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Biomarkers in Medicine

Biomarkers for *Helicobacter pylori* infection and gastroduodenal diseases

Seiji Shiota[1] & Yoshio Yamaoka*[,1,2]

[1]Department of Environmental & Preventive Medicine, Oita University Faculty of Medicine, Yufu City, Oita, Japan

[2]Department of Medicine–Gastroenterology, Baylor College of Medicine & Michael Eeast Debakey Veterans Affairs Medical Center, 2002 Holcombe Blvd, Houston, TX 77030, USA

*Author for correspondence:
Tel.: +81 97 586 5740
Fax: +81 97 586 5749
yyamaoka@oita-u.ac.jp

Helicobacter pylori infection is a major cause of gastric cancer. Although identifying *H. pylori* infected subjects is the first approach for delineating the high-risk population for gastric cancer, the presence of *H. pylori* antibodies is not sufficient for gastric cancer screening. Among *H. pylori* infected subjects, only a minority of infected individuals develop gastric cancer. Serologic markers of *H. pylori* infection can serve as potential predictors for the development of gastric cancer. Serum or urinary *H. pylori* antibodies, cytotoxin-associated gene A antibodies, pepsinogen and microRNAs were reported to be associated with precancerous lesions or gastric cancer. In this review, we summarized the utilities and limitations of each strategy.

Keywords: antibody • biomarkers • *Helicobacter pylori* • miRNA

Gastric cancer is the fifth most common cancer and third leading cause of cancer-related mortality in the world (this is also available in International Agency for Research on Cancer; GLOBOCAN2012 [1]). *Helicobacter pylori* infection is the major cause of chronic gastritis, peptic ulcers and gastric cancer [2]. Although approximately half of the world's population is infected with this bacterium, only a minority of infected individuals develop gastric cancer [3]. One possible reason for the varying outcomes of *H. pylori* infection is related to differences in the virulence of *H. pylori* strains in addition to host, environmental and dietary factors. The identification of risk markers for classifying *H. pylori* infected patients into high- and low-risk groups is highly desirable for personalized prevention. In particular, serologic markers of *H. pylori* infection can serve as potential predictors of the development of gastric cancer. In this review, we focused on serological and urinary biomarkers of *H. pylori* infection.

Anti-*H. pylori* antibody

Helicobacter pylori infection almost constantly induces a specific systemic immune response, which is followed by antibody production. Serological testing is widely used in epidemiological studies; in fact, the prevalence of *H. pylori* antibodies was significantly higher in patients with gastric cancer than in control patients [4]. Therefore, the detection of *H. pylori* infected subjects is the first approach to delineate the high-risk population for gastric cancer. In addition to serological tests, urine-based tests are more convenient and easier to use as a noninvasive method in clinical trials, especially at the point of primary care. Although the concentration of anti-*H. pylori* antibodies in urine is approximately 10,000-fold lower than that in serum, serum and urinary levels have been found to correlate well for the antibody [5]. In fact, urine antibody levels were reported to be useful for examining the prevalence of *H. pylori* infection as a screening tool [6,7]. Two urinary tests, an enzyme immunoassay method (URINELISA, Otsuka Pharmaceutical, Tokyo, Japan) and an immunochromatographic method (RAPIRUN, Otsuka Pharmaceutical), have been used for the detection of *H. pylori* infection. RAPIRUN exhibited high sensitivity (85.7–95.9%) and specificity (87.9–97.4%) in Japan (summa-

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rized results are described in [8]). The original RAPIRUN kit was developed as a plate-type test. In 2011, a stick-type kit for rapid urine testing was developed in Japan [9]. Compared to conventional RAPIRUN and URINELISA, this kit exhibited high agreement rates of 98.4 and 88.8%, respectively, for Japanese subjects. This kit can facilitate easier and more rapid testing. Urinary detection kits are also available for children. Okuda *et al.* examined the availability of urinary tests for *H. pylori* infection in Japanese children [10]. They found that RAPIRUN displayed lower sensitivity than URINELISA (78.4 vs 91.9%). In particular, the sensitivity of RAPIRUN in children aged <10 years was lower than that of URINELISA (75.0 vs 89.3%). On the contrary, the specificity was equal (>95%). This suggests that to reduce false-negative case, other diagnostic tests such as the urea breath test or stool antigen tests are necessary to identify *H. pylori*-positive children.

