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Serum *Helicobacter pylori* CagA antibody as a biomarker for gastric cancer in east-Asian countries

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Abstract

Aims—In east-Asian countries, while almost all *Helicobacter pylori* strains possess the cytotokine-associated gene A (*CagA*) gene, serum CagA antibody is not detected in some infected subjects. We aimed to clarify the association between anti-CagA antibody and gastric cancer in east-Asian countries.

Materials & methods—We performed a meta-analysis of case–control studies with age- and sex-matched controls, which provided raw data in east-Asian countries.

Results—Ten studies with a total of 4325 patients were identified in the search. Some reports from Japan, Korea and China showed a positive association between the presence of anti-CagA antibody and gastric cancer; however, the results differed in their various backgrounds. The disparate findings appeared to result from the use of different methods or from variations in the antigens used to detect the anti-CagA antibody. CagA seropositivity was associated with an increased risk of developing gastric cancer.

Conclusion—Anti-CagA antibody can be used as a biomarker for gastric cancer even in east-Asian countries.

Keywords

anti-CagA antibody; east Asia; gastric cancer; *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) infection is now accepted as the major cause of chronic gastritis. Several epidemiological studies have shown that *H. pylori* infection is also linked to the severe gastritis-associated diseases, including peptic ulcer and gastric cancer [1]. In 1994, the International Agency for Research on Cancer categorized *H. pylori* infection as a group I carcinogen [2]. Although gastric cancer is one of the most common cancers, only a minority of individuals with *H. pylori* infection ever develop it. The prevalence of gastric cancer is approximately 3% in *H. pylori*-positive patients [3]. One possible reason for the

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varying outcomes of *H. pylori* infection relates to differences in the virulence of *H. pylori* strains in addition to host, environmental and dietary factors.

The best-studied virulence factor of *H. pylori* is the cytotokine-associated gene A (CagA) protein. CagA-producing strains are reported to be associated with severe clinical outcomes, especially in Western countries [4–7]. CagA is a highly immunogenic protein with a molecular weight between 120 and 140 KDa [8,9]. Variation in the size of CagA is due to the presence of a variable number of repeat sequences located in the 3′ region of the gene [8,10–12]. The repeat regions contain the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif. Recently, sequences were annotated using segments (20–50 amino acids) flanking the EPIYA motifs (i.e., segments EPIYA-A, -B, -C or -D) [13–16]. Recent studies show that the east-Asian-type CagA, containing EPIYA-D segments, exhibits a stronger binding affinity for Src homology-2 domain-containing phosphatase (SHP)2 and a greater ability to induce morphological changes in epithelial cells than Western-type CagA, which contains segments EPIYA-C segments [13,16,17]. Another recent study showed that *H. pylori* strains possessing east-Asian-type CagA induce higher amounts of IL-8 from gastric epithelial cells than those possessing Western-type CagA [18]. Accordingly, east-Asian strains are believed to be more virulent than Western strains, and this might be the reason why the incidences of gastric cancer are relatively higher in east-Asian countries than in Europe, North America and Australia (data available at [101]). In addition, in Western countries the incidence of gastric cancer is higher in patients infected with strains carrying multiple EPIYA-C repeats compared with those infected with strains with a single repeat [10,11,19–21].

In 2003, Huang *et al.* performed meta-analysis of the association between CagA seropositivity and gastric cancer [22]. They concluded that CagA-positive strains of *H. pylori* increase the risk of gastric cancer. The odds ratios (ORs) for the risk of gastric cancer by anti-CagA antibody positive in fixed-models were 1.49 in part of a *H. pylori*-infected population and 2.64 irrespective of *H. pylori* status. However, because they included studies from both Western and Asian countries, it is not clear whether an association between CagA seropositivity and gastric cancer really exists in east-Asian countries. In east-Asian countries, it is difficult to prove the importance of the *cagA* gene in clinical outcomes because almost all *H. pylori* strains possess the *cagA* gene. For example, we previously examined 491 Japanese strains from a region in the middle of Japan (Kyoto) and found that 96.3% of the strains were *cagA* gene-positive, irrespective of disease [23]; similar results have been published for different regions in Japan [24–26] and other countries in east Asia [27,28].

