UNITED EUROPEAN GASTROENTEROLOGY ueg journal

Original Article

United European Gastroenterology Journal
2019, Vol. 7(3) 343–348

DOI: 10.1177/2050640619825947
journals.sagepub.com/home/ueg
SAGE

A combination of serum anti-*Helicobacter pylori* antibody titer and Kyoto classification score could provide a more accurate diagnosis of *H pylori*

Toshihiro Nishizawa[1,2], Kosuke Sakitani[1,3], Hidekazu Suzuki[4], Tadahiro Yamakawa[1], Yoshiyuki Takahashi[1], Nobutake Yamamichi[3], Hidenobu Watanabe[6], Yasuyuki Seto[5], Kazuhiko Koike[3] and Osamu Toyoshima[1]

Abstract

Background: We previously showed that the endoscopic Kyoto classification for gastritis could predict *Helicobacter pylori* infection in individuals with a high negative titer of serum anti-*H pylori* antibodies. This study evaluated *H pylori* infection and the Kyoto classification score in patients with a low negative titer (<3 U/ml), high negative titer (3–9.9 U/ml), low positive titer (10–49.9 U/ml), and high positive titer (≥50 U/ml).

Methods: Serum antibody levels, Kyoto classification score and histology were investigated in 870 individuals with no history of *H pylori*-eradication therapy. Urea breath tests (UBTs) were additionally conducted for patients with a low negative titer and a Kyoto score ≥1 or an antibody titer ≥10 U/ml and a Kyoto score of 0 or 1. UBTs and/or histological studies were conducted for participants with a high negative titer.

Results: False diagnoses based on anti-*H pylori* antibody titers were observed in 0.3% of the low-negative-titer group, 11.7% of the high-negative-titer group, 18.9% of the low-positive-titer group and 2.2% of the high-positive-titer group. Surprisingly, false diagnoses based on antibody titers were noted in 63.2% of patients with a low positive titer and a Kyoto score of 0 and in 62.5% of patients with a high negative titer and a Kyoto score ≥2, respectively.

Conclusions: Endoscopic findings could predict false diagnoses determined using serum antibody titers.

Keywords

Antibody titer, *H pylori*, Kyoto classification score

Received: 17 October 2018; accepted: 15 December 2018

Key summary

Summarize the established knowledge on this subject:

- Serum anti-*Helicobacter pylori* antibody tests yield accuracies of 89% to 95%, sensitivities of 88% to 96%, and specificities of 86% to 96%.
- The Kyoto classification is scored from 0 to 8 and is believed to provide an estimate of the risk of gastric cancer.

[1]Gastroenterology, Toyoshima Endoscopy Clinic, Tokyo, Japan
[2]Department of Gastroenterology, National Hospital Organization, Tokyo Medical Center, Tokyo, Japan
[3]Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[4]Medical Education Center, Keio University School of Medicine, Tokyo, Japan
[5]Department of Surgical Oncology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[6]Pathology and Cytology Laboratory Japan, Tokyo, Japan

Corresponding author:
Osamu Toyoshima, Gastroenterology, Toyoshima Endoscopy Clinic, 6-17-5 Seijo, Setagaya-ku, Tokyo 157-0066, Japan.
Email: t@ichou.com

What are the new findings of this study?

- A high rate of false anti-*H pylori* antibody test results was noted in patients with low positive titers as well as in those with high negative titers.
- If the diagnoses according to an antibody assay and the Kyoto classification score are inconsistent, further examinations such as a urea breath test should be undertaken for a more accurate diagnosis.

Introduction

Helicobacter pylori infection is one of the most prevalent infectious diseases worldwide, with 40% to 50% of the global human population estimated to be infected.[1] *H pylori* is associated with the development of atrophic gastritis and gastric cancer.[2–6] Eradication of *H pylori* infection has been reported as an effective strategy for treating atrophic gastritis and peptic ulcer and preventing gastric cancer.[7–11]

The recently developed endoscopic Kyoto classification score is based on the summation of the following endoscopic findings: atrophy, intestinal metaplasia (IM), enlarged folds, nodularity and redness.[12] The Kyoto classification is scored from 0 to 8 and is believed to provide an estimate of the risk of gastric cancer.[13]

