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Epstein–Barr Virus Antibody Titers Are Not Associated with Gastric Cancer Risk in East Asia

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Electronic supplementary material

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Abstract

Background—Epstein–Barr virus (EBV)-positive gastric cancers represent a distinct subtype of gastric cancers and account for nearly 10% of the gastric cancer burden, yet risk detection strategies for this cancer subtype are lacking.

Methods—We conducted a nested case–control study where we assayed 4 EBV antigens [viral capsid antigen (VCA), early antigen (EA), Epstein–Barr nuclear antigen (EBNA), and BZLF1-encoded replication activator protein (ZEBRA)] in either sera or plasma from 1447 gastric cancer cases and 1797 controls obtained from seven prospective cohorts representing individuals from the high gastric cancer-risk countries of China, Japan, and Korea.

Results—The prevalence of EBV sero-positivity was universal with the exception of one sero-negative individual, and the highest titers of the EBV antigens VCA (OR 0.95, 95% CI 0.78–1.17), EBNA (OR 0.88, 95% CI 0.72–1.08), EA (OR 0.97, 95% CI 0.79–1.19), and ZEBRA (OR 0.87, 95% CI 0.71–1.07) were not associated with risk of incident gastric cancer. When we stratified these data by *H. pylori* status, there was no change in the association.

Conclusions—Multiplex serology of the aforementioned EBV antigens in serum may not be a suitable biomarker for predicting gastric cancer risk in East Asian populations.

Keywords

Epstein–Barr virus; Gastric cancer; Multiplex serology

Introduction

Epstein–Barr virus (EBV) infects over 90% of the global adult population and persists for the lifetime of the host. While infection with EBV is asymptomatic in the majority of infected persons, it has been classified as a class I carcinogen due to its capacity to induce several types of malignancies including B cell neoplasia, nasopharyngeal carcinoma (NPC), and gastric cancer [1].

Gastric cancer is the fifth most common cancer and the third leading cause of cancer-related death worldwide [2]. While the majority of gastric cancers result from infection with the gastric bacterial pathogen, *Helicobacter pylori (H. pylori)*, approximately 7–10% of gastric adenocarcinomas are also positive for EBV infection and have distinct histological features [3, 4]. Notably, given the high global prevalence of gastric cancers, EBV-associated gastric cancer may be the most common form of EBV-associated malignancies [5]. While only a subset of gastric cancers may be directly associated with EBV, this virus can also induce chronic inflammation that may serve as an indirect cause of gastric carcinomas either independently of, or in concert with *H. pylori* [6, 7].

Although EBV prevalence is nearly ubiquitous, elevated pre-diagnostic antibody titers to EBV antigens have been shown to be an effective biomarker for risk of NPC [8–10]. To date, few pre-diagnostic serological studies have been conducted to examine the utility of elevated EBV antibodies as a marker of gastric cancer risk. Of the existing studies, each quantified differing combinations of EBV antigens and antibodies, making cross-study comparisons

difficult [11–14]. Therefore, in this study, we aim to expand upon this field by examining the association of EBV antibody titers and gastric cancer risk in seven prospective cohorts of East Asian origin using the same, noninvasive, serology assay to detect both EBV and *H. pylori* antigens [15, 16].

Methods

Study Population

The *H. pylori* Biomarker Cohort Consortium (HpBCC) has been described previously [16]. Briefly, the HpBCC comprises eight prospective cohort studies from the highest gastric cancer-risk countries within East Asia: China (Shanghai Men's Health Study, $n = 66$ cases and 132 controls; Shanghai Women's Health Study, $n = 295$ cases and 579 controls; and the Nutrition Intervention Trial, $n = 326$ cases and 326 controls), Japan (Japan Public Health Center-based Prospective Study I, $n = 207$ cases and 207 controls; and Japan Public Health Center-based Prospective Study II, $n = 195$ cases and 195 controls), and Korea (Korean Cancer Prevention Study II, $n = 169$ cases and 169 controls; Korean Multicenter Cancer Cohort I, $n = 189$ cases and 189 controls; and the Korean National Cancer Screenee Cohort, $n = 161$ cases and 161 controls). At baseline, these cohorts collected demographic and lifestyle information, including age, sex, smoking status, level of education, previous diagnosis of gastritis, body mass index, and family history of gastric cancer, as well as blood samples from healthy individuals, prior to cancer diagnosis. One study, the Korean National Cancer Screenee Cohort, was excluded from the present analyses due to its short median follow-up time (0.7 years). The outcome of gastric cancer was defined using the International Classification for Disease Oncology codes (C16.0–C16.6, C16.8, and C16.9).

