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results of the Kyushu and Okinawa Population
Study, a retrospective observational cohort
study

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BMJ Open Analyses of the association between *Helicobacter pylori* antibody titre and pathogenicity before and after eradication: results of the Kyushu and Okinawa population study, a retrospective observational cohort study

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ABSTRACT

Objectives To assess the utility of *Helicobacter pylori* antibody testing, we evaluated the correlation between the *H. pylori* antibody titre and *H. pylori*-associated pathogenicity and the changes in antibody titre after *H. pylori* eradication therapy.

Design A retrospective observational cohort study.

Setting and participants From 2004 to 2016, medical check-ups were performed in different regions of Japan. In total, 324 subjects infected with *H. pylori* who received *H. pylori* eradication therapy were enrolled; *H. pylori* was eradicated in 266 of these subjects. We examined the associations between *H. pylori* antibody titre with pepsinogen and the presence or absence of *H. pylori*-associated pathogenic proteins, such as cytotoxin-associated gene A and vacuolating cytotoxin gene A, at baseline and after *H. pylori* eradication therapy.

Results The *H. pylori* antibody titre showed a positive correlation with pepsinogen II and a negative correlation with the pepsinogen I/II ratio. Moreover, the *H. pylori* antibody titre significantly correlated with the positive rates of *H. pylori*-associated pathogenic protein before eradication therapy. Antibody titres decreased after eradication, the pepsinogen I/II ratio increased and the *H. pylori*-associated pathogenic protein-positive rate decreased in patients with successful eradication. The determination of eradication using the decline in antibody titre 6 months after eradication therapy was useful (area under the receiver operating characteristic curve: 0.98).

Conclusions Our data indicate that the *H. pylori* antibody titre may represent the degree of pathogenicity. The *H. pylori* antibody titre was associated with attenuation of pathogenicity in patients with *H. pylori* eradication, indicating the clinical utility of *H. pylori* antibody testing.

INTRODUCTION

Chronic and persistent *Helicobacter pylori* infection causes various gastrointestinal diseases (eg, atrophic gastritis, gastric ulcer and gastric cancer); therefore, eradication

STRENGTH AND LIMITATIONS OF THIS STUDY

- ⇒ The association between *Helicobacter pylori* antibody titre with pepsinogen and virulence factors (eg, CagA) was demonstrated before and after eradication therapy.
- ⇒ Long-term changes in *Helicobacter pylori* antibody titre after eradication therapy in a community-based population were evaluated.
- ⇒ Changes in *Helicobacter pylori* antibody titre after eradication are presented separately for successful and unsuccessful eradication.
- ⇒ The present study did not perform pathological evaluation or show an association with gastric cancer.

therapy is recommended.^{1 2} Various *H. pylori* diagnostic tests, such as the urea breath test, rapid urease test, serum and urine antibody tests and faecal antigen test, are available. These tests are appropriately used based on their strengths and weaknesses. Antibody testing can be used as a diagnostic test for *H. pylori*; however, it is not recommended for evaluating successful eradication. For the assessment of *H. pylori* eradication, the current clinical practice guidelines recommend the urea breath test and faecal antigen test.^{3 4} However, both of these tests are limited by cost, complexity and the influence of medication on the test results.⁵ Compared with the faecal antigen test, serum antibody tests are inexpensive, simple to perform and not affected by drugs such as proton pump inhibitors.⁶

Cytotoxin-associated gene A (CagA) and vacuolating cytotoxin gene A (VacA) are known pathogenic factors associated with *H. pylori*. Infection with CagA-positive and/or

VacA-positive *H. pylori* strains increases the risk of peptic ulcers and gastric cancer.^{7–9} Additionally, various factors, such as the urease gene (UreA), leucocyte activators (eg, neutrophil-activating protein, NAP) and cell adhesins (eg, *H. pylori* adhesin A, HpaA), are involved in infection and pathogenicity.¹⁰ In the present study, we term these pathogenic proteins, *H. pylori*-associated pathogenic proteins (HAPPs).