Diagnostic methods of *H pylori* infection include ^{13}C-urea breath tests (UBTs), measuring serum levels of anti-*H pylori* antibodies, stool antigen tests, rapid urease tests, culture and pathology.[14,15] UBT is regarded as the gold standard because of its higher degree of accuracy. Serum anti-*H pylori* antibody tests are easy and inexpensive, with commercial test kits yielding accuracies of 89% to 95%, sensitivities of 88% to 96%, and specificities of 86% to 96%.[16] In Japan, E-plate (Eiken Chemical, Tokyo, Japan) is the most commonly used commercial serology kit in daily clinical practice with an accuracy of 94.0%, a sensitivity of 95.2%, and a specificity of 92.6% based on the recommended cutoff point of 10 U/ml.[17] We previously reported that 17% of individuals with a high negative titer (3–9.9 U/ml) were positive for *H pylori* infection.[13] Furthermore, we determined that the Kyoto classification score could be a useful predictor of *H pylori* infection in patients with a high negative titer; the endoscopic Kyoto classification score could detect false-negative antibody test results. A combination of the antibody test and the Kyoto classification score might provide a more accurate diagnosis of *H pylori* infection. This study evaluated the effectiveness of combining the antibody test and the Kyoto classification score. Although our previous study focused on individuals with high negative titers (3–9.9 U/ml),[13] this study included patients with low negative titers (<3 U/ml), low positive titers (10–49.9 U/ml) and high positive titers (≥50 U/ml).

Methods

Patients

Consecutive patients who underwent an upper gastrointestinal endoscopy and a serum antibody test between September 2016 and August 2017 in the Toyoshima Endoscopy Clinic were retrospectively reviewed. We included patients who were evaluated for *H pylori* infection for the first time. We excluded patients with a history of eradication treatment, gastric cancer, or gastrectomy. Upper gastrointestinal endoscopy was used for screening, surveillance for upper gastrointestinal diseases, examination for abnormal findings of upper gastrointestinal radiography, or symptoms such as epigastric pain. Serum antibody levels were measured on the day of upper gastrointestinal endoscopy. This study was approved by the ethical review committee of Hattori Clinic on September 7, 2017. Written informed consent was obtained from all participants. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki.

Serum anti-H pylori antibody

Antibody titers were measured using an enzyme immunoassay kit with antigens derived from *H pylori* isolated from Japanese individuals (E-plate Eiken *H pylori* antibody II; Eiken Chemical, Tokyo, Japan). A cutoff value of 10 U/ml was determined to indicate *H pylori* positivity.[18,19] According to previous studies, we divided participants into four groups according to their serum antibody titers: < 3 U/ml (low negative), 3–9.9 U/ml (high negative), 10–49.9 U/ml (low positive) and ≥50 U/ml (high positive).[13,20]

Esophagogastroduodenoscopy, pathology and UBT

Upper gastrointestinal endoscopy was performed by expert physicians using the Olympus Evis Lucera Elite system with the GIF-HQ290 or GIF-H290Z endoscope (Olympus Corporation, Tokyo, Japan). Physicians met and discussed all endoscope images before this study. Furthermore, upper gastrointestinal endoscopy images were retrospectively reviewed by other expert physicians. Discrepancies in diagnosis between the two sets of physicians were resolved through discussion.

The Kyoto classification of gastritis is based on the sum of scores of the following five endoscopic findings, which are scored from 0 to 8: atrophy, IM, enlarged folds, nodularity and redness. A high score indicates an increased risk of gastric cancer.[21,22] Gastric atrophy was classified according to the extent of mucosal atrophy as described by Kimura and Takemoto.[23] C-II and C-III of the Kimura-Takemoto classification were scored as 1, and O-I to O-III as 2. IM is observed as grayish-whitish and slightly opalescent patches. IM within the antrum was scored as 1, and IM extending into the corpus as 2. The presence of folds enlarged over 5 mm or more was scored as 1. Nodularity is characterized by the appearance of multiple whitish, elevated lesions mainly in the pyloric gland mucosa. The presence of nodularity was scored as 1. Diffuse redness refers to uniform redness involving the entire fundic gland mucosa. The presence of redness with regular arrangements of collecting venules (RACs) was scored as 1, and without RACs as 2.

Pathological evaluation was conducted for a Kyoto classification score of gastritis ≥1. Pathological findings were evaluated using the updated Sydney System score.[24] Biopsy specimens were obtained from the greater curve of the corpus and antrum. Histological diagnosis was performed by an expert gastrointestinal pathologist (H.W.).