For studies conducted outside of China, incidence density sampling was used to select one control chosen at random for each gastric cancer case who was alive, free of cancer, and had no history of gastrectomy at the time of diagnosis of the index case (excluding studies from Japan, which did not collect information on gastrectomy). Controls were matched within cohorts based on birth date, sex, and date of blood collection. For studies conducted in Shanghai, the same control selection was implemented except two controls were selected per index case and further matched by antibiotic use at time of blood draw. For the Nutrition Intervention Trial, from Linxian, China, controls were frequency matched based on sex.

All procedures performed in this study involving human subjects were in accordance with the ethical standards of the institutional review boards of all participating institutions [16–18] and the ethical standards of the Declaration of Helsinki and its later amendments or comparable ethical standards.

Multiplex Serology

We utilized a novel multiplex serology assay to detect serum or plasma antibody titers to 15 *H. pylori* antigens and 4 EBV antigens: viral capsid antigen (VCA), early antigen (EA), Epstein–Barr nuclear antigen (EBNA) and BZLF1-encoded replication activator protein (ZEBRA) developed by the German Cancer Research Center in collaboration with the German National Reference Center for *H. pylori* as described previously [16–21]. Briefly,

antigens were fused with a glutathione *S*-transferase (GST) tag and recombinantly expressed, affinity purified and loaded onto glutathione–casein-coupled fluorescence-labeled polystyrene beads (Luminex). Labeled beads were mixed and incubated in 96-well plates with an equal volume of serum dilutions. Antibodies bound to the beads were stained with biotinylated goat anti-human IgA, IgM, IgG, and followed by the reporter conjugate R-phycoerythrin-labeled streptavidin. Plates were subsequently read using a Luminex 100 analyzer to identify the internal bead color and thus the antigen carried by the bead. The quantity of bound antibodies was determined as the median reporter fluorescence intensity (MFI) of at least 100 beads per bead set per serum [21]. Since the median fluorescence intensity (MFI) values obtained from the assay were not normally distributed, the MFI values were log transformed to calculate the coefficients of variation (CV) for each of the EBV antigens. The CV values for VCA, EBNA, EA and ZEBRA were 5.3, 3.7, 22.2, and 16.4%, respectively. Sero-positivity to EBV was defined by previously established MFI values exceeding cutoffs (100 for ZEBRA, EA, and VCA, and 250 for EBNA) to at least two EBV antigens [16].

Statistical Analysis

To determine the associations of pre-diagnostic EBV antigen-specific antibody titers by group based on distribution among controls (< 25th, 25–75th, > 75th percentile of MFI values) with gastric cancer incidence, we performed conditional logistic regression, stratified by cohort and adjusted for age, sex, smoking, and *H. pylori* status to produce odds ratios and 95% confidence intervals. Tests for trend were performed three ways: by considering the antigen levels as continuous variables; or by entering the antigen-specific categorical variables as continuous parameters in the models either as linear categorical variables (0, 1, 2) or by median value of each category. No differences were found by trend assessment method; thus, trend by linear categorical variable is reported in the results. Secondary analyses were stratified by *H. pylori* status (SAS v9.4, Cary NC). We further examined the data for potential differences in association by cohort, time of baseline blood draw, tumor stage, or anatomical subsite and found no evidence of effect modification by these variables.