In areas with a low prevalence of *H. pylori* infection, screening for *H. pylori*-infected subjects might be sufficient to narrow the high-risk population for gastric cancer. However, in areas, with a prevalence of *H. pylori* infection, especially east Asian countries, only a minority of *H. pylori*-infected subjects develop severe gastroduodenal diseases including gastric cancer; therefore, the presence of *H. pylori* antibodies is not sufficient to identify the high-risk population for gastric cancer. Additional screening tools are necessary to identify the high-risk population for gastric cancer.

A large-scale cohort study was conducted to examine the association between *H. pylori* antibody titers and gastric cancer in Japan, in which the incidence of gastric cancer is high (29.9 cases/100,000 per year) [1,11]. A total of 36,745 subjects were included from the Japan Health Center-based Prospective Study and followed up for 15 years. Among *H. pylori* seropositive subjects with no mucosal atrophy, subjects with high *H. pylori* antibody titers were at the highest risk for gastric cancer. However, among seropositive subjects with mucosal atrophy defined on the basis of pepsinogen (PG) levels, those with low *H. pylori* antibody titers had the highest risk for gastric cancer. Therefore, patients with low *H. pylori* antibody titers in addition to mucosal atrophy were considered an extremely high-risk population for gastric cancer.

However, we should pay attention to the use of *H. pylori* antibody levels for screening for *H. pylori* infected subjects. *Helicobacter pylori* antibody titers varied greatly depending on the test kit used [12]. Burucoa *et al.* evaluated the performances of 29 commercial kits for the serological diagnosis of *H. pylori* infection (17 ELISA) tests and 12 rapid tests such as immunochromatography) in France [13]. A patient was considered to be infected with *H. pylori* on the basis of a positive culture of the gastric biopsies taken during endoscopy. If the culture was negative, then the patient was considered *H. pylori* positive when histology and rapid urease test or urea breath test findings were positive. As a result, the accuracy was 73.9–97.8% for the 17 ELISA tests. Meanwhile, the sensitivity was 57.8–100%, and the specificity was 57.4–97.9%. Four ELISA tests presented excellent results with five criteria (sensitivity, specificity, positive-predictive value, negative-predictive value and accuracy) with values of >90%. Twelve rapid tests displayed lower performances and more number of heterogeneous findings. These data are useful for selecting the most optimal kit. However, these findings were based on results from French populations; therefore, further studies should be conducted in other countries. The E-plate Eiken ELISA kit (Eiken Co., Ltd., Tokyo, Japan) was developed by using Japanese *H. pylori* isolates. For Japanese subjects, the sensitivity and specificity of this kit were reported to be 95.2–100% and 76.2–80.0%, respectively [14,15]. In our previous study, 334 Bhutanese samples were examined via *H. pylori* culture, rapid urease test and histology. We found that the sensitivity and specificity of the E-plate Eiken ELISA kit for the Bhutanese population were 94.8 and 70.7%, respectively [16], comparable to the results obtained in Japan. This might be attributable to the similarity in *H. pylori* strains between Japan and Bhutan, although a detailed examination is in progress. At present, *H. pylori* strains can be divided into seven population types on the basis of geographical associations and designated as follows: hpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica and hpSahul [17]. Similar *H. pylori* strains might induce similar immune responses including antibody production. However, it might be preferable to develop a domestic ELISA kit by using local *H. pylori* strains for future studies to obtain the best results.

In addition to serum antibodies, it is better to determine the availability of urinary tests before local application. Nguyen *et al.* examined the utility of the RAPIRUN test in Vietnamese individuals [8]. Compared with the results from Japan, the sensitivity of the test was low (79.5%) in Vietnamese subjects, although the specificity of the test was 90.7%. The researchers used the test kit developed by using Japanese isolates, but the test kit might not always react well with the urinary *H. pylori* antibodies produced by Vietnamese patients. Alternatively, the higher false-negativity rate may have resulted from the extremely low urinary levels of anti *H. pylori* antibodies in their population. In fact, serum *H. pylori* antibodies were absent in five and present at extremely low levels in six of the 17 false-

negative cases, in their study. Therefore, it is better to develop a test kit by using local strains as the source of antigen to obtain the best performance. The urinary tests were also evaluated in Turkish subjects [18]. The sensitivity and specificity of URINELISA were 74.4 and 81.0%, respectively, and those of RAPIRUN were 73.2 and 78.6%, respectively. They found that the optimal cut-off value in the receiver operating characteristic curves was 0.530 for URINELISA in Turkish subjects. When they used the optimal cut-off value of 0.530 for URINELISA, the sensitivity and specificity were 90.2 and 71.4%, respectively. The authors suggested that the cut-off value of the URINELISA test should be evaluated and considered separately for each patient group and country.