UBTs were additionally conducted for individuals with a low negative titer (<3 U/ml) and a Kyoto score ≥1 and those with an antibody titer ≥10 U/ml and a Kyoto score of 0 or 1. The cutoff value for UBT was 3.0%. If proton pump inhibitors (PPIs) were used, UBT was performed two weeks after discontinuation of the PPI treatment. If a diagnosis according to serum antibody levels and UBT was inconsistent, the UBT was adopted as the gold standard. For patients with a high negative titer (3–9.9 U/ml), UBTs and/or histological examinations for *H pylori* were conducted. If the results of at least one test were positive in participants with a high negative titer, patients were considered positive for *H pylori*. Individuals with an antibody titer <3 U/ml and a Kyoto score of 0 were considered negative for *H pylori* without confirmation using the UBT. Participants with an antibody titer ≥10 U/ml and a Kyoto score ≥2 were considered positive for *H pylori* without confirmation using the UBT (Figure 1).

The white area was diagnosed as negative *Helicobacter pylori* without performing a urea breath test (UBT). The black area was diagnosed as positive *H pylori* without performing a UBT. The gray area was further investigated by UBT and/or histology.

Results

A total of 919 patients were enrolled. We investigated 870 patients, following the exclusion of 41 patients with a history of eradication therapy, four patients with past gastric cancer, and four patients with past gastrectomy. Among the 870 patients, 612, 139, 74 and 45 patients had antibody titers <3 U/ml (low negative), 3–9.9 U/ml (high negative), 10–49.9 U/ml (low positive) and ≥50 U/ml (high positive), respectively (Table 1).

In the low-negative-titer group, 5.7% (35/612) had a Kyoto score ≥1. Among them, 5.9% (2/34) were diagnosed as *H pylori* positive following a UBT, after excluding one patient who did not undergo a UBT. In the low-negative-titer group, 0.3% (2/612) were diagnosed as *H pylori* positive according to the UBT (Table 2). In these two cases, the histological findings also showed *H pylori* and neutrophil activity. One case (76 year-old female) involved O-III gastric atrophy and IM extending into the corpus (Kyoto score 4). The other case (58 year-old male) involved O-II gastric atrophy, IM within the antrum and diffuse redness without RACs (Kyoto score 5). False diagnoses based on

	Antibody titer <3 (negative low)	Antibody titer 3–9.9 (negative high)	Antibody titer 10–49.9 (positive low)	Antibody titer ≥50 (positive high)
Kyoto score 0	*H. pylori* negative	UBT and/or histology	UBT	UBT
Kyoto score 1	UBT	UBT and/or histology	UBT	UBT
Kyoto score ≥2	UBT	UBT and/or histology	*H. pylori* positive	*H. pylori* positive

Figure 1. Diagnosis algorithm for *Helicobacter pylori* infection.

Table 1. Demographic data of patients undergoing upper gastrointestinal endoscopy and *Helicobacter pylori* antibody test.

Antibody titer (U/ml)	< 3 (negative-low)	3–9.9 (negative-high)	10–49.9 (positive-low)	≥ 50 (positive-high)
Patient number (%)	612 (70.3%)	139 (16.0%)	74 (8.5%)	45 (5.2%)
Male:Female (male %)	241:371 (39.4%)	47:92 (33.8%)	32:42 (43.2%)	14:31 (31.1%)
Age (mean ± SD)	47.8 ± 13.1	47.0 ± 14.8	52.5 ± 15.8	50.9 ± 14.3
Purposes for endoscopy				
Screening	165	39	14	9
Surveillance for upper GI diseases	92	23	14	10
Examination for upper GI radiography	60	14	12	7
Examination for symptoms	295	63	34	19

GI: gastrointestinal.

Table 2. Evaluation of *Helicobacter pylori* antibody.

