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ORIGINAL ARTICLE

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The histologic detection of *Helicobacter pylori* in seropositive subjects is affected by pathology and secretory ability of the stomach

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Abstract

Background: *Helicobacter pylori* is unevenly distributed in hypochlorhydric environments. The study aim was to elucidate the risk factors for a negative Giemsa staining finding in seropositive subjects by measuring the secretory ability of the stomach.

Methods: Subjects aged over 18 years were included consecutively after endoscopic biopsy at gastric lesions with color or structural changes. Blood was sampled for the serum pepsinogen (PG) assay and *H. pylori* serology test. After excluding the subjects with past *H. pylori* eradication, the risk factors for a negative Giemsa staining finding in seropositive subjects were analyzed.

Results: Among 872 included subjects, a discrepancy between the serum anti-*H. pylori* IgG and Giemsa staining findings was found in 158 (18.1%) subjects, including 145 Giemsa-negative, seropositive subjects. Gastric adenocarcinoma/adenoma (OR = 11.090, 95% CI = 3.490-35.236) and low serum PG II level (OR = 0.931, 95% CI = 0.899-0.963) were the independent risk factors for a negative Giemsa staining finding in seropositive subjects. The cutoff value of serum PG II level was 7.45 ng/mL (area under curve [AUC] = 0.904, 95% CI = 0.881-0.927). Follow-up studies of Giemsa staining at different sites of the stomach revealed that 75% of the Giemsa-negative seropositive subjects with adenocarcinoma are positive, whereas none of those with low serum PG II level of <7.45 ng/mL revealed positive findings.

Conclusions: The risk of a negative Giemsa staining finding in seropositive subjects is increased in gastric adenocarcinoma/adenoma specimens and in subjects with a diminished gastric secretory ability with low serum PG II level of <7.45 ng/mL. A false-negative Giemsa staining finding is common in subjects with adenocarcinoma, and therefore, additional biopsies at different sites should be performed in these subjects.

KEYWORDS
biopsy, giemsa, *Helicobacter pylori*, pepsinogen, serology

1 | INTRODUCTION

For the diagnosis of *Helicobacter pylori* (*H. pylori*) infection, noninvasive tests are often recommended to decrease the risks of false-negative, invasive test findings.[1,2] Combining the serum anti-*H. pylori* immunoglobulin G (IgG) assay is useful in hypochlorhydric environments including chronic atrophic gastritis (CAG) and intestinal metaplasia (IM).[3-6] A seronegative finding is useful in excluding precancerous conditions; therefore, endoscopic biopsy and serology test are often performed on the same day.[7]

The serum anti-*H. pylori* IgG test is used for gastric cancer screening with the serum pepsinogen (PG) assay.[8,9] The secretory ability of the human stomach can be measured using the serum PG assay, which is known to be useful in detecting high-risk subjects for Lauren's intestinal-type gastric cancer.[10] The serum PG I and II levels usually increase during an active *H. pylori* infection and are thus useful in detecting current infections when combined with a serology test.[11,12] Conversely, the serum PG I and PG II levels decrease when there is a gradual loss of secretory ability with the progress of CAG and IM.[13] Low serum PG I levels indicate corpus-predominant pangastritis with an impaired acid secretion from the fundus.[14] Furthermore, low serum PG II levels indicate a more severe form of pangastritis derived from antrum-predominant CAG and IM because PG II is secreted throughout the stomach.[15,16] The serum PG I level of <70 ng/mL and PG I/II ratio of <3.0 are often used for the diagnosis of gastric corpus atrophy.[8,9,17]

As *H. pylori* tend to be unevenly distributed in unfavorable hypochlorhydric environments, it is important to discriminate false Giemsa-negative, seropositive subjects with true *H. pylori* infection among the seropositive subjects.[1-3,5,6] False-negative of Giemsa staining should be considered more seriously than false-positive of serology test, not only to reduce risk of malignancy by minimizing falsely untreated individuals, but also to prevent bacterial dissemination between people. Nonetheless, there is limited knowledge on the serum PG assay findings in subjects showing discrepancies between invasive and noninvasive *H. pylori* test findings. The aim of this study was to elucidate independent risk factors for a negative Giemsa staining finding in seropositive subjects by measuring the secretory ability of the stomach. We further tried to elucidate rates of the discrepancy in each pathologic subgroup in relation to serum PG levels, showing how changes in the gastric secretory function have effect on the rate of discrepancy. Furthermore, by finding risk factors, we tried to provide clues to discriminate the false Giemsa-negative, seropositive subjects with true *H. pylori* infection among the seropositive subjects.

