【 ORIGINAL ARTICLE 】

The Optimal Cut-off of the Latex Immunoassay (LZ Test) for *Helicobacter pylori* Infection Based on the Stool Antigen Test and *Helicobacter pylori*-associated Gastritis

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Abstract:

Objective *Helicobacter pylori* antibody kits using the latex immunoassay (LIA) are widely used in Japan. However, the optimal cut-off of the LIA remains unclear. This study clarified the optimal cut-off of the LIA for assessing the current infection status of patients (currently infected, never infected, spontaneously eradicated) in clinical practice.

Methods In total, 482 subjects with no history of *H. pylori* eradication therapy who underwent a medical examination at our hospital were enrolled. The infection status was ascertained using a stool antigen test, and the endoscopic findings of *H. pylori*-associated gastritis. *H. pylori* antibody levels were measured using the LIA.

Results In total, 414, 38, and 30 subjects were categorized into the never-infected, currently infected, and spontaneously eradicated groups. The optimal cut-off based on receiver operating characteristic curve analysis was 4 U/mL, whereas the area under the curve, sensitivity, and specificity for differentiating never-infected and currently infected subjects were 0.95, 92.1%, and 94.7%, respectively. When applying the cut-off of 4 U/mL to the judgment of current infection in all subjects, the sensitivity and specificity were 92.1% and 92.6%, respectively.

Conclusion Our findings suggest that 4 U/mL was the optimal cut-off for differentiating current infection from no prior infection, and the value may be stable because of the exclusion of subjects with spontaneous eradication. The cut-off may be useful in initial screening for current *H. pylori* infection.

Key words: *Helicobacter pylori*, latex immunoassay, optimal cut-off

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Introduction

Serology is a noninvasive diagnostic method for the detection of *Helicobacter pylori* infection (1). It is convenient because it is not strongly affected by proton pump inhibitors or antibiotics, is less expensive than the urea breath test (UBT), and does not require dietary restriction before the test (2, 3). For *H. pylori* antibody detection, several serum antibody measurement kits are used in Japan, and kits based on the latex immunoassay (LIA) have begun to replace

those of the enzyme-linked immunosorbent assay (ELISA), as the former is easier and faster to conduct than the latter (4, 5).

However, one limitation associated with the LIA is that the optimal cut-off value is unclear. To solve the issue, we attempted to clarify the optimal cut-off of the LZ test (Eiken Chemical, Tokyo, Japan) based on the LIA between currently infected and never-infected individuals using a receiver operating characteristic (ROC) curve analysis (6-9).

According to the instructions of the LZ test, the recommended cut-off (10 U/mL) was based on the results of the
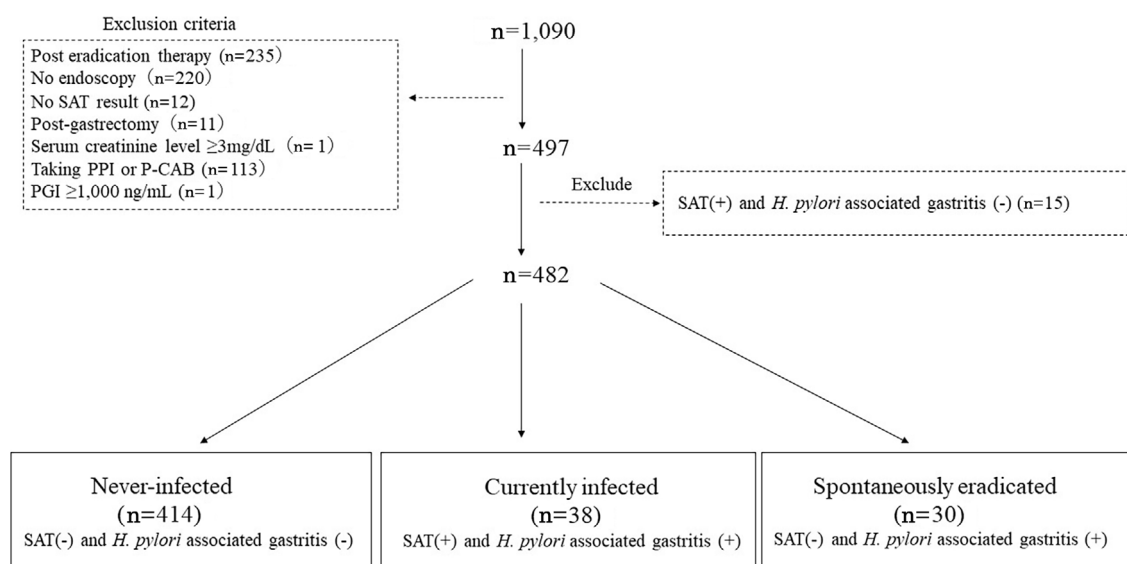
Figure 1. **The flowchart of the study.**