Antibody titer (U/ml)	< 3 (negative-low)			3–9.9 (negative-high)		10–49.9 (positive-low)			≥ 50 (positive-high)		
Patient number	612			137		74			45		
Kyoto score	0	≥1				≤1		≥2	≤1		≥2
Urea breath test		(−)	(+)	(−)	(+)	(−)	(+)		(−)	(+)	
H pylori diagnosis	(−)	(−)	(+)	(−)	(+)	(−)	(+)	(+)	(−)	(+)	(+)
Patient number	577	32	2	121	16	14	18	42	1	12	32
Rate of false diagnosis based on antibody			0.3% (2/612)		11.7% (16/137)	18.9% (14/74)			2.2% (1/45)		

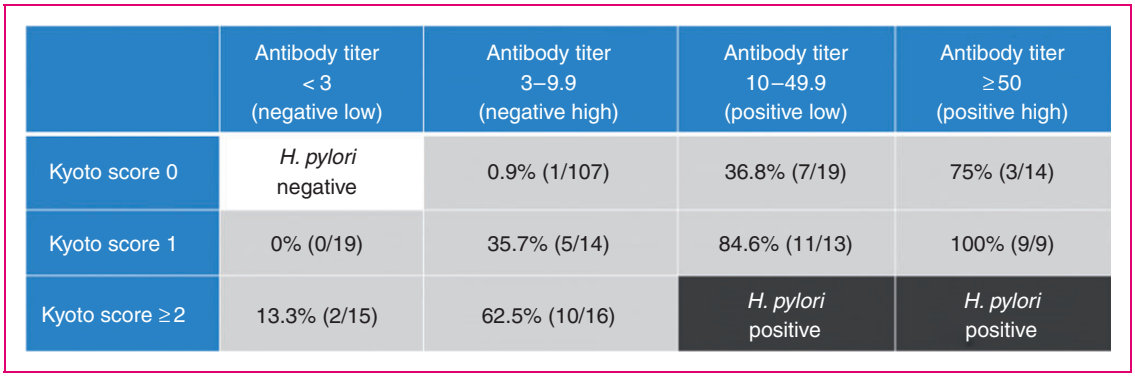

Figure 2. *Helicobacter pylori*-positive rates in subgroups based on antibody titer and Kyoto score.

antibody titers were reported in 13.3% (2/15) of patients with a low negative titer and Kyoto score ≥2 (Figure 2).

In the high-negative-titer group, two patients did not undergo a UBT or histological assessment for *H pylori*. After excluding these two patients, 137 patients in the high-negative-titer group were analyzed for the accuracy of the anti-*H pylori* antibody results. In the high-negative-titer group, 11.7% (16/137) were diagnosed as *H pylori* positive (Table 2). False diagnoses based on antibody titers were found in 35.7% (5/14) of patients with a high negative titer and a Kyoto score of 1 and in 62.5% (10/16) of patients with a high negative titer and a Kyoto score ≥2 (Figure 2).

In the low-positive-titer group, 18.9% (14/74) were diagnosed as *H pylori* negative according to the UBT

(Table 2), and 25.7% (19/74) and 17.6% (13/74) were determined to have a Kyoto score of 0 and 1, respectively. According to the UBT results, 63.2% (12/19) of the low-positive-titer group with a Kyoto score of 0 and 15.4% (2/13) of the low-positive-titer group with a Kyoto score of 1 were diagnosed as *H pylori* negative (Figure 2). The two patients with a Kyoto score of 1 were given that score according to their Kimura-Takemoto C-II or C-III classification.

In the high-positive-titer group, 2.2% (1/45) were diagnosed as *H pylori* negative according to the UBT (Table 2), and 8.9% (4/45) and 20% (9/45) were determined to have a Kyoto score of 0 and 1, respectively. Furthermore, 25% (1/4) of the high-positive-titer group with a Kyoto score of 0 were diagnosed as *H pylori* negative according to the UBT (Figure 2). This case involved a 47-year-old woman who showed a titer of 88.9 U/ml.

In this study, anti-*H pylori* antibody titers had an accuracy of 96.2%, a sensitivity of 85.2%, a specificity of 98.0%, a positive predictive value of 87.4% and a negative predictive value of 97.6% for the diagnosis of *H pylori* infection.

Discussion

False diagnoses based on anti-*H pylori* antibody titers were reported in 0.3% of the low-negative-titer group, 11.7% of the high-negative-titer group, 18.9% of the low-positive-titer group and 2.2% of the high-positive-titer group. The rate of false anti-*H pylori* antibody test results was relatively high in the high-negative-titer group and even higher in the low-positive-titer group. Therefore, it appears that it is not only high negative titers but also low positive titers that fall into the gray diagnostic zone. The results of our study therefore highlight to physicians the importance of not relying solely on anti-*H pylori* antibody titers for diagnosing *H pylori* infection.