Subjects infected with *H. pylori* containing the *cagA* gene do not always induce serum CagA antibody. For example, although most Japanese *H. pylori* possess the *cagA* gene, serum CagA antibody is detected in only 43.1–45.5% of infected subjects [29]. This suggests that serum CagA antibody may be a more useful marker in east-Asian countries than the *cagA* gene. In this study, we performed a meta-analysis for the relationship between anti-CagA antibody and gastric cancer in east-Asian countries.

Materials & methods

A literature search was performed using the PubMed database for articles published from January 1990 to March 2010, using the following words: ‘CagA’, ‘enzyme linked immunosorbent assay (ELISA)’ or ‘serological’ or ‘seropositive’ or ‘seropositivity’ or ‘serum antibody’, and ‘Japan’ or ‘China’ or ‘Korea’ or ‘east Asia’.

Inclusion criteria

The following criteria were applied to select fully published case–control studies examining the relationship between anti-CagA antibody of *H. pylori* and gastric cancer in adult populations: healthy controls were matched with cases by age and sex; the presence of anti-CagA antibody was examined by ELISA, immunoblot or both, and all original articles were published in English. *H. pylori* status was examined serologically by ELISA or immunoblot. Studies were excluded if raw data were not presented, or a histological diagnosis of gastric cancer was not confirmed. When it appeared that the same subjects were presented in multiple reports, the earliest paper was selected. All potentially relevant articles were reviewed by two investigators (Seiji Shiota and Yoshio Yamaoka) independently and disagreement was resolved by discussion.

Data extraction

Data were extracted from each study by investigators independently and entered into a computerized database. The information retrieved covered countries where the study was performed, characteristics of cases and controls and matching techniques, number of subjects, *H. pylori* infection and anti-CagA status.

Statistical analysis

Summary ORs and 95% confidence intervals (CIs) were calculated from the raw data of the selected studies using the DerSimonian and Laird method in a random effects model because of significant heterogeneity. In this article, some ORs were different to those shown in original articles. Although some previous papers regarded *H. pylori*-negative/CagA-positive as false-positive, we counted them as a *H. pylori*-negative/CagA-positive group. Nevertheless, the difference was minor and did not change the main result.

Publication bias was assessed by funnel plots and regression test by Egger *et al.* [30]. A p-value of less than 0.05 was considered as statistically significant in all meta-analyses. All analyses were performed using Comprehensive Meta-analysis software (version 2, Biostat, Englewood, NJ, USA).

Results

The literature searches generated 83 potentially relevant citations. Of these, ten studies with a total of 4325 patients met the inclusion criteria.

CagA seropositivity in the *H. pylori*-infected east-Asian population

CagA seropositivity in the *H. pylori*-infected population was examined in seven studies (five from Japan, one from China, and one from Korea) (Table 1) [31–37]. One study examined the CagA seropositivity by two different methods (ELISA and immunoblot) [32]. The prevalence of CagA antibodies ranged from 61.1 to 96.6% in gastric cancer patients and 52.5 to 90.3% in controls.

A positive association between anti-CagA antibody and gastric cancer was detected in three studies [31,36,37]. A statistically significant positive association was observed in only one study [19] by our analysis, although two studies described a significant relationship. The study examined 57 gastric cancer patients and 57 sex- and age-matched controls. Serum CagA antibodies were assayed by ELISA using purified recombinant CagA protein.

Contrary to the published reports, five studies showed no significant difference in patients with and without gastric cancer (Table 1) [32–35]. For example, we examined sera from 110 gastric cancer patients and sex- and aged-matched *H. pylori*-positive asymptomatic controls

[32]. The CagA status was assessed by ELISA using the 65 KDa fragment protein from OraVax Inc. (Cambridge, MA, USA) and immunoblotting (Chiron Corp., CA, USA). Serum CagA antibodies were present in similar proportions in patients with and without gastric cancer, with no significant differences in histological classification, clinical stage or location.

In the seven studies, the overall OR was 1.26 (95% CI: 1.05–1.52) as determined by meta-analysis in a random-effects model. Therefore, the prevalence of CagA antibody in *H. pylori*-positive gastric cancer patients was higher than that of *H. pylori*-positive controls; however, the OR in east-Asian countries was smaller than it was in the meta-analysis that included Western countries (1.26 vs 1.49).

The titer of CagA antibody has been related to gastric cancer risk. Suzuki *et al*. reported that the risk of gastric cancer is different between high and low CagA antibody titers [37]. They examined 299 noncardia gastric cancers and 1048 matched controls. Among seropositive subjects for *H. pylori* antibody, those with low CagA antibody titers had higher and more significant risk (relative risk [RR]: 3.9; $p < 0.001$) for future noncardia gastric cancer than those with CagA-seronegative (RR: 2.2; $p = 0.0052$) or high CagA antibody titers (RR: 2.0; $p = 0.0022$). These data suggest that attention should be paid to the antibody titer, in addition to seropositivity.

CagA seropositivity in both *H. pylori*-infected & uninfected east-Asian population

CagA seropositivity in both *H. pylori*-infected and -uninfected population was reported in eight studies (five from Japan, two from China and one from Korea) (Table 2) [31,33–39]. Overall, the prevalence of anti-CagA antibody ranged from 38.1 to 92.5% in gastric cancer patients, and 29.2 to 85.7% in controls.

A positive association between anti-CagA antibody and gastric cancer was detected in seven studies [31,33–38]. Among them, five studies showed a statistically significant positive association [31,33,35,37,38]. Interestingly, Maeda *et al.* reported that the CagA seropositivity (92.5%) was higher than that of the anti-*H. pylori* antibody (65.0–77.5%) in patients with gastric cancer [38]. These data show that anti-*H. pylori* antibodies may be undetectable in patients with advanced atrophy or intestinal metaplasia. Moreover, Kikuchi *et al*. reported that the prevalence of CagA antibody in patients aged under 40 years was higher in gastric cancer than in healthy controls [33]. These data may also reflect the advanced atrophy that occurs even in young patients with gastric cancer.

Only one study showed that the CagA sero-positivity did not differ in patients with and without gastric cancer, although the number of subjects was small [39]. In China, Mitchell *et al.* examined the seroprevalence of CagA antibody by Helico-blot 2.0. This immunoblotting kit consists of Western blot membrane strips prepared using a surface antigen-enriched preparation of *H. pylori*. This preparation is known to contain several serologically important antigens of *H. pylori*, including CagA, VacA and the urease subunits. No significant difference was found to exist between the seroprevalence of CagA antibody in asymptomatic subjects compared with gastric cancer patients (85.7 vs 83.3%) [39].

In eight studies, the pooled prevalence of CagA seropositivity was 71.6% (1019 out of 1423) in cases and 62.7% (1595 out of 2542) in controls. The estimated overall OR was 1.50 (95% CI: 1.30–1.72). In meta-analysis in a random-effects model, overall OR was 1.81 (95% CI: 1.30–2.11). This shows that the gastric cancer risk for CagA-positive cases was higher overall than in *H. pylori*-infected subjects; however, the OR in east-Asian countries was smaller than the result of the meta-analysis that included Western countries (1.81 vs 2.64).

In our study, significant heterogeneity was evident (Q: 30.6 with 7 degrees of freedom, $p < 0.0001$) but publication bias did not exist (intercept: 1.78; $p = 0.32$).

CagA seropositivity in the *H. pylori*-negative east-Asian population

The previous conclusion allowed us to examine the prevalence of anti-CagA antibody in the *H. pylori*-negative population. Four studies provided raw data on CagA seroprevalence in the *H. pylori*-negative population [31,33,35,36]. The prevalence of anti-CagA antibodies ranged from 18.2 to 81.8% in gastric cancer patients, and 9.8 to 60.2% in controls (Table 3). The pooled prevalence of CagA seropositivity was 50.6% (40 out of 79) in cases and 34.1% (107 out of 314) in controls. The estimated overall OR was 1.98 (95% CI: 1.20–3.26). In meta-analysis in a random-effects model, the overall OR was 1.95 (95% CI: 1.05–3.64). Therefore, in the *H. pylori*-negative population, the presence of anti-CagA antibodies increases the risk of gastric cancer. This evidence confirms that CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody. Accordingly, anti-CagA antibody was related to gastric cancer in both *H. pylori*-positive and -negative populations.

Which is a better biomarker for the risk of gastric cancer?

We calculated the OR of the anti-*H. pylori* antibody for the risk of gastric cancer from the eight studies discussed above. The pooled prevalence of the seropositive *H. pylori* was 86.7% (1548 out of 1785) in cases and 71.5% (1656 out of 2317) in controls. The estimated overall OR was 2.61 (95% CI: 2.21–3.07). In meta-analysis of a random-effects model, the overall OR was 2.59 (95% CI: 2.15–3.12). Therefore, the OR of anti-*H. pylori* antibody was bigger than that of the anti-CagA antibody.