Pepsinogen (PG) is the precursor of pepsin, a proteolytic enzyme produced specifically in the stomach. The serum PG method is used to screen for gastric cancer,¹¹ and PG levels have been reported to be associated with the degree of gastric mucosal atrophy.¹²

Few studies have reported the relationship between serum *H. pylori* antibody titre and PG and HAPP status. Furthermore, although successful eradication therapy leads to a decrease in antibody titre over time^{13–15} and changes in PG after eradication have been reported,¹³ changes in HAPPs after eradication are yet to be fully elucidated. In the present study, our aims were as follows: (1) to assess the association between the *H. pylori* antibody titre and PG and HAPP levels; (2) to examine the relationship between changes in *H. pylori* antibody titre and HAPP levels after successful *H. pylori* eradication therapy and (3) to demonstrate the clinical utility of *H. pylori* antibody testing for the evaluation of *H. pylori* eradication.

METHODS

Subjects

This retrospective observational cohort study was performed as part of the Kyushu and Okinawa Population Study, a community-based prospective epidemiological study of lifestyle-related diseases and cancers that has been ongoing since 2004.^{16–19} Each year, medical check-up is performed for residents ≥20 years of age. From 2004 to 2016, 5692 subjects underwent upper gastrointestinal endoscopy and the serum *H. pylori* antibody titre was measured as part of this screening. Of these subjects, 2735 (48.0%) were positive for serum antibodies against *H. pylori*. Among these 2735 subjects, 589 with chronic *H. pylori* infection, who were positive for *H. pylori* antibody and the urea breath test, received *H. pylori* eradication therapy after providing informed consent. Data for 324 subjects who underwent a follow-up after eradication therapy were available for analysis in the present study (online supplemental figure 1).

This study used the following exclusion criteria: (1) subjects who had undergone *H. pylori* eradication therapy before enrolment; (2) subjects with gastric cancer; (3) subjects who regularly received non-steroidal anti-inflammatory drugs; (4) subjects who regularly received proton pump inhibitors; (5) subjects who underwent partial or total gastrectomy and (6) subjects with renal dysfunction (serum creatinine >1.1 mg/dL) because renal dysfunction affects serum PG levels.²⁰

Serum antibody testing

Serum samples were stored at –80 °C until measurement. The measurement of *H. pylori* antibody was performed using the *H. pylori*-latex ‘Seiken’ kit (Denka, Tokyo, Japan), which employs a latex-enhanced immunoturbidimetric method with a cut-off value of >10 IU/mL for clinically-positive *H. pylori* status. The latex-enhanced immunoturbidimetric method can process a greater number of samples more easily and quickly compared with previously used enzyme immunoassays. The detection accuracy of the test is equivalent to or higher than that of enzyme immunoassays.²¹

H. pylori eradication and its evaluation

Triple combination therapy was initiated for subjects who were diagnosed with chronic *H. pylori* infection and who agreed to receive *H. pylori* eradication therapy. Therapy comprised a 1-week course of oral triple-drug therapy with lansoprazole (30 mg two times per day), amoxicillin (750 mg two times per day) and clarithromycin (400 mg two times per day). The subjects underwent a urea breath test 3 months after the start of therapy to evaluate the results of *H. pylori* eradication. *H. pylori* antibody titres were measured before 3 months and 6 months after, and yearly for 5 years after *H. pylori* eradication therapy to follow the changes before and after *H. pylori* eradication. The changes in antibody titre and the rate of decline in the titre in subjects with and without successful *H. pylori* eradication were evaluated. The reduction rate (%) was calculated using the following formula: (1—antibody titre after *H. pylori* eradication therapy/antibody titre before *H. pylori* eradication therapy) × 100.

Pepsinogen (PG) testing and *H. pylori*-associated pathogenic proteins (HAPPs)

The serum PG level was measured to evaluate the degree of gastric mucosal atrophy caused by *H. pylori* infection. PG I and II levels and the PG I/II ratio were measured using the LZ test ‘Eiken’ PG I, II (Eiken Chemical, Tokyo, Japan). The Blot-Line *Helicobacter* IgG kit (TestLine Clinical Diagnostics sro, Brno, Czech Republic), which is a western blotting-based testing method, was used to examine the presence or absence of serum CagA, VacA, UreA, NAP, HpaA, *Helicobacter* cysteine-rich protein C (HcpC) and chaperonin (heat shock protein (Hsp 60) and GroEL). The levels of HAPPs, as determined by western blotting, were classified as ‘intensive’, ‘weak’ or ‘no band’. In this study, ‘intensive’ was defined as HAPP-positive, whereas ‘weak/no band’ was defined as HAPP-negative. We measured PG and HAPP levels before and 6 months after eradication.