UBT. However, one problem is that study populations might include individuals with spontaneous eradication and a variety of *H. pylori* antibody titers. Because the endoscopic findings of *H. pylori*-associated gastritis can be altered by *H. pylori* eradication (10), discriminating currently infected individuals from those with spontaneous eradication is difficult. This study was thus performed to determine the optimal cut-off value for distinguishing currently infected and never-infected individuals after excluding those with spontaneous eradication.

We applied this optimal cut-off value to two situations: the judgement of the need for eradication therapy (currently infected vs. never-infected and spontaneously eradicated) and the identification of a high-risk group for gastric cancer screening (currently infected and spontaneously eradicated vs. never-infected). The ABC classification, which is based on the *H. pylori* antibody titer and serum pepsinogen (PG) level, is widely used for gastric cancer risk stratification during medical examinations in Japan (11, 12). However, Group A under this classification system, which is considered the low-risk group, includes individuals with current infection or spontaneous eradication. Individuals with spontaneous eradication are said to be at a high risk of gastric cancer (13, 14). To resolve the issue, the Japanese Society of Helicobacter Research conducted a multicenter study and concluded that the most reliable cut-off for the E-plate test for assessing gastric cancer risk was 3.0 U/mL (6). However, the optimal cut-off of the LIA for gastric cancer risk stratification remains controversial.

Therefore, we compared the sensitivity and specificity of our optimal cut-off value with the manufacturer-recommended cut-off value to identify individuals with current infection and spontaneous eradication who are at a high risk of gastric cancer.

Materials and Methods

In total, 1,090 individuals who visited Tsukuba Preventive Medical Research Center, University of Tsukuba Hospital from January 2019 to February 2020 were analyzed. We used all available data and did not conduct a sample size calculation because our study was an exploratory retrospective study. Serum *H. pylori* antibody and PG assessments and the stool antigen test (SAT) were requested for all individuals. We excluded 235 individuals who had a history of *H. pylori* eradication therapy because their *H. pylori* antibody titers were expected to vary based on the duration since *H. pylori* infection had disappeared. We also excluded 220 individuals who did not undergo endoscopy, 12 who did not undergo SAT, 11 who had a history of gastrectomy, 1 with renal dysfunction (serum creatinine level ≥3 mg/dL), 113 who were taking proton pump inhibitors or potassium-competitive acid blockers, and 1 with implausible results (PGI ≥1,000 ng/mL) (Fig. 1).

The *H. pylori* antibody levels were measured using the LZ test. PG levels were measured using the chemiluminescence immunoassay (LSI Medience, Tokyo, Japan). The SAT was performed using the Testmate Pylori Antigen enzyme immunoassay (EIA, Wakamoto Pharmaceutical, Tokyo, Japan). The SAT is known to be a reliable test with high diagnostic accuracy. A previous study found that the accuracy of the Testmate Pylori Antigen EIA was 100% using fecal samples from 111 patients. In addition, no cross-reactivity was observed with other *H. pylori* species or intestinal bacteria. In total, 1,342 of 1,344 clinical strains tested positive, resulting in a sensitivity of 99.9% (15). For this reason, we set the SAT as the gold standard.

We defined *H. pylori*-associated gastritis as findings of atrophy (C-2 or higher) based on the Kimura-Takemoto classification (16) or metaplasia according to the Kyoto Classifi-

Table 1.   Baseline Data according to *H. Pylori* Infection Status.

Variables	Never-infected (n=414)	Currently infected (n=38)	Spontaneously eradicated (n=30)	Never-infected vs. Currently infected	Never-infected vs. Spontaneously eradicated	Currently infected vs. Spontaneously eradicated
Gender, Male (%)	213 (51.4)	22 (57.9)	11 (36.7)	0.45	0.12	0.08
Age †	51.0 (42.3-63.0)	60.5 (50.5-68.8)	68.0 (53.0-70.8)	0.0039	<0.001	0.12
H. pylori antibody †	1.5 (1.5-1.5)	28.0 (13.3-52.0)	1.5 (1.5-10.3)	<0.001	<0.001	<0.001
	<3 U/mL (90.6%)	<3 U/mL (7.9%)	<3 U/mL (53.3%)			
PGI †	48.1 (40.1-59.8)	57.8 (46.3-76.5)	41.2 (30.3-53.0)	0.0044	0.0022	<0.001
PGII †	7.0 (5.7-8.7)	16.9 (13.5-23.1)	7.0 (5.7-9.2)	<0.001	0.51	<0.001
PGI/II ratio †	7.1 (6.1-8.1)	3.4 (2.5-5.1)	6.0 (4.5-6.6)	<0.001	<0.001	0.0012

†: Median (IQR)

cation (17), which was confirmed by two gastroenterologists. Using endoscopic findings and SAT data, we categorized patients into four groups: SAT (-)/*H. pylori*-associated gastritis (-), SAT (+)/*H. pylori*-associated gastritis (+), SAT (+)/*H. pylori*-associated gastritis (-), and SAT (-)/*H. pylori*-associated gastritis (+). We excluded 15 SAT (+)/*H. pylori*-associated gastritis (-) patients because their infection status was difficult to determine. *H. pylori*-associated gastritis (-)/SAT (-) subjects comprised the never-infected group, and *H. pylori*-associated gastritis (+)/SAT (+) individuals were categorized into the currently infected group. In addition, SAT (-)/*H. pylori*-associated gastritis (+) individuals were considered to have undergone spontaneous eradication because the group did not include individuals with predominant gastric mucosal atrophy of the corpus and fornix (autoimmune gastritis). Ultimately, 482 patients (414 never-infected, 38 currently infected, and 30 with spontaneous eradication) were enrolled in our observational study (Fig. 1).

First, we performed the Mann-Whitney U test for quantitative variables (e.g. age) and the chi-squared test for categorical variables (e.g. gender) to compare variables among the three *H. pylori* infection statuses. Next, we conducted an ROC curve analysis to determine the optimal cut-off. We defined the point on the ROC curve with the shortest distance from the upper left corner (0, 1) as the optimal cut-off value. This point was calculated as an integer because *H. pylori* antibody titers were reported as integers for all patients in our center.

All statistical analyses were performed using the Bell Curve plugin, version 3.20 (Social Survey Research Information, Tokyo, Japan) for the Microsoft Excel software program (Microsoft Japan, Tokyo, Japan), and $p<0.05$ was considered statistically significant. To discriminate between *H. pylori* antibody titers of <3.0 and 3 U/mL, titers of <3.0 U/mL were calculated as 1.5 U/mL for convenience.

The study was approved by the University Hospital of Tsukuba Ethics Committee. All data were fully anonymized before we accessed them, and informed consent was obtained via the opt-out method under approval from the ethics committee.

Results

The medians (interquartile ranges) of variables in the never-infected, currently infected, and spontaneously eradicated groups were as follows: age, 51.0 (42.3-63.0), 60.5 (50.5-68.8), and 68.0 (53.0-70.8) years old, respectively; *H. pylori* antibody titer, 1.5 (1.5-1.5), 28.0 (13.3-52.0), and 1.5 (1.5-10.3) U/mL, respectively; PGI, 48.1 (40.1-59.8), 57.8 (46.3-76.5), and 41.2 (30.3-53.0) ng/mL, respectively; PGII, 7.0 (5.7-8.7), 16.9 (13.5-23.1), and 7.0 (5.7-9.2) ng/mL, respectively; and PGI/II ratio, 7.1 (6.1-8.1), 3.4 (2.5-5.1), and 6.0 (4.5-6.6), respectively (Table 1). The never-infected group was significantly younger than the currently infected and spontaneously eradicated groups (p=0.0039 and p<0.001, respectively). The *H. pylori* antibody and PGI levels and the PGI/II ratio differed significantly among the three groups, whereas no significant difference in gender was observed. In the never-infected group, the range of PGI levels, which is correlated with gastric atrophy and inflammation, was similar to that in previous reports (18-20).

The distributions of individuals in the never-infected, currently infected, and spontaneously eradicated groups according to *H. pylori* antibody titers are presented in Fig. 2. Never-infected individuals had titers of <3 to 14 U/mL, and 375 individuals in this group (90.6%) had titers of <3 U/mL. In contrast, 7 currently infected individuals (18.4%) had antibody titers of 100 U/mL. Meanwhile, individuals with spontaneous eradication had *H. pylori* antibody titers of <3 to 100 U/mL, with most (53.3%) having titers of <3 U/mL.