Discussion

In east-Asian countries, different CagA sero-positivity has been reported despite almost all *H. pylori* possessing the *cagA* gene. The association of anti-CagA antibody with gastric cancer also varied in each study. In meta-analysis, CagA seropositivity was higher in gastric cancer patients than controls, even in east-Asian countries, although the OR in east-Asian countries was smaller than in studies that included Western countries. The OR was higher in whole subjects, irrespective of *H. pylori* status, than *H. pylori*-infected subjects. This suggests that the *H. pylori*-negative/CagA-positive subject is the highest risk group for gastric cancer. Asaka *et al.* reported that *H. pylori* antibody titer is significantly higher in early gastric cancer than in advanced cancer [40]. The lower frequency of higher-titer IgG antibody in advanced cancer may be due to the increasing extent of intestinal metaplasia associated with transition from the intestinal type of early gastric cancer to advanced cancer, such that the local environment is no longer ideal for the growth of *H. pylori* [40,41]. CagA antibodies may be positive in patients who have a negative *H. pylori* serologic test [42,43] since CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [44,45]. Therefore, a negative *H. pylori* serologic test does not rule out the possibility of a previous exposure to infection. At this point, anti-CagA antibody alone is not a superior biomarker to the anti-*H. pylori* antibody alone. It is necessary to evaluate the availability of anti-*H. pylori* antibody plus anti-CagA antibody for screening the risk of gastric cancer.

As described above, the relationship between anti-CagA antibody and gastric cancer varied in each study. We found a significant heterogeneity in a meta-analysis. This heterogeneity appeared to result from the use of different populations or different methods, or from differences in the antigens used to detect anti-CagA antibodies. In support of this, CagA seropositivity in gastritis ranged from 53.7 to 83.3%, even in Japan [31,37].

We previously examined the relationship between anti-CagA antibody and gastric cancer in a Japanese population using two different recombinant CagA antigens [32]: one was evaluated by ELISA using a 65 KDa fragment protein from OraVax Inc. and the other was evaluated by immunoblot using a 25.5 KDa fragment protein from Chiron Corp. CagA positivity was 82% by OraVax antigen and 72% by Chiron antigen, irrespective of the existence of gastric cancer, when determining the cutoff value by the population living in the same region (Kyoto). This suggests that numerical results from studies using different antigens and different protocols may not be comparable [46,47]. Likewise, Basso *et al.* reported different properties from two ELISA kits (RADIM [Pomezia, Italy] and EUROSPITAL [Helori CTX IgG, Trieste, Italy]) [48].

We studied the efficacy of Helicoblot 2.1 for serum samples from 222 patients (120 patients from Japan and 102 from the USA) [49]. With these criteria, the sensitivity was 100% in both countries, but the specificity was 88.8% in Japanese samples and 44.4% in US samples. This study illustrates the variance of assays in different populations. Because many strains of recombinant CagA used as coating antigen in ELISA were derived from European strains, serum containing east-Asian CagA may not react enough with the specific structure of Western CagA. Therefore, ELISA using recombinant CagA derived from an east-Asian strain is more suitable to use in east-Asian countries. As described above, *H. pylori* strains possessing east-Asian-type CagA are more virulent than Western-type CagA. east-Asian-type CagA or Western-type CagA status may also affect the anti-CagA antibody titer and/or sensitivity of the assay. At present, there are no reports that examine the prevalence of east-Asian-type CagA-specific antibody in serum. Recently, Yasuda *et al.* reported the development of monoclonal antibody against east-Asian-type CagA for developing a sandwich-ELISA system [50]. However, this is a system for detecting east-Asian-type CagA strains but not serum antibody. To detect east-Asian-type CagA-specific antibody, the development of an ELISA assay using east-Asian-type CagA-specific antigen will be required.