Results

The population for our nested case–control study was composed of 7 of the 8 prospective cohorts contained within the HpBCC. These included two studies from Japan, two from Korea, and three from China. Cumulatively, these studies provided a median follow-up time of 5.9 years, a median age of entry of 58.7 years, and 52.2% male sex [16]. The prevalence of *H. pylori* within this consortium is higher than that of the current East Asian populations today; however, this is due to the dates of enrollment for the prospective studies, ranging from 1984 to 2006. Among 1447 prospectively ascertained cases of gastric cancer and 1797 controls, all participants, with the exception of one individual, were sero-positive for EBV (previously defined as sero-positivity to at least 2 EBV antigens [15]). Gastric cancer incidence was significantly associated with male sex, current smoking, and *H. pylori* status (defined dichotomously or by sero-positivity to two specific *H. pylori* virulence markers: a bacterial outer membrane protein, Omp and the protein encoded by the gene *HP0305* as

described previously [16–18]). However, there was no association between gastric cancer risk and level of EBV antigen-specific antibody titers (Table 1).

When comparing these same gastric cancer-risk factors with EBV antigen-specific antibody levels, female sex and older age were strongly associated with higher titers to the EBV antigens VCA, EA, and ZEBRA (all *p* values for the difference in titers by sex and age < 0.0001, except for sex and VCA, with a p-value of 0.003). Never smoking was associated with higher titers to EBNA, EA, and ZEBRA, although the strength of the association was weaker (*p* values of 0.01, 0.03 and 0.01, respectively). Similarly, higher titers against EA and ZEBRA were nominally associated with *H. pylori* sero-positivity (both *p* values 0.03), but were not associated with combined Omp and HP 0305 status (Table 2).

Overall, no associations were found between EBV antigen-specific antibody titers and incident gastric cancer. Adjustment for age, sex, smoking, and high cancer-risk *H. pylori* sero-positivity did not significantly affect the associations (Table 3). Stratification by *H. pylori* sero-positivity as well as the more specific high cancer-risk *H. pylori* markers CagA or dual Omp with HP0305 sero-positivity also showed no significant association with gastric cancer risk (Table 3). In this analysis, we did observe a statistically significant protective effect of high VCA antibody titers against gastric cancer risk only in patients negative for the *H. pylori* proteins Omp and HP0305; however, we cannot rule out that this association may be due to chance. As gastric cancer is typically asymptomatic and progresses slowly, we further stratified our analysis by within 5 years of blood draw or beyond 5 years post-blood draw to fully explore the possible longitudinal effects of EBV antibody titers on gastric cancer incidence. We again saw no significant associations between pre-diagnostic EBV antibody titers and gastric cancer risk (Supplemental Tables 1 and 2). Additionally, when we stratified the analyses by cohort, tumor stage, or anatomical subsite (cardia and non-cardia), there remained no association between EBV titers and gastric cancer incidence (data not shown).

Discussion

EBV has been previously associated with B cell malignancies and epithelial malignancies such as nasopharyngeal carcinoma (NPC) [4]. Although evidence is accumulating, its association with gastric adenocarcinoma has not been fully established. Multiple studies have identified EBV antigens within approximately 7–10% of all gastric tumor biopsies [3, 22, 23]; however, whether EBV is an etiological cause or a consequence of gastric neoplastic transformation has not been established.

Our previous work has demonstrated that serum antibody titers to select *H. pylori* antigens are important predictors of disease risk [16–18]. Therefore, in this study, we sought to examine whether serum antibody titers against EBV could also serve as a marker of disease risk, either alone or in combination with *H. pylori* serum markers. While EBV-associated gastric cancers can only be diagnosed through a biopsy, in this study we sought to utilize a noninvasive strategy to predict gastric cancer risk.

In NPC, EBV viral reactivation from its natural niche among circulating B cells may facilitate epithelial cell infection [24]. The EBV structural protein VCA is a marker of viral reactivation and elevated antibody titers against this antigen have been associated with NPC risk [25]. In this study, we examined the association between pre-diagnostic levels of four EBV antigens and gastric cancer risk in East Asia. Three of the antigens examined—viral capsid antigen (VCA), early antigen (EA) and BZLF-1 encoded replication activator protein (ZEBRA)—are markers of primary infection and lytic cycles; the fourth, Epstein–Barr nuclear antigen (EBNA), is a marker of chronic infection [16]. Although EBNA is less immunogenic than the other three antigens, it may have a critical role in promoting oncogenic transformation [6]. In addition, similar to the mechanism of *H. pylori*-induced gastric carcinogenesis [24], EBV may act indirectly to induce chronic inflammation during its viral reactivation cycle by recruiting high levels of immune cell infiltration and thereby promoting tissue damage [6].