Upper gastrointestinal endoscopy

All subjects underwent upper gastrointestinal endoscopy, which identified atrophic gastritis, gastric ulcer, duodenal ulcer and gastric cancer. In this study, multiple gastrointestinal endoscopists performed the endoscopic evaluations. The degree of gastric mucosal atrophy was assessed

during endoscopy, and subjects with atrophy were included in the evaluation, while subjects with gastric cancer were excluded.

Statistical analysis

The data are expressed as medians. The unpaired t-test or Spearman's rank correlation was used for between-group comparisons, as appropriate. The χ^2 test was used to compare the receiver operating characteristic (ROC) curves. The McNemar test was used to compare changes in the HAPP positivity rate before and after eradication. The Jonckheere-Terpstra test was used to compare changes in antibody titre and the decline rate of antibody titre after eradication. All analyses were performed using IBM SPSS Statistics for Windows, V.22.0 (IBM, Armonk, New York, USA), and $p < 0.05$ was considered statistically significant.

Patient and public involvement

Patients were involved in this study as research participants but did not contribute to the conceptualisation, design or interpretation of the study. Preliminary results were disseminated to participants through residents' briefings.

RESULTS

Characteristics of this study

In the present study, the median age of the 324 subjects analysed was 62 (IQR, 55–68), and 43.2% were male. The median *H. pylori* antibody titre before *H. pylori* eradication therapy was 57.5 U/mL (IQR, 36.5–81.5 U/mL) for the tested subjects. For these subjects, the median PG I and II levels were 53.4 ng/mL (IQR, 37.7–76.2) and 20.1 ng/mL (IQR, 15.3–27.2), respectively, and the median PG I/II ratio was 2.7 (IQR, 1.9–3.5). The positive rates of HAPP in the subjects were 74.4% for CagA, 51.2% for VacA, 21.9% for UreA, 14.2% for NAP, 30.6% for HpsA, 23.5% for HcpC and 47.2% for GroEL.

Analysis of *H. pylori* antibody titres and correlations with PG levels and the presence of HAPPs

The correlations between *H. pylori* antibody titre and each assessed factor were analysed (figure 1A–C). A positive correlation was detected between the *H. pylori* antibody titre and PG II levels before *H. pylori* eradication therapy (figure 1B) ($r = 0.26$; $p < 0.0001$), whereas a negative correlation was detected between the *H. pylori* antibody titre and the PG I/II ratio (figure 1C) ($r = -0.13$; $p = 0.010$). As shown in table 1, comparing the respective antibody titre of the HAPP-positive and negative groups, the positive group had significantly higher antibody titres than the negative group for all HAPPs.

Analysis of changes in PG levels and positive rates for HAPPs with and without successful *H. pylori* eradication

Of the 324 subjects who received *H. pylori* eradication therapy, *H. pylori* was successfully eradicated in 266 subjects and unsuccessfully eradicated in 58 subjects (online supplemental figure 1). Changes in PG levels in both successful and unsuccessful eradication subjects are shown in online supplemental table 1. PG I and II levels decreased significantly in subjects with successful *H. pylori* eradication compared with subjects with unsuccessful eradication ($p < 0.0001$); there was also a significant increase in the PG I/II ratio ($p < 0.0001$). Although there was a significant increase in the PG I/II ratio in subjects with unsuccessful *H. pylori* eradication ($p = 0.019$), there were no significant changes in the PG I or II levels of these subjects.

As shown in table 2, a significant reduction was observed in the positivity rates of all HAPPs in subjects with successful *H. pylori* eradication ($p < 0.0001$). Although a significant reduction in the positivity rates of CagA and GroEL was observed in subjects with unsuccessful *H. pylori* eradication, there were no significant changes in the other HAPPs.

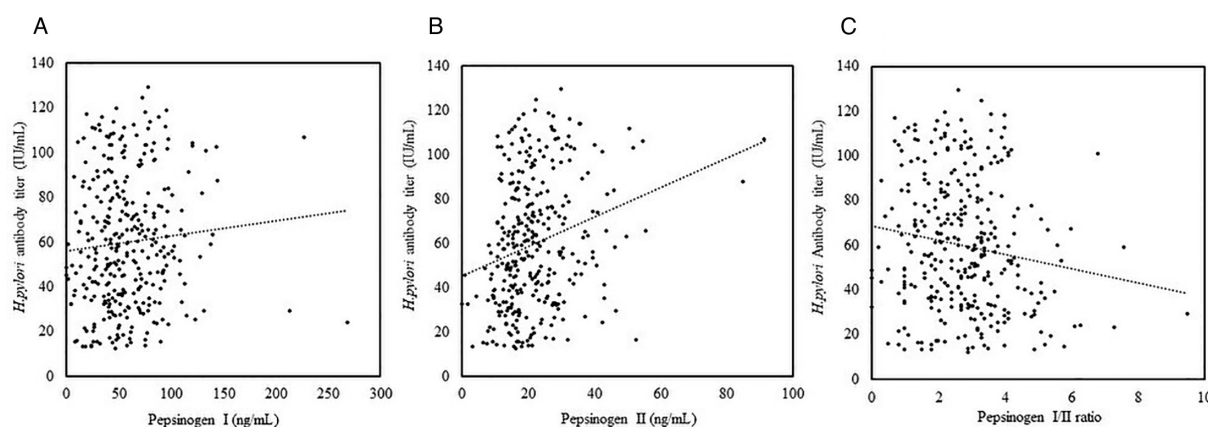


Figure 1 Correlation between *Helicobacter pylori* antibody titre and pepsinogen level. (A) Correlation between *Helicobacter pylori* antibody titre and pepsinogen I (Spearman's rank correlation); $R_s = 0.0922$; $p = 0.0969$. (B) Correlation between *Helicobacter pylori* antibody titre and pepsinogen II (Spearman's rank correlation); $R_s = 0.26$; $p < 0.0001$. (C) Correlation between *Helicobacter pylori* antibody titre and the pepsinogen I/II ratio (Spearman's rank correlation); $R_s = -0.1346$; $p = 0.0152$.

Table 1 Antibody titre for each *Helicobacter pylori*-associated pathogenic protein (HAPP) group (n=324)

HAPP	Positive		Negative		P value
	N	Antibody titre (IU/mL)*	N	Antibody titre (IU/mL)*	
CagA	241	65.32 (43.3–86.9)	83	44.28 (24.9–55.4)	<0.0001
VacA	166	65.54 (40.9–87.4)	158	54.53 (32.8–71.9)	0.0005
UreA	71	75.46 (52.3–101.7)	253	55.57 (32.5–73.7)	<0.0001
NAP	46	71.89 (42.3–100.5)	278	57.95 (35.1–76.1)	0.0011
HpaA	99	74.25 (53.4–97.5)	225	53.62 (32.0–71.3)	<0.0001
HcpC	76	72.98 (46.8–102.2)	248	55.92 (34.0–73.6)	<0.0001
GroEL	153	69.74 (48.3–94.2)	171	51.14 (31.6–65.1)	<0.0001

P-value based on a comparison between the positive and negative values for each HAPP.

*Median (IQR).

CagA, cytotoxin-associated gene; GroEL, chaperonin, heat shock protein (Hsp 60); HAPP, *Helicobacter pylori*-associated pathogenic protein; HcpC, *Helicobacter* cysteine-rich protein; HpaA, *Helicobacter pylori* adhesin A; n, number; NAP, neutrophil-activating protein; UreA, urease; VacA, vacuolating cytotoxin.

Analysis of temporal changes in *H. pylori* antibody titre with and without successful *H. pylori* eradication

As shown in figure 2, antibody titres decrease over time after eradication. There was a statistically significant decrease in both successful and failed eradication subjects. As shown in figure 3, the median decline rate in antibody titre 3 months, 6 months, 1 year, 2 years, 3 years, 4 years and 5 years after *H. pylori* eradication therapy was 55%, 73%, 80%, 87%, 88%, 92% and 89%, respectively, in subjects with successful *H. pylori* eradication (figure 3A) and 6%, 4%, 7%, 8%, 35%, 5% and 17%, respectively, in subjects with failed *H. pylori* eradication (figure 3B) (compared with pre-*H. pylori* eradication therapy). In subjects with successful eradication, antibody titres decreased by more than 80% within 1 year after eradication and achieved approximately 90% after 2 years. Regarding the rate of decrease in antibody titres, there was a significant trend towards a decrease in the successful eradication group ($p<0.001$), but not in the unsuccessful eradication group ($p=0.0550$).

ROC analysis of the utility of the *H. pylori* antibody test for predicting successful or unsuccessful *H. pylori* eradication therapy

The cut-off value in the ROC curves for differentiating success and failure of *H. pylori* eradication therapy is shown in online supplemental table 2. In the evaluation of *H. pylori* eradication within 1 year after the start of therapy, the area under the curve (AUC) was significantly higher 6 months after *H. pylori* eradication therapy than at other time points. As shown in online supplemental figure 2A, when an antibody titre of 30 U/mL 6 months after *H. pylori* eradication therapy was used as the cut-off value, the sensitivity, specificity and AUC were 83%, 70% and 0.83, respectively. As shown in online supplemental figure 2B, when the rate of decline in antibody titre of 46% 6 months after *H. pylori* eradication therapy was used as the cut-off value, the sensitivity, specificity and AUC were 94%, 96% and 0.98, respectively, demonstrating the utility of this method of evaluation of *H. pylori* eradication.

Table 2 Changes in the positive rate for HAPPs after *Helicobacter pylori* eradication therapy (n=276)

HAPP	Successful eradication (n=230)			Unsuccessful eradication (n=46)		
	pre-eradication	Posteradication	P value	pre-eradication	post-therapy	P value
	N (%)	N (%)		N (%)	N (%)	
CagA	172 (74.8)	64 (27.8)	<0.0001	29 (63)	22 (47.8)	0.0348
VacA	124 (53.9)	47 (20.4)	<0.0001	22 (47.8)	19 (41.3)	0.1797
UreA	51 (22.2)	7 (3.0)	<0.0001	6 (13.0)	5 (10.9)	0.7389
NAP	34 (14.8)	7 (3.0)	<0.0001	7 (15.2)	6 (13.0)	0.3173
HpaA	76 (33.0)	14 (6.1)	<0.0001	11 (23.9)	8 (17.4)	0.2568
HcpC	59 (25.7)	16 (7.0)	<0.0001	9 (19.6)	8 (17.4)	0.5637
GroEL	113 (49.1)	29 (12.6)	<0.0001	23 (50)	15 (32.6)	0.0114

P value based on a comparison between the pre-eradication and posteradication therapy values for each HAPP.

CagA, cytotoxin-associated gene; GroEL, chaperonin heat shock protein (Hsp 60); HAPP, *Helicobacter pylori*-associated pathogenic protein; HcpC, *Helicobacter* cysteine-rich protein; HpaA, *Helicobacter pylori* adhesin A; n, number; NAP, neutrophil-activating protein; UreA, urease; VacA, vacuolating cytotoxin.