In addition, the staging of gastric cancer affects the anti-CagA antibody titer. We examined the prevalence of anti-CagA antibodies in both early and advanced gastric cancer in *H. pylori*-positive patients [32]. Seropositivity of CagA tended to be higher in 65 early gastric cancer cases than in 25 advanced gastric cancer cases (87.7 vs 72.0%; $p = 0.07$). Anti-CagA antibody may be used to detect gastric cancer in the earlier stage. Several other variables of gastric cancer can influence the outcome of studies. For example, previous studies have reported that although CagA seropositivity was associated with increased risk of intestinal-type gastric cancer, there was no association with the diffuse type of gastric cancer [4,7]. The subsite of gastric cancer also affects the results. In a meta-analysis by Huang, *H. pylori* infection significantly increased the risk for noncardia gastric cancer, with an overall OR of 2.71 (95% CI; 1.74–4.21), but did not increase the risk for gastric cancer for cardia gastric cancer (OR: 1.13; 95% CI: 0.75–1.70) [22]. In *H. pylori*-positive populations, CagA seropositivity increased the risk of noncardia gastric cancer 2.01-fold (95% CI: 1.21–3.32), but not the risk for gastric cancer at the cardia (OR: 0.70; 95% CI: 0.44–1.10). In this article, we reviewed the availability of anti-CagA antibody for all gastric cancers including noncardia and cardia due to insufficient data according to subsite in east-Asian countries. Further study is needed to clarify the relationship between anti-CagA antibody and gastric cardia cancer in east-Asian countries.

In the latest article, Wada *et al.* examined the expression level of tyrosine phosphorylation of the EPIYA motif in CagA (CagA-P) in diffuse-type gastric cancer patients [51]. The serum titer of anti-CagA-P antibody was significantly higher in the gastric cancer group than in the matched atrophic gastritis group, especially in females. These data show that in addition to east-Asian-type and Western-type CagA, various epitopes of CagA may be

related to the risk of gastric cancer. Recently, Klimovich *et al.* developed the ELISA system with monoclonal antibody inducing by several recombinant fragments of CagA, and each immunochemical property were different [52]. There was also a report using full-length recombinant CagA [53]. It is necessary to consider the antigen of ELISA system for detecting anti-CagA antibody.

Recent reports show the availability of anti-CagA antibody as a biomarker for other diseases, in addition to gastric cancer. Although partial or even complete remission of thrombocytopenia has been reported in some chronic idiopathic thrombocytopenic purpura (ITP) patients after eradication of *H. pylori* [54,55], it has been controversial whether *H. pylori* eradication therapy in chronic ITP patients is effective in increasing the platelet count or not. In Japan, it is reported that the cure of *H. pylori* infection resulted in a 40–60% increase in the platelet count compared with that in pretreatment or that in *H. pylori*-positive chronic ITP patients [54]. As a result, *H. pylori* eradication therapy was approved for ITP in Japan in 2010. Two reports from east Asia have shown an association between anti-CagA antibody and ITP. Suzuki *et al.* performed randomized controlled trials of ITP patients and examined the association of the anti-CagA antibody with response to therapy in Japan [56]. *H. pylori*-positive chronic ITP patients were randomly assigned to either the eradication or the noneradication group. The eradication group was divided into responder and nonresponder groups, according to the increase of platelet count. In the results, the ELISA titers of serum anti-CagA antibody of the responders were significantly higher than those of the nonresponders. Kodama *et al.* also examined the relationship between anti-CagA antibody titer and response to eradication therapy in Japan. Although anti-*H. pylori* or anti-CagA antibody titer was not different between responders and nonresponders, the serum anti-CagA antibody titer decreased significantly after eradication therapy only in responders [57]. By contrast, anti-*H. pylori* antibody titer decreased equally in both responders and nonresponders.

These phenomena were also observed in gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Sumida *et al.* examined the relationship between gastric MALT lymphoma and the effect of *H. pylori* eradication therapy in Japan [58]. In responders, the serum anti-*H. pylori* antibody titer and the anti-CagA antibody titer were significantly higher than those in the non-responders; this difference was more significant for anti-CagA antibody than for anti-*H. pylori* antibody. On the contrary, Delchier *et al.* reported that there was no relationship between the prevalence of anti-CagA antibody and the response to *H. pylori* eradication therapy in MALT lymphoma [59]; however, they did not measure the anti-CagA antibody titer. These data suggest that anti-CagA antibody titer (not the presence of antibody) is possibly a better biomarker for the response to eradication therapy than anti-*H. pylori* antibody titer.

Conclusion & future perspective

In meta-analysis, CagA seropositivity was higher in gastric cancer patients than controls, even in east-Asian countries, although the OR in east-Asian countries was smaller than that in studies that included Western countries. It is necessary to evaluate the availability of anti-*H. pylori* antibody plus anti-CagA antibody for screening for-risk of gastric cancer. Moreover, it is necessary to develop anti-CagA antibody diagnostic methods dependent on CagA phenotype, especially east-Asian CagA.