Our analyses indicate that there is no association between these specific EBV antigens and incident gastric cancer risk within this population. While previous studies have examined this association in limited numbers of gastric cancer cases within individual countries, the strength of our study is derived from examining over 1400 cases and 1700 controls across multiple high gastric cancer-risk countries with a uniform methodology. Additionally, we have included the EBV antigen ZEBRA in our analysis, which has not been previously investigated for its pre-diagnostic association with gastric cancer incidence. Although we observed some p values less than 0.05 within our stratified analysis of the association between pre-diagnostic EBV antibody titers and gastric cancer risk in cases diagnosed at least 5 years beyond the time of blood draw, we do not believe that these associations are significant because we have no a priori hypothesis for the observation and we cannot rule out that the association may be due to multiple testing.

These findings are largely in agreement with Kim et al. [11] who found null associations between IgG and IgA serum antibodies to EA, EBNA, and VCA with gastric cancer incidence in the Korean Multi-Center Cohort. However, our findings differ from earlier investigations in East Asian populations that found either a suggestive increased risk of gastric adenocarcinoma with elevated levels of pre-diagnostic VCA and EBNA serum IgG antibody titers [13] or an inverse association of gastric cancer risk with elevated levels of VCA IgG antibodies [12]. This discordance highlights one potential limitation of our study in that we were not able to discriminate between IgG/IgA/IgM isotypes.

As mentioned previously, EBV-associated gastric cancer can only be diagnosed through the presence or absence of virus found directly within tumor tissue. While our study sought to identify noninvasive markers of gastric cancer risk through the examination of select EBV antibody titers, our study was limited in that we did not have tumor tissue to examine whether these antibody titers were associated with EBV-associated gastric cancer. EBV has classically been considered a direct transforming agent through regulation of its own cell survival and proliferation genes. Therefore, based on our results, pre-diagnostic EBV serum antibody levels may be a more useful biomarker for EBV^+ tumors and not overall gastric cancer risk, as suggested by two previous studies from Japan which found increased IgG and IgA serum titers to VCA and EBNA in EBV^+ gastric cancer cases compared to EBV^-

gastric cancer cases [13, 14]. In addition, recent studies have also demonstrated that circulating microRNAs in plasma samples may be a potential noninvasive alternative for detecting EBV microRNAs to aid in characterizing gastric cancers and therefore may also be useful in pre-diagnostic screening [25].

Unlike the association between EBV and NPC, our results indicate that elevated antibody titers against EBV antigens are not a useful measure for estimating gastric cancer risk in East Asia. Future work is necessary to examine how this nearly ubiquitous pathogen can augment cancer risk in only a subset of infected individuals, as the results of these studies may lead to a more successful predictive tool for gastric cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Niedobitek G, Meru N, Delecluse HJ. Epstein–Barr virus infection and human malignancies. Int J Exp Pathol. 2001; 82:149–170. [PubMed: 11488990]
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136:E359–E386. [PubMed: 25220842]
3. Murphy G, Pfeiffer R, Camargo MC, Rabkin CS. Meta-analysis shows that prevalence of Epstein–Barr virus-positive gastric cancer differs based on sex and anatomic location. Gastroenterology. 2009; 137:824–833. [PubMed: 19445939]
4. Coghill AE, Hildesheim A. Epstein–Barr virus antibodies and the risk of associated malignancies: review of the literature. Am J Epidemiol. 2014; 180:687–695. [PubMed: 25167864]
5. Young LS, Yap LF, Murray PG. Epstein–Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer. 2016; 16:789–802. [PubMed: 27687982]
6. Fuentes-Panana EM, Morales-Sanchez A. Epstein–Barr virus-associated gastric cancer and potential mechanisms of oncogenesis. Curr Cancer Drug Targets. 2016; 16:1–21.
7. