

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

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**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.<sup>(1,2)</sup> The eradication of this pathogen reduces ulcer recurrence

and the methods for doing this are cost effective.<sup>(3,4)</sup> Therefore, the development of accurate tests to detect this organism is important. Current guidelines

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suggest that *H. pylori* can be diagnosed by several invasive and noninvasive methods.<sup>(5)</sup> 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.<sup>(5-8)</sup>

For histological examination to detect *H. pylori*, biopsy specimens should be obtained from a nonulcer part of the stomach.<sup>(5,9)</sup> In patients with gastric ulcers (GUs), however, multiple biopsy specimens must be obtained from the ulcer margin to distinguish benign from malignant ulcers.<sup>(10,11)</sup> *H. pylori* status has been recommended to be included in the diagnostic phrase if they are detected in any of the biopsy specimens.<sup>(9)</sup> Therefore, this histological examination of ulcer margins (HEUM) will also reveal *H. pylori* infection in patients with GU, but there few studies 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.<sup>(12)</sup> 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.<sup>(13)</sup> Therefore, the aim of this study was to evaluate the effectiveness of HEUM in detecting *H. pylori* infection in GU patients.

## 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 find-

ings, 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.

### Rapid urease test and ulcer biopsy

Pronto Dry (Medical Instruments Corporation, ZI Nord, Brignais, France), a biopsy urease test, was used for the RUT. The clinician and endoscopist varied. The RUT was carried out only upon clinician's request before the endoscopy. It was our practice to obtain two specimens from the nonulcer part of the antrum for the RUT. The tests were carried out until positive results were obtained or for up to one hour. A color change from yellow to pink was considered a criterion for the presence of *H. pylori* infection.

Biopsy specimens for histological examination were obtained from the GU margin (that is, HEUM). 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

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  $\chi^2$ -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

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

Test results	Initial HEUM (n = 284)	Revised HEUM (n = 284)
HEUM (+) RUT (+)	119	137
HEUM (+) RUT (-)	16	9
HEUM (-) RUT (+)	36	18
HEUM (-) RUT (-)	113	120

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

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

	Overall (n = 164)			Antrum ulcers (n = 105)			Angulus ulcers (n = 28)			Proximal stomach ulcers (n = 21)		
	HEUM		RUT	HEUM		RUT	HEUM		RUT	HEUM		RUT
	Initial	Revised		Initial	Revised		Initial	Revised		Initial	Revised	
Detection Rate (n)	78.0% (128)	89.0% (146)	94.5% (155)	81.0% (85)	92.4% (97)	93.3% (98)	78.6% (22)	85.7% (24)	100% (28)	61.9% (13)	81.0% (17)	90.4% (19)

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

(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.

### 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

**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.

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

\*: 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.

negative HEUMs than in patients with true positive HEUMs.

## 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*.<sup>(5,14,15)</sup> 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.<sup>(12,13)</sup> 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.<sup>(16,17)</sup>

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.<sup>(15,18,19)</sup> 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.<sup>(9)</sup> 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.<sup>(14)</sup> 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.<sup>(14)</sup>

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.<sup>(20)</sup>

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.<sup>(17)</sup> Specimens from the corpus did not increase the diagnostic yield unless extensive intestinal meta-

plasia 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*.<sup>(5)</sup> 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%.<sup>(6-8,16,21)</sup> 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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# 胃潰瘍邊緣生檢法診斷幽門螺旋桿菌感染之臨床應用

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**背景：** 目前很少有利用胃潰瘍邊緣切片以診斷幽門螺旋桿菌感染的研究。本研究的目的是在於評估用胃潰瘍邊緣組織切片以診斷非出血性胃潰瘍患者是否有幽門螺旋桿菌感染的臨床應用性。

**方法：** 本研究為一回溯性研究，包含 284 位非出血性胃潰瘍患者。所有患者都同時接受潰瘍邊緣切片的組織學檢查 (histological examination of ulcer margin, HEUM) 及快速尿素酶試驗來檢驗其是否有幽門螺旋桿菌的感染。其中有 52 位患者潰瘍邊緣切片的組織學檢查和快速尿素酶試驗的結果並不一致，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試驗的定義為病理組織學檢查陽性及 / 或快速尿素酶試驗陽性，據此可計算出個別檢驗法 (病理組織學檢查或快速尿素酶試驗) 的診斷正確率。一些變項，包括年紀、性別、潰瘍位置及大小、切片數目以及病理組織學發現，例如是否有惡性細胞、腸上皮化生、及胃黏膜萎縮等都將在病理組織學檢查幽門螺旋桿菌真陽性以及偽陰性的患者之間做比較。

**結果：** 根據本研究的定義總共有 164 位患者有幽門螺旋桿菌的感染。原始的潰瘍邊緣切片的組織學檢查 (initial HEUM)，重新判讀的病理組織學檢查 (revised HEUM)，及快速尿素酶試驗三種不同的檢驗法其幽門螺旋桿菌的檢測率分別為 78.0% (128/164)，89.0% (146/164)，以及 94.5% (155/164)。對於不同潰瘍部位的分析結果如下：胃竇部的潰瘍，上述的三種檢查其個別的檢測率分別為 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，只有惡性腫瘤這一項因子在病理組織學檢查幽門螺旋桿菌呈偽陰性的患者明顯多於病理組織學檢查真陽性的患者。

**結論：** 對於胃潰瘍的患者，使用潰瘍邊緣切片的組織學檢查來檢測其是否有幽門螺旋桿菌可能不夠準確，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，當胃潰瘍為惡性腫瘤時，用潰瘍邊緣切片的組織學檢查來診斷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：** 潰瘍生檢之組織學檢查，幽門螺旋桿菌感染，快速尿素酶試驗

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