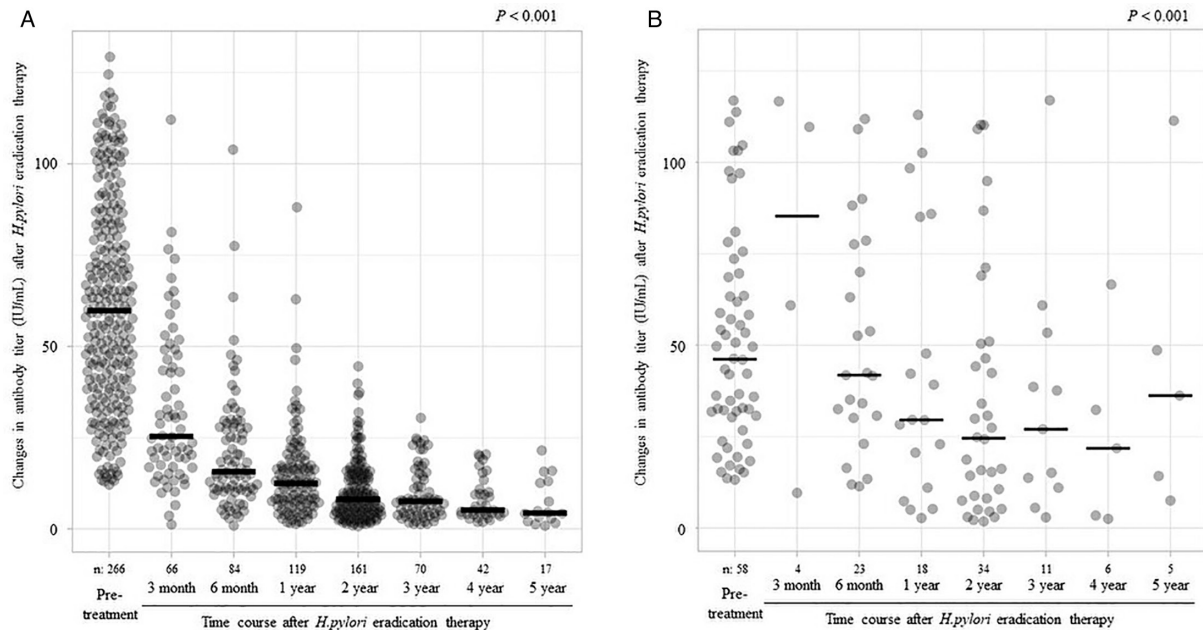


Figure 2 Change in *Helicobacter pylori* antibody titre over time after eradication therapy. Each subjects are shown as beeswarm plot, at each point. The horizontal bars indicate the median value. n indicates the number of subjects measured at each time point. (A) Successful *Helicobacter pylori* eradication group. $p < 0.001$; Jonckheere-Terpstra test. (B) Unsuccessful *Helicobacter pylori* eradication group. $p < 0.001$; Jonckheere-Terpstra test.

DISCUSSION

The present study showed the following: (1) the positive association between the actual antibody titre and the pathogenicity of *H. pylori* before eradication therapy in a well-defined population; (2) the changes in antibody titre after *H. pylori* eradication and (3) the clinical utility of the *H. pylori* antibody titre through ROC analysis.

In Japan, gastric fluoroscopy has predominantly been used for gastric cancer screening.²² Currently, gastric cancer risk classification is performed using *H. pylori* antibody testing and by the measurement of serum PG.^{11 23} Although this classification is useful in determining the risk of gastric cancer using only serologic testing, in reality, some high-risk subjects may be included in the group that

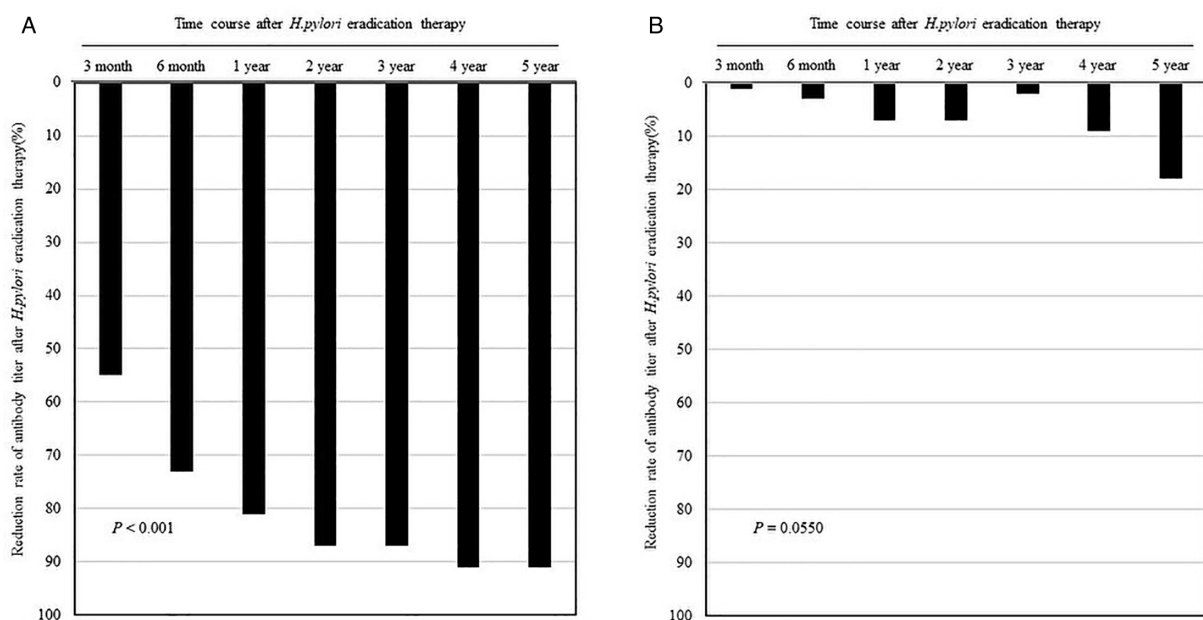


Figure 3 The reduction rate of *Helicobacter pylori* antibody titre over time after eradication therapy. The reduction rate of antibody titre after *Helicobacter pylori* eradication at each time point, as calculated by the relevant formula. (A) Successful *Helicobacter pylori* eradication group. $p < 0.001$; Jonckheere-Terpstra test. (B) Unsuccessful *Helicobacter pylori* eradication group. $p = 0.0550$; Jonckheere-Terpstra test.

is determined to be low risk.²⁴ Therefore, caution must be exercised in making such a determination, but there is also a method to discriminate false low-risk groups,²⁵ and this method should be used to avoid overlooking high-risk subjects. In the present study, all subjects underwent upper gastrointestinal endoscopy, as well as the urea breath test and the measurement of serum *H. pylori* antibody. Although no consensus has been reached on the association between *H. pylori* antibody titre and *H. pylori* pathogenicity, the present study showed an association between high antibody titre and high PG II levels and a low PG I/II ratio. Previous studies have reported that high PG II levels and a low PG I/II ratio are associated with gastric atrophy,^{12 26 27} suggesting a correlation between antibody titre and gastric atrophy. The antibody titre is particularly high in subjects with CagA-positive *H. pylori*, which is considered a risk factor for gastric cancer.⁸ A high antibody titre may indicate *H. pylori* pathogenicity. Patients with a high antibody titre before *H. pylori* eradication therapy may have a high risk of developing gastric atrophy and gastric cancer; therefore, early *H. pylori* eradication therapy is recommended. Regarding the stratification of gastric cancer risk for patients with *H. pylori* infection, our results suggest that the *H. pylori* antibody titre levels might be useful for risk classification.

In the present study, after successful *H. pylori* eradication therapy, the positive detection rate of all HAPPs was significantly lower, with an improvement in serum PG levels, the degree of gastric mucosal atrophy and the attenuation of pathogenicity. According to Formichella *et al*,²⁸ VacA and other HAPPs turned negative after successful eradication, but CagA remained positive even after successful eradication. However, in the present study, all HAPPs, including CagA, showed a significant reduction in positive detection rates after successful *H. pylori* eradication therapy. The disappearance of CagA, which is considered an oncogenic protein,²⁹ implied recovery from pathogenic infection. Our results may also indicate an association between a decline in antibody titre and the attenuation of pathogenicity after successful *H. pylori* eradication therapy, and furthermore, the decline in antibody titre after eradication might be associated with the reduced incidence of gastric cancer.³⁰

Although several studies have focused on the changes in antibody titre after *H. pylori* eradication,^{13–15} the duration and rate of decline remained unclear. The present study demonstrated a significant decline in antibody titre over time in subjects with *H. pylori* eradication. Antibody titres also decreased in the eradication failure group. However, there is a report of spontaneous decline in antibody titres over time even without eradication,³¹ suggesting that the decrease might be due to natural fluctuations. Nonetheless, the rate of decline in antibody titres was significant only in the successful eradication group. The rate of decline in antibody titre 6 months after successful *H. pylori* eradication therapy was approximately 70%. Additionally, the results of the analysis of the ROC curves (sensitivity: 91%, specificity: 96% and AUC:

0.95) suggested that serum antibody testing may be useful with an antibody titre reduction rate of 45.6% as a cut-off value. These results indicate that the serum antibody titre could be used for the screening and evaluation of *H. pylori* eradication, such as in a community-based study.

This study has some limitations. First, all subjects were Japanese. Second, no pathological evaluation was performed. Third, not all *H. pylori*-positive patients underwent *H. pylori* eradication therapy. Furthermore, this study could not show an association between *H. pylori* antibody titre and the development of gastric cancer, and did not examine the relationship between clinical and endoscopic findings and antibody titre. However, this study is useful as it involved a large sample size of subjects recruited from a well-defined population. Future studies should also investigate the risk of gastric cancer.

In conclusion, *H. pylori* antibody titres were higher in CagA-positive subjects, and there was a negative correlation with the PG I/II ratio. Thus, the measurement of *H. pylori* antibody may provide additional information for gastric cancer risk. Furthermore, a decline in antibody titre after *H. pylori* eradication was associated with the attenuation of *H. pylori* pathogenicity. These findings may demonstrate the clinical utility of *H. pylori* antibody titre in the evaluation of *H. pylori* eradication.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, conduct, reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the institutional review board of Kyushu University Hospital (approval numbers: 28-320 (this study) and 590-04 (KOPS)). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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