Cytotoxin-associated gene A antibodies

The best studied virulence factor of *H. pylori* is the cytotoxin-associated gene A (CagA) protein [19]. Several reports indicated that CagA-positive strains were significantly associated with severe clinical outcomes, especially in western countries [20–23]. Huang *et al.* examined the association between CagA seropositivity and gastric cancer by performing a meta-analysis [24]. They found that serum CagA antibodies were significantly associated with gastric cancer when they included studies from both western and Asian countries. In east Asian countries, almost all *H. pylori* strains possess *cagA* and produce CagA protein; therefore, it is difficult to illustrate the significance of CagA for gastric cancer. For example, our previous report revealed that 96.3% of Japanese strains were *cagA*-positive irrespective of clinical outcomes [25]. Similar results have been found in different regions in Japan [26–28] and other east Asian countries [29,30]. However, subjects infected with CagA-positive *H. pylori* do not always produce serum CagA antibodies, even in east Asian countries. Our previous study indicated that serum CagA antibodies were found in 75.0% of Japanese subjects [31]. This suggests that serum CagA antibodies rather than the presence of *cagA* might represent a suitable biomarker.

Intriguingly, we previously reported that the presence of serum CagA antibodies was significantly associated with gastric cancer, even in east Asian countries, in a meta-analysis [32]. In this analysis, ten studies with a total of 4325 patients were included in the final analysis. The prevalence of CagA antibodies in *H. pylori*-positive patients with gastric cancer was significantly higher than that of the *H. pylori*-positive controls, although the odds ratio (OR) for east Asian countries was smaller than that for both east Asian and western countries (1.26 vs 1.49). We also examined the prevalence of anti-CagA antibodies in the *H. pylori*-negative population, although none of the references mentioned whether patients with a previous history of *H. pylori* eradication therapy were included. Even in the *H. pylori*-negative population, the presence of serum CagA antibodies was significantly associated with gastric cancer. This demonstrates that serum CagA antibodies can potentially remain present for a longer period than serum *H. pylori* antibodies. According to the development of severe atrophy and intestinal metaplasia, the bacterial load will decline in the stomach, after which serum *H. pylori* antibodies will not be detected in these patients [33,34]. On the contrary, other antigenic proteins might have stronger antigenicity, and their antibodies might persist long after the clearance of infection. After our publication, a study elucidated the positive association between CagA seropositivity and gastric cancer in China [35]. When we included this additional study in our meta-analysis, the prevalence of serum CagA antibodies was significantly associated with gastric cancer (OR: 1.91; 95% CI: 1.42–2.57; random effects model structured by Biostat comprehensive meta-analysis software) (Figure 1).

In our previous study, we examined the relationship between serum CagA antibody titers and histological scores in Japan [31]. Interestingly, serum CagA antibody titers were significantly correlated with gastric mucosal inflammation in the corpus. This suggests that both the presence of antibodies and antibody titers are biomarkers for delineating the high-risk population. Meanwhile, Suzuki *et al.* examined the risk of gastric cancer according to CagA antibody titers in Japan [36]. They included 299 patients with noncardia gastric cancer and 1048 matched controls. Among the *H. pylori*-seropositive subjects, those with low CagA antibody titers had a greater risk for future noncardia gastric cancer than those with serum CagA negativity or high CagA antibody titers. These data suggest that attention should be paid to the antibody titer in addition to seropositivity.

In addition to serum and urinary *H. pylori* antibodies, serum CagA antibody titers can also differ according to the test kit used. We identified significant heterogeneity in our meta-analysis [32]. The differences of populations studied and differences in the antigens used to detect anti-CagA antibodies may have contributed to this heterogeneity. We previously reported that the CagA seropositivity rate was 82% by using the OraVax antigen and 72% by using the Chiron antigen, even when we used same samples from Japan [37]. This suggests that data obtained by using different antigens may not be comparable with each other [38,39]. CagA can be divided into two types, east Asian-type CagA and western-type CagA, according to the amino acid

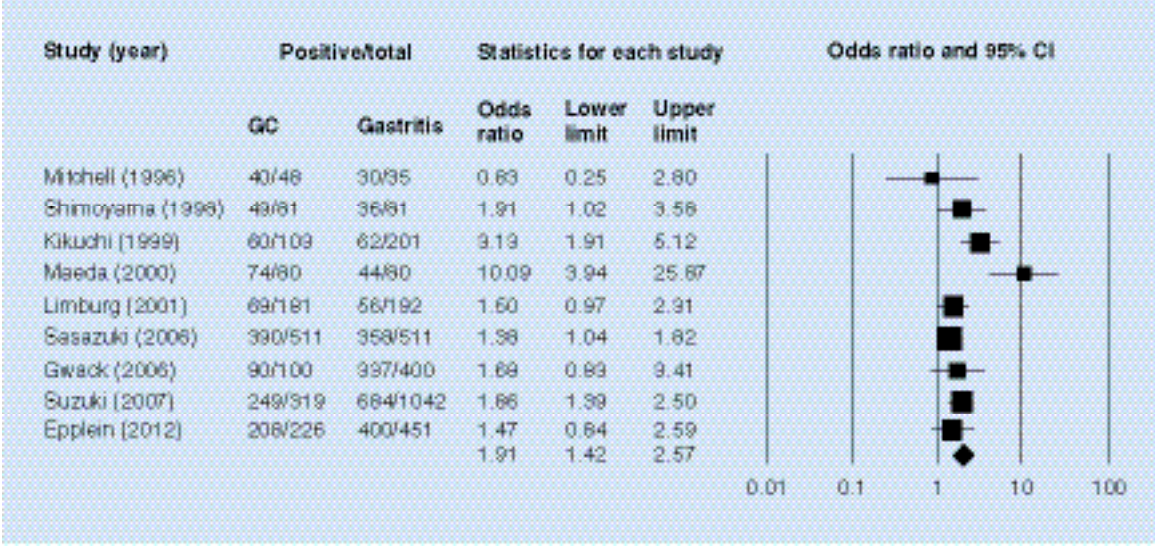

Figure 1. Association between serum cytotoxin-associated gene A antibodies and gastric cancer. The presence of serum cytotoxin-associated gene A antibodies was significantly associated with gastric cancer in a random-effect model in a meta-analysis.

sequences of the C-terminus of CagA [40]. Infection with east Asian type CagA strains was reported to be associated with peptic ulcers and gastric cancer compared with infection with western-type CagA strains [41,42]. As recombinant CagA from European strains was used as the coating antigen in ELISA systems, recombinant CagA derived from east Asian strains may be better for ELISA systems in east Asian countries. At present, there are no reports that examined the prevalence of east Asian type CagA-specific antibodies in sera although there is a sandwich ELISA system to detect east Asian type CagA strains [43]. In addition, Wada *et al.* examined the level of tyrosine phosphorylation of the EPIYA motif in CagA (CagA-P) in patients with diffuse-type gastric cancer in Japan [44]. The serum titer of anti-CagA-P antibodies was significantly higher in the gastric cancer group than in the matched control group. In addition to the CagA status based on east Asian-type and western-type CagA, other epitopes of CagA may be associated with the risk of gastric cancer. Furthermore, an ELISA system with monoclonal antibodies induced by several recombinant fragments of CagA was developed, and the immunochemical properties of the antibodies were different [45]. It is necessary to consider the proper antigens for ELISA system for detecting anti-CagA antibodies.