We previously showed that a Kyoto classification score of 2 or more could predict *H pylori* infection in high-negative-titer patients.[13] On the other hand, a Kyoto classification score of 1 or 0 indicates that a patient is negative for *H pylori* infection. If the diagnoses according to an antibody assay and the Kyoto classification score are inconsistent, further examinations such as a UBT should be undertaken for a more accurate diagnosis.

The Kyoto classification score is believed to provide an estimate of the risk of gastric cancer. Sugimoto et al. showed that the Kyoto classification score in patients with gastric cancer was significantly higher than that in patients with gastritis alone.[25] Our study showed that the Kyoto classification score might be useful not only for estimating the risk of gastric cancer but also for predicting false diagnoses following anti-*H pylori* antibody test results.

In the low-negative-titer group, 0.3% (2/612) were diagnosed as *H pylori* positive according to the UBT. These two patients had severe atrophic gastritis with IM. Both patients were in their seventies. These two patients were similar to Group D according to the ABC method.[26] Severe atrophic gastritis with IM could induce a significant decrease in the *H pylori* count, resulting in a decreased antibody titer.

Individuals with positive anti-*H pylori* antibody levels and negative UBT results could include those in whom the infection resolved spontaneously. *Helicobacter* infections other than *H pylori* could also result in positive serum antibody levels.[27] These cases would not require *H pylori* eradication treatment. Our study showed that a combination of serum anti-*H pylori* antibody titer and a Kyoto classification score could avoid unnecessary eradication treatment. This study could serve as a warning against unnecessary eradication treatment based on just the anti-*H pylori* antibody test.

There are some limitations to this study. First, we used the UBT as the gold standard for diagnosing *H pylori* infection; however, its accuracy is not 100%. Second, we did not perform a UBT in individuals with an antibody titer <3 U/ml and a Kyoto score of 0 or in participants with an antibody titer ≥ 10 U/ml and a Kyoto score ≥ 2. Kyoto score ≥ 2 has been reported to predict *H pylori* infection in high-negative-titer patients.[13] Therefore we judged that individuals with an antibody titer ≥ 10 U/ml and a Kyoto score ≥ 2 had *H pylori* infection. Furthermore, we considered that conducting UBTs for these cases would be excessive in daily clinical practice. Further study should be conducted to analyze the association between *H pylo*ri infection and the Kyoto classification score.

In conclusion, a high rate of false anti-*H pylori* antibody test results was noted in patients with low positive titers as well as in those with high negative titers. If the diagnoses according to the antibody assay and endoscopic findings are inconsistent, further examination such as a UBT should be added.

Acknowledgments

We would like to thank Editage for English language editing. Author contributions include the following: T.N. analyzed data and wrote the manuscript; K.S. critically revised the manuscript; H.S. was responsible for interpretation of data; T.Y and Y.T. collected data; N.Y., Y.S. and K.K. supervised the study; H.W. was responsible for pathological analysis; and O.T. developed the study concept and design.

Declaration of conflicting interests

None declared.

Ethics approval

This study was approved by the ethical review committee of Hattori Clinic on September 7, 2017. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Informed consent

Written informed consent was obtained from all participants.