2 | MATERIALS AND METHODS

2.1 | Study subjects

Koreans aged over 18 years who visited our center for gastric cancer screening were enrolled consecutively from 2010-2014 in a retrospective and a prospective manner after the previous study registered at ClinicalTrials.gov (NCT01824953).[9] Among the subjects who underwent upper gastrointestinal (UGI) endoscopy, serum PG assay, and *H. pylori* serology test on the same day, those who underwent endoscopic biopsy at a gastric lesion with either a color or a structural change were included in this study. Subjects who underwent gastrectomy or those with an equivocal serology test finding were excluded.

After the initial enrollment, follow-up gastric biopsy findings before June 2017 were added to elucidate *H. pylori* infection in the Giemsa-negative, seropositive subjects. All of the subjects provided informed consent before the tests, and the study was approved by the Institutional Review Board of Konkuk University Medical Center (KUH1010854).

2.2 | Serum assays

After 12 hours of fasting for UGI endoscopy, venous blood was sampled for the serum PG assay and *H. pylori* serology test. To measure the secretory ability of the gastric mucosa, the serum PG assay was performed using the latex-enhanced turbidimetric immunoassay (HBi, Anyang, Korea) as described in a previous study.[9] The serum PG I and PG II levels were provided with the serum PG I/II ratio in each subject. Gastric corpus atrophy was diagnosed when the serum PG I/II ratio was below 3.0, and the serum PG I level was below 70 ng/mL.[8,9,17]

For the measurement of the serum anti-*H. pylori* IgG, either the Chorus *H. pylori* IgG (DIESSE Diagnostica Senese, Siena, Italy) or the Vidas *H. pylori* IgG (BioMérieux, Marcy-l'Etoile, France) was used. Both exhibited a high concurrence rate and a similar diagnostic accuracy with the Genedia *H. pylori* ELISA kit (Green Cross Medical Science, Korea) in Koreans.[18] Using the urea breath test as a gold standard for the diagnosis for *H. pylori* infection, the sensitivities and the specificities of the Vidas, Chorus, and Genedia assays were 89.7% and 100%, 100% and 85.5%, and 75.4% and 80.7%, respectively.

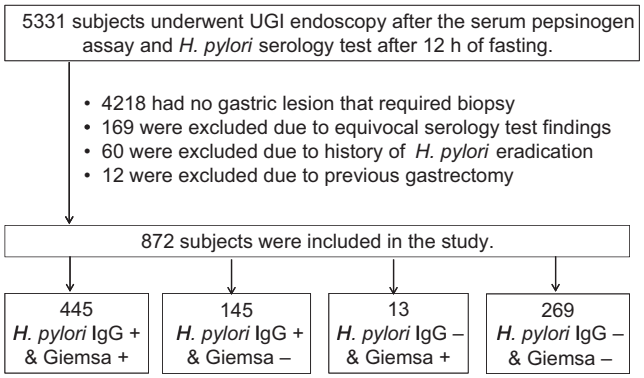


FIGURE 1 Flow of the study. Among the subjects who underwent gastric cancer screening test, 782 subjects satisfied the inclusion criteria. The subjects are classified into four groups according to their serum anti- *Helicobacter pylori* IgG assay and Giemsa staining findings. A discrepancy between the tests is found in the 145 seropositive subjects and in the 13 seronegative subjects. UGI, upper gastrointestinal; IgG, immunoglobulin G