Novel *H. pylori* multiplex serology was used to determine the seroprevalence of 15 specific *H. pylori* antigens in Germany [46]. The presence of chronic atrophic gastritis was determined according to the status of PG. In subjects with chronic atrophic gastritis, 11 antibodies were present in >50% of the subjects. The prevalence of all 15 antibodies was significantly higher in patients with chronic atrophic gastritis than in the controls. In particular, CagA, vacuolating toxin (VacA), Helicobacter cysteine-rich protein C (HcpC) and the chaperonin GroEL were the independent significant predictors of chronic atrophic gastritis. Furthermore, dose–response relationships between antibody levels and mild-to-moderate chronic atrophic gastritis were observed for these specific antigens. GroEL is a molecular chaperone that is required for the proper folding of many proteins in bacteria. Additionally, it was reported that GroEL might play a role in gastrointestinal homeostasis owing to its ability to bind to components of the gastrointestinal mucosa and aggregate *H. pylori*. The biological roles of HcpC are unclear. Further studies are required to clarify the association between these factors and gastroduodenal disease in other countries. In a subsequent study, the authors examined the association between the presence of 15 individual *H. pylori* proteins and gastric cancer in Germany [4]. CagA and GroEL seropositivity were significantly associated with gastric cancer. Intriguingly, the increase in risk was particularly pronounced in subjects who were *H. pylori* antibody negative but positive for both CagA and GroEL. This evidence also suggests that the organism can be lost from the stomach owing to alterations in the internal environment, with the development of advanced gastric disease [33,34]. Unfortunately, this study did not mention whether subjects who received previous treatment for *H. pylori* infection were excluded. When including *H. pylori*-eradicated subjects, it is important to clarify whether these markers are available for predicting the future development of gastric cancer. Another report

from China also revealed that seropositivity for six proteins including outer membrane protein, HP0305, hydantoin utilization protein A, flagellar sheath adhesion lipoprotein (HpaA), CagA and VacA, was significantly associated with gastric cancer when using the same multiplex serology [35].

Recently, Pan *et al.* examined the relationship between seropositivity for CagA, VacA, GroEL, urease subunit A (UreA), γ-glutamyl transpeptidase (gGT) and HcpC and the development of gastric lesions during the follow-up period in patients with a risk for precancerous gastric lesions at baseline [47]. The presence of antibodies for each specific antigen was examined by using the recomLine *H. pylori* test system, which is a line immune assay. The seroprevalences of CagA, VacA, GroEL, UreA, HcpC and gGT were 83.9, 38.9, 66.1, 17.8, 59.7 and 43.3%, respectively. Seropositivity for CagA was an independent predictor for advanced gastric lesions. In addition, they found that seropositivities for CagA and GroEL were independent predictors for the progression of gastric lesions in a follow-up study. These findings can be helpful for stratifying the *H. pylori* infected population into high- and low-risk populations for gastric cancer.

PG & ABC classification

The serum PG status has been used as a marker of gastric mucosal atrophy and inflammation [12]. The chief and mucus neck cells in the gastric fundus and corpus produce two forms of PG: PG I and PG II. PG II is also produced by the pyloric glands in the antrum and Brunner glands in the proximal duodenum. The assessment of gastric mucosal atrophy via endoscopic random biopsy can include sampling errors because atrophy of the gastric mucosa can be irregular. PG can be used as a surrogate marker for gastric mucosal status [48]. *Helicobacter pylori* infection induces higher serum levels of PG I and PG II. According to the greater increment of PG II levels than PG I levels, the PG I/II ratio is lower in the presence of *H. pylori*. Over time, as the fundic gland mucosa is reduced, PG I levels gradually decrease, whereas those of PG II remain fairly constant. Therefore, the PG I/II ratio will decline according to the progression from normal gastric mucosa to severe atrophic gastritis. Low PG I levels and PG I/II ratios have been associated with severe gastric atrophy and used as a screening tool to detect gastric cancer [12,48]. A meta-analysis illustrated that a PG I level ≤70 ng/ml and a PG I/II ratio ≤3 displayed a sensitivity of 57% and specificity of 80% for screening for atrophic gastritis to detect gastric cancer [49].

The risk of gastric cancer can be classified by the presence of *H. pylori* infection and the status of gastric mucosal atrophy [50]. In particular, the combination of *H. pylori* serology, PG I levels and the PG I/II ratio can be used for gastric cancer screening named as ABC method in Japan [12,51]. In the ABC method, subjects can be classified into four groups on the basis of the presence of *H. pylori* infection and PG as follows: Group A (*H. pylori* negative and PG negative), Group B (*H. pylori* positive and PG negative), Group C (*H. pylori* positive and PG positive) and Group D (*H. pylori* negative and PG positive) [12]. Group D is generally considered the highest risk group for the development of gastric cancer, followed by Groups C, B and A [12].