Next, we performed an ROC curve analysis to determine the optimal cut-off for differentiating currently infected and never-infected individuals. The area under the curve (AUC), sensitivity, and specificity for differentiating these groups were 0.95 [95% confidence interval (CI)=0.91-1.00], 4 U/mL, 92.1%, and 94.7%, respectively (Fig. 3).

Finally, we set a cut-off value of 4 U/mL for judging current infection. The sensitivities of cut-off values of 4 and 10 mL for discriminating currently infected individuals from other subjects were 92.1% and 86.8%, respectively, whereas the specificities were 92.6% and 97.1%, respectively (Ta-

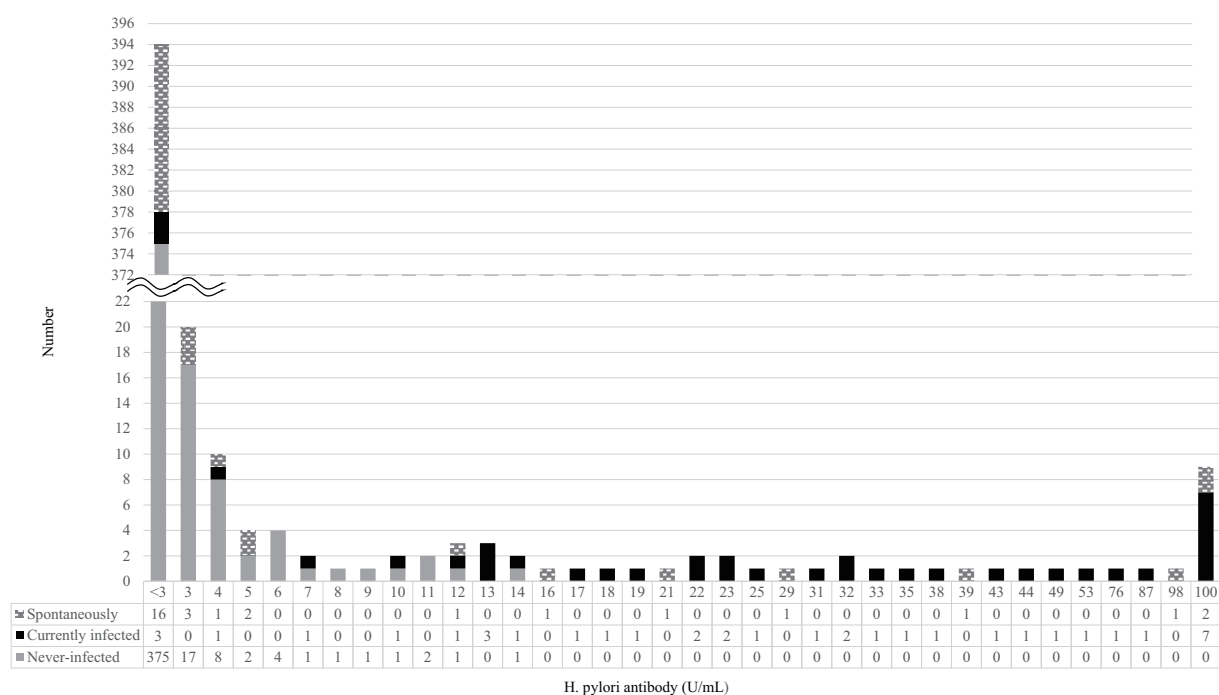| H. pylori antibody (U/mL)	<3	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	19	21	22	23	25	29	31	32	33	35	38	39	43	44	49	53	76	87	98	100
Spontaneously	16	3	1	2	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2
Currently infected	3	0	1	0	0	1	0	0	1	0	1	3	1	0	1	1	1	0	2	2	1	0	1	2	1	1	1	0	1	1	1	1	1	1	0	7
Never-infected	375	17	8	2	4	1	1	1	1	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 2.   The distribution of the never-infected, currently infected, and spontaneously eradicated groups according to the *Helicobacter pylori* antibody titer.
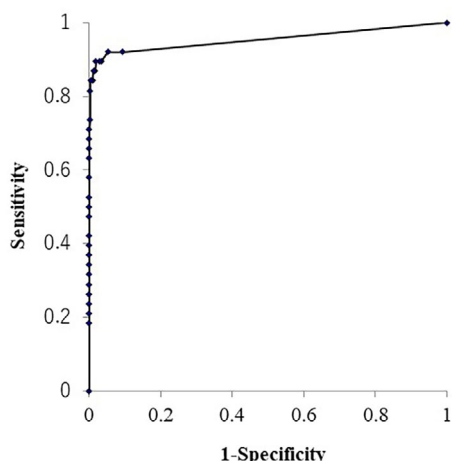

Figure 3.   Receiver operating characteristic curve for currently infected versus never-infected individuals.

ble 2). In the context of using our optimal cut-off value to identify individuals at a high risk of gastric cancer, the sensitivities of the cut-off values of 4 and 10 U/mL for discriminating subjects with current infection or spontaneous eradication from never-infected individuals were 67.6% and 60.3%, respectively, whereas the specificities were 94.7% and 98.8%, respectively (Table 3).

Discussion

This study was an observational study of healthy individuals who underwent medical examinations at our hospital. Consequently, we determined that the optimal cut-off

value for the LZ test based on an ROC curve analysis was 4 U/mL.

According to the manufacturer's instructions, the recommended cut-off of the LZ test of 10 U/mL was based on the results of UBTs. Kawai et al. assessed the diagnostic accuracy of the LZ test based on the UBT result using the manufacturer-recommended cut-off of 10 U/mL. The AUC, sensitivity, and specificity were 0.86 (95% CI=0.79-0.94), 98.1%, and 78.0%, respectively. They concluded that the reason for the low specificity was the inclusion of patients with prior *H. pylori* infection who tested negative for *H. pylori* infection according to the UBT but positive for infection according to the LZ test (5). To determine a more appropriate cut-off by removing individuals who experienced spontaneous eradication, the present study used the SAT and endoscopic findings as the gold standards. As a result, we succeeded in excluding individuals with spontaneous eradication in advance. Some reports described the optimal cut-off of the LZ test after removing individuals with spontaneous eradication as well as in populations with small numbers of such individuals. Aoyama et al. reported that the optimal cut-off for discriminating never-infected and currently infected individuals according to an ROC curve analysis was 6.485 (AUC=0.988, sensitivity=95.96%, specificity= 95.96%) (8). They used endoscopic findings, the rapid urease test, and a pathological examination as gold standards and excluded patients with past infection in advance. Furthermore, Tsutsumi et al. screened a cohort of junior high school students believed to include few individuals with spontaneous eradication. They reported that the cut-off of the LZ test in screening adolescents based on the SAT was

Table 2.   Diagnosis of Current Infection.

a				b		
H. pylori antibody (U/mL)	Currently infected (n=38)	Spontaneously (n=30) Never-infected (n=414) (n=444)		*H. pylori* antibody (U/mL)	Currently infected (n=38)	Spontaneously (n=30) Never-infected (n=414) (n=444)
≥4	35	Spontaneously 11 Never-infected 22		≥10	33	Spontaneously 8 Never-infected 5
<4	3	Spontaneously 19 Never-infected 392		<10	5	Spontaneously 22 Never-infected 409

c		
Cut-off (U/mL)	Sensitivity (%)	Specificity (%)
4	92.1	92.6
10	86.8	97.1

Table 3.   Diagnosis of High-risk Group for Gastric Cancer.

a				b		
H. pylori antibody (U/mL)	Currently infected (n=38) Spontaneously (n=30) (n=68)	Never-infected (n=414)		*H. pylori* antibody (U/mL)	Currently infected (n=38) Spontaneously (n=30) (n=68)	Never-infected (n=414)
≥4	Currently 35 Spontaneously 11	22		≥10	Currently 33 Spontaneously 8	5
<4	Currently 3 Spontaneously 19	392		<10	Currently 5 Spontaneously 22	409

c		
Cut-off (U/mL)	Sensitivity (%)	Specificity (%)
4	67.6	94.7
10	60.3	98.8

3.1 (AUC=0.88, sensitivity=87.5%, specificity=91.5%) (7). The present and previous findings thus suggest that the reliable cut-off for discrimination in the absence of subjects with prior infection may be lower than the manufacturer-recommended value.