TABLE 1 Characteristics of the 872 included subjects

Variables	Total (n = 872)	Giemsa-negative, seronegative (n = 269)	Giemsa-positive, seronegative (n = 13)	Giemsa-negative, seropositive (n = 145)	Giemsa-positive, seropositive (n = 445)	*P*-value
Age (year-old)	53.9 ± 10.2	52.9 ± 10.9	54.4 ± 13.3	57.9 ± 10.2	53.2 ± 9.4	<.001
Gender (male, %)	540 (61.9)	135 (50.2)	11 (84.6)	103 (71.0)	291 (65.4)	<.001
Comorbidity (%)						
Diabetes mellitus	48 (5.5)	17 (6.3)	3 (23.1)	6 (4.1)	22 (4.9)	.115
Hypertension	120 (13.8)	33 (12.3)	3 (23.1)	24 (16.6)	60 (13.5)	.316
Coronary heart disease	15 (1.7)	4 (1.5)	0	3 (2.1)	8 (1.8)	.886
Cerebrovascular disease	10 (1.1)	1 (0.4)	1 (7.7)	2 (1.4)	6 (1.3)	.165
Recent drug intake (%)						
Antithrombotics	96 (11.0)	25 (9.3)	2 (15.4)	21 (14.5)	48 (10.8)	.281
NSAID	57 (6.5)	18 (6.7)	1 (7.7)	10 (6.9)	28 (6.3)	.982
Antacid	4 (0.5)	2 (0.7)	0	0	2 (0.4)	.751
Serum PG assay						
PG I (ng/mL)	60.3 ± 28.5	51.2 ± 22.5	61.6 ± 36.4	53.2 ± 30.6	68.3 ± 28.5	<.001
PG II (ng/mL)	16.3 ± 10.3	9.8 ± 6.9	15.0 ± 10.5	14.7 ± 10.7	20.8 ± 9.6	<.001
PG I/II ratio	4.3 ± 1.9	5.6 ± 1.6	4.5 ± 1.8	4.5 ± 2.4	3.5 ± 1.3	<.001
Biopsied site (n, %)						<.001
Antrum	473 (54.3)	133 (49.4)	5 (38.4)	96 (66.2)	255 (57.3)	
Angle	71 (8.1)	7 (2.6)	0	12 (8.3)	55 (12.4)	
Low-body	97 (11.1)	29 (10.8)	1 (7.7)	28 (19.3)	45 (10.1)	
Mid-body	75 (8.6)	31 (11.5)	3 (23.1)	16 (11.0)	25 (5.6)	
High-body	73 (8.4)	32 (11.9)	3 (23.1)	16 (11.0)	28 (6.3)	
Cardia	44 (5.0)	18 (6.7)	1 (7.7)	4 (2.8)	22 (4.9)	
Fundus	39 (4.5)	19 (7.1)	0	6 (4.1)	15 (3.4)	
Pathology (n, %)						<.001
Adenoca./adenoma	24 (2.8)	5 (1.9)	0	15 (10.3)	4 (0.9)	
Hemorrhage	11 (1.3)	7 (2.6)	0	3 (2.1)	1 (0.2)	
Intestinal metaplasia	367 (42.1)	86 (31.9)	4 (30.7)	71 (49.1)	206 (46.4)	
Foveolar hyperplasia	115 (13.2)	66 (24.5)	1 (7.7)	19 (13.1)	29 (6.5)	
Hyperplastic polyp	44 (5.0)	5 (1.9)	1 (7.7)	7 (4.8)	31 (7.0)	
Ulcer/erosion	77 (8.8)	12 (4.5)	2 (15.4)	9 (6.2)	54 (12.1)	

(Continues)

TABLE 1 (Continued)

Variables	Total (n = 872)	Giemsa-negative, seronegative (n = 269)	Giemsa-positive, seronegative (n = 13)	Giemsa-negative, seropositive (n = 145)	Giemsa-positive, seropositive (n = 445)	*P*-value
Fundic gland polyp	82 (9.4)	68 (25.3)	1 (7.7)	7 (4.8)	6 (1.3)	
Atypical/regenerating	44 (5.0)	8 (3.0)	1 (7.7)	5 (3.4)	30 (6.7)	
Lymphoid follicles	91 (10.4)	7 (2.6)	3 (23.1)	8 (5.5)	73 (16.4)	
Atrophy	13 (1.5)	3 (1.1)	0	1 (0.7)	9 (2.0)	
Others[a]	4 (0.5)	2 (0.7)	0	0	2 (4.5)	

SD, standard deviation; NSAID, nonsteroidal anti-inflammatory drug; PG, pepsinogen; Adenoca., adenocarcinoma; Atypical/regenerating; atypical cells or regenerating glands.
[a]Others consist of three inflammatory polyps and one xanthoma.

The test value (TV) of ≥1.0 was defined as a seropositive finding, and the TV of <0.75 was defined as a seronegative finding for the Vidas assay. For the Chorus assay, the IgG titer of ≥12.0 arbitrary units (AU)/mL was defined as a seropositive finding, and the titer of <8.0 AU/mL was defined as a seronegative finding. Values between the seropositive and seronegative titers were defined as equivocal test findings. Genedia was not used in this study because it is not available at the outpatient clinic due to the manufacturer's policy.

2.3 | UGI endoscopy

One of the board-certified gastroenterologists performed the endoscopic procedure using either GIF-H260 (Olympus, Tokyo, Japan) or EG-2990i (Pentax, Tokyo, Japan) at our center. When there was an abnormal gastric lesion with either a color or a structural change, endoscopic biopsy was performed during the procedure. The location of the biopsied site was classified into the antrum, angle, low-body, mid-body, high-body, cardia, and fundus.

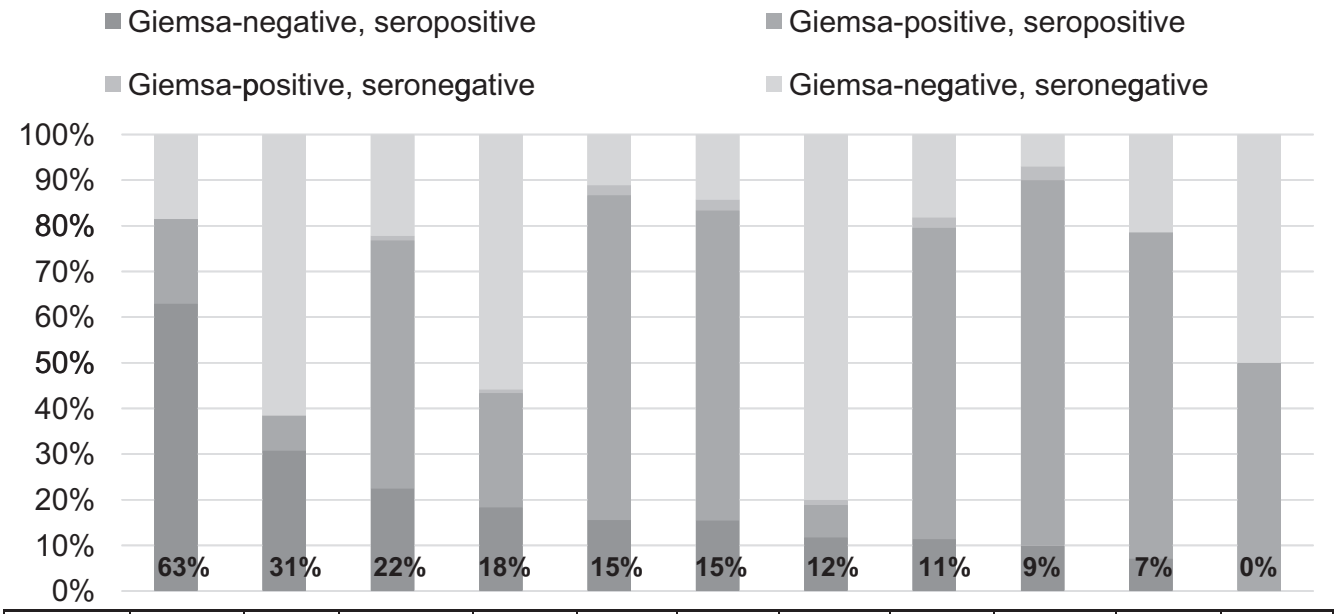
2.4 | Pathology evaluation

The gastric biopsied specimens were fixed with 10% neutral buffered formalin and were embedded in a paraffin block. The specimens were sliced into 4-μm-thick sections for Hematoxylin & Eosin and Giemsa staining. The stained specimens were examined under 40-400 high-power field using a microscope to determine *H. pylori* infection and histology. The final pathology findings of the gastric biopsied specimens were classified into 10 categories: (1) adenocarcinoma and adenoma, (2) atypical cells and regenerating glands, (3) IM, (4) atrophy, (5) ulcer and erosion, (6) hemorrhage, (7) lymphoid cell aggregations and lymphoid follicles, (8) hyperplastic polyp, (9) fundic gland hyperplasia and fundic gland polyp, and (10) foveolar hyperplasia and foveolar polyp (ie, inflammatory polyp and xanthoma).

2.5 | Statistical analysis

The PASW statistics software version 17.0 (Chicago, IL, USA) was used, and statistical significance was defined as $P < .05$. Differences between the (1) Giemsa-positive, seropositive subjects, (2) Giemsa-negative, seropositive subjects, (3) Giemsa-positive, seronegative subjects, and (4) Giemsa-negative, seronegative subjects were analyzed by ANOVA with Bonferroni correction for continuous variables and chi-square test with Bonferroni correction for categorical variables. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were presented as percentage.

A logistic regression analysis was done to verify risk factors for a negative Giemsa staining finding in seropositive subjects. The values were presented as odds ratio (OR) with 95% confidence intervals (CI) after adjusting age and sex. A receiver operating characteristic (ROC) curve analysis was performed by plotting true-positive rate (sensitivity) against false-positive rate (1-specificity) over all possible thresholds of the serum PG assay findings that are related to a negative Giemsa staining finding in seropositive subjects. Cutoff



Pathology findings	Adenoma /adenocarcinoma (n = 24)	Hemorrhage (n = 11)	Intestinal metaplasia (n = 367)	Foveolar hyperplasia (n = 115)	Hyperplastic polyp (n = 44)	Ulcer /erosion (n = 77)	Fundic gland polyp (n = 82)	Atypical cells (n = 44)	Lymphoid follicle (n = 91)	Atrophy (n = 13)	Others (n = 4)
PG I (ng/mL)	37.1 ± 25.3	44.7 ± 11.2	60.7 ± 29.5	57.2 ± 25.7	65.7 ± 37.4	65.4 ± 28.3	52.8 ± 21.6	69.7 ± 32.5	66.2 ± 21.0	65.8 ± 32.0	67.9 ± 42.2
PG II (ng/mL)	14.2 ± 5.5	8.9 ± 4.5	17.1 ± 10.1	12.3 ± 7.0	22.9 ± 11.6	17.2 ± 11.9	9.2 ± 3.8	19.7 ± 10.8	19.3 ± 9.4	23.5 ± 14.8	24.3 ± 10.7
PG I/II ratio	2.6 ± 1.6	5.6 ± 1.6	4.1 ± 1.8	5.1 ± 1.7	3.3 ± 2.0	4.4 ± 1.7	6.0 ± 1.6	4.0 ± 1.4	3.9 ± 1.5	3.7 ± 1.4	2.7 ± 0.9

FIGURE 2 *Helicobacter pylori* test findings in the different pathology groups. The proportion of Giemsa-negative, seropositive subjects is shown in percentages for each pathology findings. The discrepancy rate is highest (62.5%) in the subjects who exhibited gastric adenocarcinoma or adenoma on the biopsied specimen ($P < .001$). The serum PG I and PG II levels are increased in the subgroups with a higher prevalence of Giemsa-positive, seropositive subjects (lymphoid cell aggregations/follicles, hyperplastic polyp, ulcer/erosion, atypical cells/regenerating glands, and atrophy). PG, pepsinogen

values were provided with area under curve (AUC), standard error (SE), and 95% CI for significant serum PG assay findings. As the study subjects are from endemic area of *H. pylori* infection, prevalence-independent likelihood ratios were calculated to provide a better estimation for predicting a negative Giemsa staining finding in seropositive subjects using the significant cutoff values. The positive likelihood ratio was calculated as sensitivity/(1-specificity), and the negative likelihood ratio was calculated as (1-sensitivity)/specificity with the aid of MEDCALC software (https://www.medcalc.org/calc/diagnostic_test.php).

3 | RESULTS

3.1 | Discrepancy between the *Helicobacter pylori* test findings

Among 5331 Korean adults who underwent UGI endoscopy and serum assays for gastric cancer screening, 872 subjects satisfied the inclusion criteria (Figure 1). Among them, a discrepancy between the Giemsa staining and serology test findings was found in 158 (18.1%) subjects. Most of the discrepancies (145/158, 91.8%) were found in the seropositive subjects with a negative Giemsa staining finding (Table 1). Recent intake of acid suppressant was found in two Giemsa-positive, seropositive subjects and in two Giemsa-negative, seronegative subjects.

3.2 | Link between the discrepancies and gastric biopsy findings

Of all the pathology subgroups patients, 10 with gastric adenocarcinoma and 14 with adenoma (15/24, 62.5%) showed the highest discrepancy rate between the serology test and Giemsa staining findings (Figure 2). Negative Giemsa staining findings were common in the seropositive subjects when the biopsied specimen exhibited hemorrhage or IM.

The antrum was the most common biopsied site in all groups. The proportion of the biopsies performed in the gastric body was higher in the 145 Giemsa-negative, seropositive subjects than in the

TABLE 2 Significant variables for a negative Giemsa staining in the seropositive subjects

Variables	Adjusted OR[a]	95% CI	*P*-value
Pathology findings			<.001
Gastric adenoma or adenocarcinoma	11.090	3.490-35.236	
Other gastric pathology findings	1 (Reference)		
Serum PG I level	0.996	0.985-1.006	.427
Serum PG II level	0.931	0.899-0.963	<.001
Serum PG I/II ratio	1.456	1.285-1.648	<.001
Biopsied site of the stomach			
Antrum	1.869	0.468-7.462	.376
Angle	0.863	0.181-4.111	.853
Low-body	2.962	0.685-12.811	.146
Mid-body	4.407	0.963-20.169	.056
High-body	1.894	0.401-8.949	.420
Cardia	2.018	0.359-11.358	.426
Fundus	1 (Reference)		

PG, pepsinogen; OR, odds ratio; CI, confidence intervals.
[a]Adjusted OR for age and gender.

445 Giemsa-positive, seropositive subjects (Table 1). Conversely, the proportion of the biopsies performed in the angle and cardia was lower in the Giemsa-negative, seropositive subjects than the Giemsa-positive, seropositive subjects ($P < .001$).

3.3 | Gastric secretory ability as reflected by the serum PG assay findings

The serum PG I and PG II levels were the highest in the Giemsa-positive, seropositive subjects and were the lowest in the Giemsa-negative, seronegative subjects (Table 1). The serum PG I and PG II levels were significantly lower in the 145 Giemsa-negative, seropositive subjects than in the 445 Giemsa-positive, seropositive subjects. The proportion of gastric corpus atrophy did not differ between the groups ($P = .524$).

3.4 | Risk factors for a negative Giemsa staining finding in the seropositive subjects

The presence of gastric adenocarcinoma or adenoma (OR = 11.090, 95% CI = 3.490-35.236), low serum PG II level (OR = 0.931, 95% CI = 0.899-0.963), and high serum PG I/II ratio (OR = 1.456, 95% CI = 1.285-1.648) were the independent risk factors for a negative Giemsa staining in the seropositive subjects after adjusting age and sex (Table 2). The cutoff value of the serum PG II level was 7.45 ng/mL (AUC = 0.904, 95% CI = 0.881-0.927, SE = 0.012, $P < .001$), and that of the serum PG I/II ratio was 3.05 (AUC = 0.857, 95% CI = 0.827-0.887).

To verify whether these cutoff values are useful in predicting a negative Giemsa staining finding in the seropositive subjects, the proportion of the subjects with the serum PG II level of <7.45 ng/mL and PG I/II ratio of >3.05 was analyzed. There was a significant difference between the Giemsa-negative and Giemsa-positive seropositive subjects when the cutoff value was serum PG II levels of <7.45 ng/mL (Figure 3). Using these criteria, the positive likelihood ratio was 71.65 (95% CI = 26.81-191.43), and the negative likelihood ratio was 0.39 (95% CI = 0.33-0.47) for detecting a negative Giemsa staining finding in the seropositive subjects. The positive and negative predictive values were 96.5% and 87.1%, respectively.

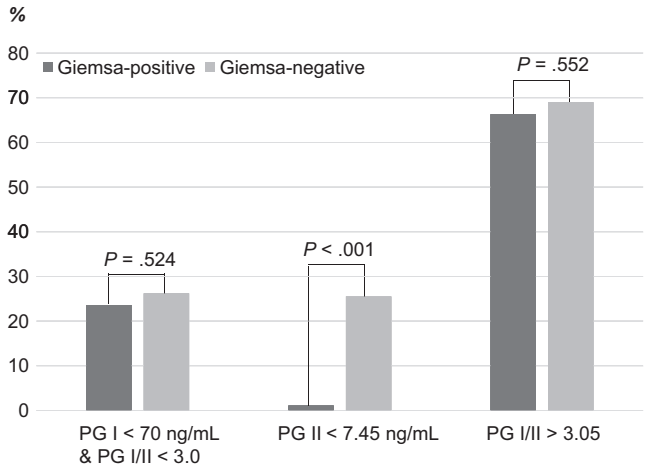


FIGURE 3 Different serum PG assay findings between the Giemsa-negative and the Giemsa-positive, seropositive subjects. Using the significant cutoff values found in this study, the proportion of the subjects with low serum PG II levels below 7.45 ng/mL was significantly higher in the Giemsa-negative, seropositive subjects (37/145, 25.5%) than the Giemsa-positive, seropositive subjects (5/445, 1.1%, $P < .001$). The proportion of the subjects with high serum PG I/II ratio above 3.05 was similar between the Giemsa-negative, seropositive subjects (100/145, 69.0%) and the Giemsa-positive, seropositive subjects (295/445, 66.3%, $P = .552$). The conventional criteria for the gastric corpus atrophy (serum PG I level of <70 ng/mL and PG I/II ratio of <3.0) did not differ between the two groups (26.2% vs 23.6%, $P = .524$). PG, pepsinogen

3.5 | Follow-up Giemsa staining finding in the Giemsa-negative, seropositive subjects

Forty-six seropositive subjects with a negative Giemsa staining finding underwent follow-up gastric biopsies at our center. Thirteen (28.3%) subjects showed a positive Giemsa staining finding, indicating that their initial Giemsa staining findings were false-negative. None of these seropositive subjects with false-negative Giemsa staining findings showed a diminished gastric secretory ability fulfilling the criteria of serum PG II level below 7.45 ng/mL (Table 3).

Among the 15 subjects with gastric adenoma or adenocarcinoma showing seropositive and Giemsa-negative staining findings, 11 underwent follow-up Giemsa staining findings at our center. Three (75%) of the four Giemsa-negative seropositive subjects with adenocarcinoma showed positive findings on the follow-up Giemsa staining, whereas none of the Giemsa-negative seropositive subjects with adenoma showed positive findings (Table 4).