We previously examined the status of the ABC classification in Bhutan, where the incidence of gastric cancer is high [52]. The proportions of patients in Groups B (53.8%) and C (17.3%) were higher in Bhutanese subjects than those obtained in Japan, where the values were 16.3 and 9.3%, respectively [12]. Even in younger subjects, the proportion of Group C subjects was approximately 20% in Bhutan, which was higher than that in Japan (≤5%) [53]. This suggests that high incidence of severe gastric atrophy in Bhutan might have contributed to the high incidence of gastric cancer in Bhutan.

Yanaoka *et al.* used PG to examine the preventive effect of *H. pylori* eradication therapy on the development of gastric cancer in a longitudinal cohort study [54]. A total of 473 *H. pylori* eradicated subjects and 3656 subjects with persistent infection were followed up for approximately 10 years. Subjects were divided into three groups according to the presence of chronic atrophic gastritis based on PG. In the PG-negative group with mild chronic atrophic gastritis, the incidence of gastric cancer was significantly lower in the *H. pylori* eradicated group than in the noneradicated group. On the contrary, a difference in the gastric cancer incidence rate was not found in the PG-positive group between *H. pylori* eradicated and noneradicated subjects. This suggests that *H. pylori* eradication therapy can reduce the incidence of gastric cancer in subjects without severe chronic atrophic gastritis. This finding also supports that PG might be available for identify subjects at high risk for developing gastric cancer.

However, the best cut-off value of PGs might vary according to the patient background [55]. In fact, Indian subjects displayed lower PG levels than Chinese and Malaysian populations, even after adjustment for sex and *H. pylori* infection rates [56]. Therefore, serum PG levels cannot be used for gastric cancer screening in the Indian population [57]. The levels of PG I and PG II might be influenced by other factors, such as age, sex, height, body weight, body surface area, smoking and drinking habits [48]. In the Chinese

population, the cut-off values of PG I and the PG I/II ratio for detecting atrophic gastritis were reported to be 82.3 ng/ml and 6.05, respectively [58]. The proper cut-off values of PGs should be determined in each population [51,55].

Other factors associated with gastric cancer

miRNAs are 18–25 nucleotide noncoding RNA sequences that are transcribed but not translated into proteins. Several papers reported that circulating miRNAs can serve as noninvasive biomarkers for the detection of gastric cancer. Liu *et al.* performed genome-wide miRNA expression profiling by using a human miRNA microarray and serum samples from patients with gastric or colorectal cancer and controls in China [59]. Three miRNAs (miR-187, miR-371-5p and miR-378) were significantly upregulated in the sera of patients with gastric cancer. Multivariate logistic regression analyses revealed that miR-378 was a potential biomarker for detecting gastric cancer. Furthermore, there was no difference in the serum levels of miR-378 according to the stage of gastric cancer, which suggests that elevated serum miR-378 levels can be detected during early stages of gastric cancer. In addition, Li *et al.* examined the association between several miRNAs and gastric cancer in China [60]. Seventy patients with gastric cancer and 70 healthy control subjects were included. The researchers found that the plasma levels of miR-223 and miR-21 were significantly higher in patients with gastric cancer than in healthy controls, whereas miR-218 levels were significantly lower in patients with gastric cancer. Furthermore, miR-223 levels were significantly higher in *H. pylori* infected patients with gastric cancer than in uninfected patients. Among *H. pylori* infected patients, the plasma levels of miRNAs were significantly higher in those with gastric cancer than in healthy controls. This finding suggests that plasma miRNA levels can serve as novel non-invasive biomarkers for detecting gastric cancer in *H. pylori* infected subjects. Zhu *et al.* also examined the circulating miRNA profiles of 48 patients with gastric cancer, 54 subjects with precancerous lesions such as intestinal metaplasia and 48 healthy subjects [61]. As a result, five miRNAs (miR-16, miR-25, miR-92a, miR-451 and miR-486-5p) were upregulated in the patients with gastric cancer irrespective of the stage of the disease. Interestingly, the combination of the five miRNAs displayed enhanced sensitivity and specificity for gastric cancer.