To compare the sensitivity and specificity between cut-offs of 4 and 10 U/mL in clinical practice, we evaluated them in two situations: the judgment of current infection to assess the eradication therapy (Table 2) and the identification of the high-risk group in gastric cancer screening (Table 3). Table 2c shows that by reducing the cut-off value from 10 to 4 U/mL, the sensitivity improved by 5.3%, meaning that we were able to identify two more currently infected individuals. Because low false-negative rates are important for diagnosing *H. pylori* infection, a high sensitivity rather than a high specificity is required. Therefore, it is more appropriate to use 4 U/mL as the optimal cut-off. However, by focusing only on individuals with spontaneous eradication, we would misdiagnose 11 subjects as having current infection (Table 2a). Thus, we recommend using a cut-off value of 4 U/mL for the first screening of *H. pylori* infection, after which test-positive patients should be re-evaluated using the UBT to account for the low diagnostic accuracy for individuals with spontaneous eradication.

In Table 3c, the sensitivity, which is required for gastric cancer screening, was increased by 7.3% when the cut-off value was decreased from 10 to 4 U/mL. However, 32.4% of subjects with current infection or spontaneous eradication would be incorrectly assigned to the never-infected group under such conditions, which remains unacceptable. Therefore, we concluded that it is difficult to detect individuals at high risk of gastric cancer using *H. pylori* antibody titers.

The main advantage of the optimal cut-off value may be the fact that it is a clear, stable, and ideal value that is not affected by individuals with spontaneous eradication. However, one disadvantage is that because we did not consider individuals with spontaneous eradication when identifying the optimal cut-off, inconsistency may arise when the value is applied in real-world situations. The diagnostic accuracy of the optimal cut-off value decreased when individuals with spontaneous eradication were included in the population (Table 2, 3). Despite this disadvantage, we believe that the optimal cut-off is more reliable than that generated using individuals with spontaneous eradication, as the former value is not affected by the ratio of individuals with spontaneous eradication. For instance, the Japanese Society of Helicobacter Research reported in a multicenter study that the optimal cut-off of the LZ test for gastric cancer screening was 6.1

U/mL (9). They calculated this value based on an ROC curve analysis comparing the never-infected and currently infected/spontaneous eradication groups. When we applied this cut-off to our study population, 38.2% of individuals in the high-risk group of gastric cancer were misdiagnosed (data not shown), which was approximately 27% higher than that reported previously. In other words, that optimal cut-off value was not useful for detecting patients at high risk of gastric cancer in our study population.

In addition, the optimal cut-off and AUC (sensitivity, specificity) for discriminating the current infection/spontaneous eradication and no prior infection groups in the present study were 3 U/mL and 0.84 (72.1%, 90.6%), respectively (data not shown). The sensitivity was 72.1%, which was insufficient for gastric cancer screening. A previous study reported that the AUC (sensitivity, specificity) for the cut-off of 6.1 U/mL was 0.97 (89%, 95%). The sensitivity in the previous report was 16.9% higher than that in the present study. One reason for this difference may be because the ratio of subjects with current infection to those with spontaneous eradication (946/89) in the previous study was higher than that in our study, as the target population contained many outpatients. As has been suggested, the optimal cut-off and diagnostic accuracy, which were determined in a study population including subjects with spontaneous eradication, may easily change.

Several limitations of our study need to be considered. First, because we used only the SAT and an endoscopic evaluation as the gold standards for assessing the infection status, we might have misclassified some individuals. Because the SAT does not have 100% sensitivity and specificity (21, 22), several individuals with spontaneous eradication but false-positive SAT results might have been misclassified into the currently infected group, whereas some currently infected individuals with false-negative SAT results might have been incorrectly classified into the spontaneous eradication group. In addition, we did not perform a histological diagnosis, which may be more accurate than an endoscopic evaluation. Second, the never-infected group might have included spontaneously eradicated cases whose atrophy improved after the disappearance of *H. pylori*. Third, there was potential bias toward favorable results because the population in which the cut-off value was calculated was also included in the validation. Finally, this study was retrospective and performed at a single center. A multicenter and prospective study is thus needed to validate the results.

In conclusion, our analysis suggested that 4 U/mL was a reliable cut-off value for the LZ test. In terms of indications for eradication therapy, it may be helpful to use a cut-off of 4 U/mL for initial screening for current *H. pylori* infection. Conversely, it may be not appropriate to use *H. pylori* antibody titers alone for gastric cancer screening.

The authors state that they have no Conflict of Interest (COI).

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