4 | DISCUSSION

In this study, one of the five seropositive subjects showed a negative Giemsa staining finding when the biopsy was performed at a gastric lesion with either a color or a structural change. The risk was increased when the biopsied specimen revealed gastric adenocarcinoma or adenoma. According to the follow-up Giemsa staining findings, a false-negative Giemsa staining finding was common in subjects with adenocarcinoma due to uneven distribution of *H. pylori*. This provides further evidence that the link between *H. pylori* and gastric adenocarcinoma may have been underestimated because *H. pylori* tend not to be detected when the biopsy specimens are obtained from the mucosa adjacent to cancer cells. Conversely, none of the subjects with adenoma or diminished gastric secretory ability with the serum PG II level of <7.45 ng/mL showed a positive Giemsa staining finding at the follow-up biopsy. Applying these to clinical management, additional biopsies at different site should be performed when there is a discrepancy between the *H. pylori* test findings in adenocarcinoma subjects.

The pathology finding of the biopsied specimen was more important than the location of the gastric biopsy in this study. The greater curvature side of the upper body has been recommended as an adequate biopsy site to prevent discrepancies between the invasive and noninvasive methods.[19,20] Nonetheless, the present study shows that a negative Giemsa staining finding can be still found in seropositive subjects with a decreased gastric secretory ability or in those with specific pathology findings (adenoma, adenocarcinoma, IM, and hemorrhage) irrespective of the biopsied site. The risk of a discrepancy was lower in IM than in adenoma or adenocarcinoma because *H. pylori* can exist on the surface of the metaplastic gastric mucosa as long as the gel structure is preserved.[21] Furthermore, unusual adherence of *H. pylori* to IM seems to be more frequent in Koreans than other population.[22] In that study, all IM adherent to *H. pylori* contained sulfomucins without a brush border, suggesting that population with a higher incidence of incomplete IM show frequent adherence of *H. pylori*. In addition to IM, the risk was also increased in hemorrhage because a tinged blood on the gastric mucosal surface may disturb *H. pylori* colonization. Histology and rapid urease test are known to be inadequate for *H. pylori* detection in acute UGI bleeding conditions owing to the low sensitivity of an invasive test.[23]

Interestingly, 28.3% of the Giemsa-negative, seropositive subjects showed a positive Giemsa staining finding on the follow-up

TABLE 3 The Giemsa-negative, seropositive subjects with a positive Giemsa staining finding on the follow-up tests

Subject (sex/age)	PG I level (ng/mL)	PG II level (ng/mL)	PG I/II ratio	Pathology finding	Biopsied site on the initial test	Biopsied site on the follow-up test
F/50	104.5	23.8	4.4	Adenoca, PD	Low-body, AW	Mid-body, GC
M/69	27.1	16.6	1.6	Adenoca, MD	Low-body, LC	Mid-body, AW
M/56	35.4	12.2	2.9	Adenoca, MD	Angle, Mid	Mid-body, GC
M/45	78.9	11.7	6.7	IM	High-body, GC	Mid-body, GC
M/72	47	12.5	3.8	IM	High-body, GC	Low-body, GC
M/62	57.2	16.3	3.5	IM	Mid-body, LC	Mid-body, GC
M/68	16.1	14.6	1.1	IM	Mid-body, GC	Low-body, GC
M/53	18.1	11.2	1.6	IM	Low-body, LC	Mid-body, GC
F/67	101.1	21.9	4.6	IM	Antrum, AW	Low-body, GC
M/45	54.1	17.7	3.1	IM	Antrum, AW	Antrum, LC
M/59	39.5	14.4	2.7	IM	Antrum, GC	Low-body, AW
M/57	85.8	20.1	4.3	IM	Antrum, GC	Low-body, GC
M/63	92.1	17.3	5.3	Foveolar hyperplasia	Antrum, GC	High-body, GC

PG, pepsinogen; F, female; M, male; Adenoca, adenocarcinoma; PD, poorly differentiated; MD, moderately differentiated; IM, intestinal metaplasia; AW, anterior wall; LC, lesser curvature; Mid, middle; GC, greater curvature.

TABLE 4 Findings of the subjects with gastric adenoma or adenocarcinoma

Group	Pathology finding	Subject (sex/age)	Serum PG I (ng/mL)	Serum PG II (ng/mL)	Serum PG I/II ratio	Biopsied site	Follow-up Giemsa staining
Giemsa-negative, seropositive	Adenoca, PD	F/50	104.5	23.8	4.4	Low-body, AW	Positive
	Adenoca, MD	M/56	35.4	12.2	2.9	Angle, Mid	Positive
	Adenoca, MD	M/69	27.1	16.6	1.6	Low-body, LC	Positive
	Adenoca, PD	M/43	68.5	21.1	3.2	Low-body, LC	Negative
	Adenoca, PD	M/58	52.5	25.7	2.0	High-body, LC	NA[a]
	Adenoca, MD	M/72	38.7	6.6	5.9	Antrum, LC	NA
	Adenoca, WD	M/62	9.2	10.6	0.9	Low-body, GC	NA
	Adenoma	M/70	30.9	7.4	4.2	Antrum, AW	Negative
	Adenoma	M/72	58.7	14.1	4.2	Antrum, AW	Negative
	Adenoma	F/73	64.0	17.5	3.7	Antrum, LC	Negative
	Adenoma	M/78	6.1	5.9	1.0	Antrum, AW	Negative
	Adenoma	M/68	76.6	19.2	4.0	Angle, Mid	Negative
	Adenoma	M/81	12.3	11.7	1.0	Angle, PW	Negative
	Adenoma	M/70	16.1	11.5	1.4	Cardia	Negative
	Adenoma	M/60	48.8	13.3	3.7	Antrum, GC	NA
Giemsa-positive, seropositive	Adenoca, MD	M/76	50.9	16.6	3.1	Antrum, LC	Positive
	Adenoca, MD	M/58	12.1	10.6	1.1	Angle, PW	Positive
	Adenoma	M/64	75.2	23.8	3.2	Mid-body, LC	Positive
	Adenoma	M/63	40.3	19.9	2.0	Antrum, PW	Positive
Giemsa-negative, seronegative	Adenoca, MD	F/46	53.0	8.8	6.0	Low-body, PW	Negative
	Adenoma	M/79	11.1	9.2	1.2	Antrum, LC	Negative
	Adenoma	F/69	15.3	13.0	1.2	Mid-body, LC	Negative
	Adenoma	M/66	13.3	11.8	1.1	Low-body, GC	Negative
	Adenoma	M/70	31.0	12.9	2.4	Antrum, GC	Negative

Adenoca, adenocarcinoma; PD, poorly differentiated; MD, moderately differentiated; WD, well-differentiated; F, female; M, male; PG, pepsinogen; AW, anterior wall; LC, lesser curvature; GC, greater curvature; Mid, middle; PW, posterior wall; NA, not available due to the lack of follow-up gastric biopsy at our center.

[a]Gastric biopsy was not available because the subject underwent total gastrectomy for the resection of advanced gastric cancer.

tests, and most of them had adenocarcinoma or IM with preserved PG II secreting ability of the stomach. The discrepancy between the initial and follow-up biopsies in adenocarcinoma and IM specimens seems to arise from uneven distribution of *H. pylori* in unfavorable hypochlorhydric environments. Conversely, none of the subjects with adenoma showed positive staining findings on the follow-up tests. Based on previous studies, disappearance of *H. pylori* in subjects with adenoma seems to be correlated with the development of a gland damage, which secretes PG.[24,25] Together, a false-negative Giemsa staining finding should be considered in subjects with adenocarcinoma and IM, whereas spontaneous regression of *H. pylori* should be considered in subjects with adenoma or those with a diminished gastric secretory ability.

The low serum PG II level increased the risk of a negative Giemsa staining finding in the seropositive subjects than the serum PG I level in this study. Irrespective of the pathology findings, most of the seropositive subjects with the serum PG II level of <7.45 ng/mL showed a negative Giemsa staining finding. Moreover, none of the subjects with a positive Giemsa staining finding on the follow-up test showed low serum PG II levels below 7.45 ng/mL. These suggest that a diminished secretory ability of the entire stomach (as reflected by low serum PG II levels) seems to be more unfavorable for *H. pylori* colonization than gastric corpus atrophy alone (as reflected by low serum PG I levels). The colonization of *H. pylori* seems to be greatly influenced by the extent of a diminished secretory ability of the gastric mucosal cells.[24-26] Therefore, spontaneous regression of *H. pylori* should be considered in Giemsa-negative, seropositive subjects with low serum PG II levels.

The limitation of this study is the lack of noninvasive tests other than the serum anti-*H. pylori* IgG assay. The fundamental characteristics of a serology assay might create discrepancies with Giemsa staining because it cannot distinguish current and past infections completely. Nonetheless, the serum anti-*H. pylori* IgG titer decreases after the regression of *H. pylori*, and a diminished bacterial load often leads to seronegative test findings[12,26-30] In the current study, one-third of the followed up, Giemsa-negative, seropositive subjects had true infection. This further suggests that a false-negative Giemsa staining finding is not uncommon in the seropositive subjects with preserved gastric secretory ability.

In conclusion, a negative Giemsa staining findings in seropositive subjects are related to the pathology of the biopsied specimen and decreased secretory ability of the stomach. The absence of *H. pylori* due to spontaneous regression should be considered in gastric adenoma specimens and those with low serum PG II levels of <7.45 ng/mL. Conversely, a false-negative Giemsa staining finding should be considered in gastric adenocarcinoma and IM specimens owing to the uneven distribution of *H. pylori*. Additional *H. pylori* tests should be performed in these conditions, so that more infected subjects could be found and eradicated.

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