Noninvasive biomarkers should be used even after *H. pylori* eradication therapy. Shiotani *et al.* examined serum miRNA levels in patients with recent histories of endoscopic resection for early gastric cancer and control subjects (*H. pylori*-positive gastric ulcer or atrophic gastritis) before and after *H. pylori* eradication therapy in Japan [62]. They found that the levels of miR-106b and let-7 were significantly higher in patients with gastric cancer than in control subjects irrespective of the history of eradication therapy. Conversely, miR-21 levels were higher in patients with gastric cancer than in the control subjects only after eradication therapy. These findings suggest that miRNA levels of can change after *H. pylori* eradication therapy. In addition, we should keep in mind the possibility that the most suitable biomarkers for detecting individuals at high risk of gastric cancer may differ depending on the populations.

Glycomics might also represent a sensitive diagnostic tool for gastric cancer. Ozcan *et al.* examined native glycans via MS in the sera of patients with gastric cancer and nonatrophic gastritis in Mexico [63]. As a result, 19 glycans were associated with gastric cancer. In particular, the levels of three groups including the high mannose-type glycans, glycans with 1 complex type antenna, and bigalactosylated biantennary glycans were lower in patients with gastric cancer than in those with nonatrophic gastritis, whereas the levels of nongalactosylated biantennary glycans were higher in patients with gastric cancer. These glycans might be suitable for detecting gastric cancer at an early stage.

Single nucleotide polymorphisms associated with *H. pylori* infection

Even in the presence of high exposure rates, approximately 5–10% of a population is never infected with *H. pylori*. Interestingly, the identification of genetic loci associated with *H. pylori* serological status was performed in a genome-wide association study in Germany [64]. A total of 10,938 subjects were included from two independent population-based cohorts. The seropositive rate was 56.3%. The Toll-like receptor (TLR) locus on 4p14 and FCGR2A encoding the Fcγ receptor 2a locus on 1q23.3 were associated with *H. pylori* seropositivity. In fact, high fecal *H. pylori* antigen titers were associated with high TLR1 expression levels, suggesting that TLR1 can be causatively associated with *H. pylori* susceptibility. These findings might contribute to the screening of high-risk populations for *H. pylori* infection.

How can we identify the high-risk population for gastric cancer?

As described previously, several biomarkers were associated with gastric cancer or precancerous legions. In areas with a low prevalence of *H. pylori* infection, it is likely sufficient to consider *H. pylori* infected subjects to be a high-risk population for gastric cancer. On

Table 1. Biomarkers for *Helicobacter pylori* infection and gastroduodenal diseases.

Biomarkers	Advantages	Disadvantage
Serum *Helicobacter pylori* antibody	Easy to use for epidemiological research	*Helicobacter pylori* antibody titers varied greatly depending on the test kit used
	Patients with low *H. pylori* antibody titers in addition to mucosal atrophy represented an extremely high-risk population for gastric cancer	
Urinary *H. pylori* antibody	More convenient and easy to use as a noninvasive method in clinical trials	The cut-off value of the URINELISA test should be evaluated and considered for each patient group and country
	The stick-type kit for rapid urine test was also developed in Japan	
Serum CagA antibody	CagA seropositivity was significantly associated with gastric cancer even in east Asian countries	Serum CagA antibody titers can vary according to the test kit used
	Multiplex serology against other *H. pylori* proteins in addition to CagA can be utilized	
Pepsinogen	Serum PG levels were identified as a marker of the gastric mucosal status including atrophy and inflammation	Serum PG levels can be affected by the subjects' ethnic background
	The combination of *H. pylori* serology and measurements of serum PG I levels and the PG I/II ratio can be applied for gastric cancer screening	
miRNAs	Circulating miRNAs can serve as noninvasive biomarkers for the detection of gastric cancer	Further studies are required to clarify the importance of miRNAs