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Table 1

CagA seropositivity and risk of gastric cancer in the *Helicobacter pylori*-infected population.

Author (year)	Country	Method	Controls			Gastric cancer			OR	95% CI	Ref.
			Positive	*Total*	*Prevalence (%)*	*Positive*	*Total*	*Prevalence (%)*			
Shimoyama *et al.* (1998)	Japan	ELISA	30	57	52.6	42	57	73.7	2.52	1.14–5.53	[31]
Yamaoka *et al.* (1999)	Japan	ELISA	73	90	81.1	75	90	83.3	1.16	0.54–2.50	[32]
Yamaoka *et al.* (1999)	Japan	Immunoblot	64	90	71.1	66	90	73.3	1.12	0.58–2.14	[32]
Kikuchi *et al.* (1999)	Japan	ELISA	50	79	63.3	58	92	63.0	0.99	0.53–1.84	[33]
Limburg *et al.* (2001)	China	ELISA	56	99	56.6	69	113	61.1	1.20	0.69–2.08	[34]
Sasazuki *et al.* (2006)	Japan	ELISA	281	383	73.4	363	478	75.9	1.15	0.84–1.56	[35]
Gwack *et al.* (2006)	Korea	Immunoblot	325	360	90.3	86	89	96.6	3.09	0.92–10.27	[36]
Suzuki *et al.* (2007)	Japan	ELISA	684	821	83.3	249	285	87.4	1.39	0.93–2.05	[37]
Overall									1.26	1.05–1.52	

CagA: Cytotokine-associated gene A protein; ELISA: Enzyme-linked immunosorbent assay; OR: Odds ratio.

Table 2

CagA seropositivity and risk of gastric cancer irrespective of *Helicobacter pylori* status.

Author (year)	Country	Method	Controls			Gastric cancer			OR	95% CI	Ref.
			Positive	*Total*	*Prevalence (%)*	*Positive*	*Total*	*Prevalence (%)*			
Mitchell *et al.* (1996)	China	Immunoblot	30	35	85.7	40	48	83.3	0.83	0.25–2.80	[39]
Shimoyama *et al.* (1996)	Japan	ELISA	36	81	44.4	49	81	60.5	1.91	1.02–3.58	[31]
Kikuchi *et al.* (1999)	Japan	ELISA	50	201	24.9	58	101	57.4	4.07	2.45–6.77	[33]
Maeda *et al.* (2000)	Japan	ELISA	44	80	55.0	74	80	92.5	10.09	3.94–25.87	[38]
Limburg *et al.* (2001)	China	ELISA	56	192	29.2	69	181	38.1	1.50	0.97–2.31	[34]
Sasazuki *et al.* (2006)	Japan	ELISA	358	511	70.1	390	511	76.3	1.38	1.04–1.82	[35]
Gwack *et al.* (2006)	Korea	Immunoblot	337	400	84.3	90	100	90.0	1.68	0.83–3.41	[36]
Suzuki *et al.* (2007)	Japan	ELISA	684	1042	65.6	249	321	77.6	1.81	1.35–2.42	[37]
Overall									1.81	1.30–2.11	

CagA: Cytotokine-associated gene A protein; CI: Confidence interval; ELISA: Enzyme-linked immunosorbent assay; OR: Odds ratio.

Table 3

CagA seropositivity and risk of gastric cancer in *Helicobacter pylori*-negative population.

Author (year)	Country	Method	Controls			Gastric cancer			OR	95% CI	Ref.
			Positive	*Total*	*Prevalence (%)*	*Positive*	*Total*	*Prevalence (%)*			
Shimoyama *et al.* (1998)	Japan	ELISA	6	24	25.0	7	24	29.2	1.24	0.34–4.42	[31]
Kikuchi *et al.* (1999)	Japan	ELISA	12	122	9.8	2	11	18.2	2.04	0.39–10.54	[33]
Sasazuki *et al*. (2006)	Japan	ELISA	77	128	60.2	27	33	81.8	2.98	1.15–7.72	[35]
Gwack *et al.* (2006)	Korea	Immunoblot	12	40	30.0	4	11	36.4	1.33	0.32–5.42	[36]
Overall									1.95	1.05–3.64	

CagA: Cytotokine-associated gene A protein; ELISA: Enzyme-linked immunosorbent assay; OR: Odds ratio.