ORCID iD

Osamu Toyoshima http://orcid.org/0000-0002-6953-6079

References

1. Suzuki H, Nishizawa T and Hibi T. *Helicobacter pylori* eradication therapy. *Future Microbiol* 2010; 5: 639–648.
2. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001; 345: 784–789.
3. Nishizawa T, Suzuki H, Sakitani K, et al. Family history is an independent risk factor for the progression of gastric atrophy among patients with *Helicobacter pylori* infection. *United European Gastroenterol J* 2017; 5: 32–36.
4. Toyoshima O, Tanikawa C, Yamamoto R, et al. Decrease in PSCA expression caused by *Helicobacter pylori* infection may promote progression to severe gastritis. *Oncotarget* 2018; 9: 3936–3945.
5. Suzuki H, Nishizawa T and Hibi T. Can *Helicobacter pylori*-associated dyspepsia be categorized as functional dyspepsia? *J Gastroenterol Hepatol* 2011; 26(Suppl 3): 42–45.
6. Nishizawa T, Suzuki H, Nakagawa I, et al. Early *Helicobacter pylori* eradication restores sonic hedgehog expression in the gastric mucosa of Mongolian gerbils. *Digestion* 2009; 79: 99–108.
7. Lee YC, Chiang TH, Chou CK, et al. Association between *Helicobacter pylori* eradication and gastric cancer incidence: A systematic review and meta-analysis. *Gastroenterology* 2016; 150: 1113–1124.e5.
8. Sakitani K, Nishizawa T, Arita M, et al. Early detection of gastric cancer after *Helicobacter pylori* eradication due to endoscopic surveillance. *Helicobacter* 2018; 23: e12503.
9. Nishizawa T, Suzuki H, Nakagawa I, et al. Gatifloxacin-based triple therapy as a third-line regimen for *Helicobacter pylori* eradication. *J Gastroenterol Hepatol* 2008; 23(Suppl 2): S167–S170.
10. Nishizawa T, Suzuki H, Takahashi M, et al. Delay of second-line eradication therapy for *Helicobacter pylori* can increase eradication failure. *J Gastroenterol Hepatol* 2013; 28: 1608–1610.
11. Toyoshima O, Yamaji Y, Yoshida S, et al. Endoscopic gastric atrophy is strongly associated with gastric cancer development after *Helicobacter pylori* eradication. *Surg Endosc* 2017; 31: 2140–2148.
12. Kato M. Endoscopic findings of *H. pylori* infection. In: Suzuki H, Warren R and Marshall B (eds) *Helicobacter pylori*. Tokyo: Springer Japan, 2016, pp. 157–167.
13. Toyoshima O, Nishizawa T, Arita M, et al. *Helicobacter pylori* infection in subjects negative for high titer serum antibody. *World J Gastroenterol* 2018; 24: 1419–1428.
14. Suzuki H, Nishizawa T, Tsugawa H, et al. Molecular approaches and modern clinical strategies for the management of *Helicobacter pylori* infection in Japan. *Keio J Med* 2012; 61: 109–119.
15. Nishizawa T and Suzuki H. Mechanisms of *Helicobacter pylori* antibiotic resistance and molecular testing. *Front Mol Biosci* 2014; 1: 19.
16. Talley NJ, Kost L, Haddad A, et al. Comparison of commercial serological tests for detection of *Helicobacter pylori* antibodies. *J Clin Microbiol* 1992; 30: 3146–3150.
17. Toyoshima O, Nishizawa T, Sakitani K, et al. Serum anti-*Helicobacter pylori* antibody titer and its association with gastric nodularity, atrophy, and age: A cross-sectional study. *World J Gastroenterol* 2018; 24: 4061–4068.
18. Nishizawa T, Suzuki H, Arano T, et al. Characteristics of gastric cancer detected within 1 year after successful eradication of *Helicobacter pylori*. *J Clin Biochem Nutr* 2016; 59: 226–230.
19. Nishizawa T, Suzuki H, Fujimoto A, et al. Effects of patient age and choice of antisecretory agent on success of eradication therapy for *Helicobacter pylori* infection. *J Clin Biochem Nutr* 2017; 60: 208–210.
20. Kishikawa H, Kimura K, Takarabe S, et al. *Helicobacter pylori* antibody titer and gastric cancer screening. *Dis Markers* 2015; 2015: 156719.
21. Kiso M, Yoshihara M, Ito M, et al. Characteristics of gastric cancer in negative test of serum anti-*Helicobacter pylori* antibody and pepsinogen test: A multicenter study. *Gastric Cancer* 2017; 20: 764–771.
22. Shichijo S, Hirata Y, Niikura R, et al. Association between gastric cancer and the Kyoto classification of gastritis. *J Gastroenterol Hepatol* 2017; 32: 1581–1586.
23. Kimura K and Takemoto T. An endoscopic recognition of the atrophic border and its significance in chronic gastritis. *Endoscopy* 1969; 3: 87–97.
24. Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; 20: 1161–1181.
25. Sugimoto M, Ban H, Ichikawa H, et al. Efficacy of the Kyoto classification of gastritis in identifying patients at high risk for gastric cancer. *Intern Med* 2017; 56: 579–586.
26. Miki K. Gastric cancer screening by combined assay for serum anti-*Helicobacter pylori* IgG antibody and serum pepsinogen levels—"ABC method". *Proc Jpn Acad Ser B Phys Biol Sci* 2011; 87: 405–414.
27. Nakamura M, Matsui H, Takahashi T, et al. Suppression of lymphangiogenesis induced by Flt-4 antibody in gastric low-grade mucosa-associated lymphoid tissue lymphoma by *Helicobacter heilmannii* infection. *J Gastroenterol Hepatol* 2010; 25: 1–6.