the contrary, other biomarkers in addition to *H. pylori* positivity are necessary to identify the high-risk population for gastric cancer in areas with a high incidence of gastric cancer. Serum CagA antibodies, the status of PG and miRNA levels might be suitable for delineating the high-risk population for gastric cancer in these regions. We should remember that the serum *H. pylori* antibody status would be negative in some high-risk populations. The latest Japanese guidelines for *H. pylori* management published by the Japanese Society for Helicobacter Research considered all *H. pylori* infected patients as having a high risk for developing *H. pylori*-related diseases. In fact, the Japanese national health insurance system approved *H. pylori* eradication therapy for all patients with '*H. pylori* related chronic gastritis' in 2013 [65]. However, the risk of gastric cancer exists even after curing *H. pylori* infection. The risk of developing gastric cancer in patients cured of *H. pylori* infection was 0.3% per year in Japan [66]. The longest interval between *H. pylori* eradication and the occurrence of cancer was 13.7 years. Follow-up endoscopic examination for more than 10 years is necessary even after curing *H. pylori* infection. In addition, each biomarker described previously was tested in specific populations. In particular, most studies were conducted in Asian countries with a high incidence of gastric cancer. Therefore, it is necessary to examine the utility of each test in each population before they can be used.

Conclusion

Helicobacter pylori infection is a major risk factor for gastric cancer. Therefore, it is important to identify *H. pylori*-positive subjects as a screening strategy. However, among *H. pylori* infected subjects, only a minority of infected individuals develop gastric cancer. The combination of serum *H. pylori* antibody and PG levels might be useful for detecting the high-risk population for gastric cancer among *H. pylori* infected subjects. In addition, other antibodies against *H. pylori* protein can be more useful for delineating the high-risk population. Recent studies revealed sig-

nificant differences in the serum/plasma levels of miRNAs between patients with gastric cancer and control subjects. We summarized the advantages and disadvantages of these tests in Table 1. Further prospective cohort studies are necessary to clarify the importance of these tests. However, we should remember that non-invasive biomarkers could change depending on the target populations and test kits. In addition, studies with positive results regarding biomarkers can be published easily compared with those with negative results, especially retrospective studies (publication bias) [67]. Standardized protocols can improve the reliability of the studies.

Future perspective

Although *H. pylori* eradication therapy might reduce the incidence of gastric cancer, it cannot completely prevent the development of gastric cancer, especially in patients with precancerous lesions. In principal, all *H. pylori* infected subjects are considered to have a high risk for gastric cancer. In fact, the Japanese health insurance system approved *H. pylori* eradication therapy for all subjects with *H. pylori* related chronic gastritis in 2013. Therefore, it is important to identify the high-risk population among patients after *H. pylori* eradication therapy. Noninvasive tests can be used for many populations owing to their greater convenience and ease of use. The optimal cut-off value of the test kit should be determined separately in each population and country. In addition, the usage of local *H. pylori* strains might improve the accuracy of local specific test kits. Recent studies illustrated that several miRNAs could be biomarkers for gastric cancer. However, we should consider the possibility of confounding effects or interactions for clarifying independent risk factors. Furthermore, prospective studies are necessary to elucidate the significance of miRNAs in detecting early gastric cancer.

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Executive summary

Serum *Helicobacter pylori* antibody

- The prevalence of *Helicobacter pylori* antibodies was significantly higher in patients with gastric cancer than in control subjects.
- Patients with low *H. pylori* antibody titers and mucosal atrophy comprised an extremely high-risk population for gastric cancer.
- It is important that *H. pylori* antibody titers varied greatly depending on the test kit used. Therefore, it is preferable to develop a domestic ELISA kit by using local *H. pylori* strains.

Urinary *H. pylori* antibody

- Two urinary tests, an enzyme immunoassay method and an immunochromatographic method, have been used for the detection of *H. pylori* infection.
- The accuracy of urinary test kits can also vary according to the patient group and country. Therefore, it is better to develop test kits by using local strains as the source of antigen to obtain the best test performance.

CagA antibody

- Subjects infected with *cagA*-positive *H. pylori* do not always produce serum CagA antibodies, even in east Asian countries.
- CagA seropositivity was significantly associated with gastric cancer, even in east Asian countries, in a meta-analysis.
- Serum CagA antibody titers can differ according to the ELISA kit used.

PG & ABC classification

- Serum PG was identified as a marker of the gastric mucosal status including atrophy and inflammation.
- The combination of *H. pylori* serology and measurements of serum PG I levels and the PG I/II ratio can be applied for gastric cancer screening.

Other biomarkers

- Serum/plasma miRNAs or glycans can serve as novel noninvasive biomarkers for the detection of gastric cancer in *H. pylori* infected subjects.

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