteen (11.0%) of the 164 patients had false negative tests on the revised HEUM. We analyzed several parameters and found that only malignancy was more prevalent in patients with negative than positive HEUMs. In fact, none of the 7 patients with gastric malignancy had a positive HEUM, compared with 4 of the 7 patients with a positive RUT. Therefore, HEUMs may be unreliable for diagnostic H. pylori infection in patients with malignant gastric ulcers. Instead, the upper body greater curvature side has been suggested to be the most sensitive and specific biopsy site for detecting H. pylori in patients with gastric cancers.

Genta et al. reported that that two antral biopsy specimens (one from the lesser and one from the greater curvature of the nonulcer part) yielded virtually 100% sensitivity for detecting H. pylori infection. Specimens from the corpus did not increase the diagnostic yield unless extensive intestinal metaplasia was present in the antrum. For HEUM, the results of the present study showed that the detection rates of H. pylori infection were comparable between using 2 to 3 biopsy specimens and 4 or more specimens. This result suggests that 2 to 3 biopsy specimens obtained from the ulcer margin may be sensitive enough to detect H. pylori infection.

The major limitation of this study was that the definition of patients with H. pylori infection was somewhat arbitrary. This is mainly because there is no single test that can be considered the gold standard for the diagnosis of H. pylori. In this study, however, most (137 of 164, 83.5%) patients were positive on both the revised HEUM and RUT. Nine (5.5%) patients were diagnosed with H. pylori infection based on only the revised HEUM. However, both of the two pathologists agreed that H. pylori were present in the biopsy specimens. In the remaining 18 (11.0%) patients, H. pylori infection was diagnosed only according to a single RUT. However, the specificity of the RUT is reported to be nearly 100%. That is, a false positive RUT is unusual. Therefore, we believed that the definition of patients with H. pylori infection in the present study was reasonable.

In conclusion, this report is one of the few studies dealing with HEUM for diagnosing H. pylori infection in GU patients. HEUM by H & E stain, a routine test for excluding malignant gastric ulcers, might be not sensitive enough for diagnosing H. pylori in GU patients. HEUM was especially insensitive when the ulcers were in proximal stomach and/or when the slides were interpreted by pathologists in a rotating manner. It was also unreliable for patients with malignant gastric ulcers.

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Diagnosing *H. pylori* from ulcer margins


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胃溃疡边缘生検法诊断幽門螺旋桿菌感染之临床应用

李沐憲 葉琦如 陳星譯 歐永寬 林政輝 連昭明

背 景：目前很少有利用胃溃疡边缘切片以诊断幽門螺旋桿菌感染的研究。本研究的目的在於评估用胃溃疡边缘组织切片以诊断非出血性胃溃疡患者是否有幽門螺旋桿菌感染的临床应用性。

方法：本研究为一回顾性研究，包含284位非出血性胃溃疡患者。所有患者都同时接受溃疡边缘切片的组织学检查 (histological examination of ulcer margin, HEUM) 及快速尿素酶試驗來検出其是否有幽門螺旋桿菌的感染。其中有52位患者溃疡边缘切片的組織学检查和快速尿素酶試驗的结果並不一致，這些患者的病理組織切片則由一位有經驗的病理科醫師重新判讀 (revised HEUM)。幽門螺旋桿菌感染在本研究的定義為病理組織學检查陽性及 / 或快速尿素酶試驗陽性，據此可計出個別検验法（病理組織検查或快速尿素酶検查）的診斷正確率；一些變項，包括年紀、性別、潰瘍位置及大小、切片数目以及病理組織學發現，例如是否有息肉性细胞、腸上皮化生、及胃黏膜萎縮等都將在病理組織學検查幽門螺旋桿菌真陽性以及偽陰性之患者之間做比較。

结果：根据本研究的定義總共有164 位患者有幽門螺旋桿菌的感染。原始的溃疡边缘切片的組織学検査 (initial HEUM)，重新判讀的病理組織学検査 (revised HEUM)，及快速尿素酶試驗三種不同的検驗法其幽門螺旋桿菌的検出率分別為 78.0% (128/164)，89.0% (146/164)，及 94.5% (155/164)。對於不同潰瘍部位的分析结果如下：胃窦部的潰瘍，上述的三種検査其個別的検出率分別為 81.0% (85/105)，92.4% (97/105)，以及 93.3% (98/105)；胃角處的潰瘍，78.6% (22/28)，85.7% (24/28)，及 100% (28/28)；胃近端部的潰瘍，61.9% (13/21)，81.0% (17/21)，及 90.4% (19/21)。所有統計的變項中，只有胃黏膜萎縮這一項因子在病理組織學検查幽門螺旋桿菌陽性之患者中明顯多於病理組織學検查偽陽性的患者。

结论：對於胃溃疡的患者，使用溃疡边缘切片的組織学検查來検査其是否有幽門螺旋桿菌可能存在不夠準確，尤其是發生在胃近端部的潰瘍，或當病理切片被依常規輪班方式的病理科醫師判讀時；另外，當胃溃疡為惡性腫瘤時，用溃疡边缘切片的組織学検查來诊断患者是否有幽門螺旋桿菌的感染並不可靠。

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關鍵詞：潰瘍生検之組織学検査，幽門螺旋桿菌感染，快速尿素酶試験

長庚醫療財團法人長庚紀念醫院 腸胃腸科系・病理科系・中醫科系・長庚大學 醫學院

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連線作者: 鄭永寬醫師。長庚醫療財團法人長庚紀念醫院 腸胃腸科系。桃園縣333龜山鄉復興街5號。

Tel: (03)3281206轉8108; Fax: (03)3272236; E-mail: flying3454@xuite.net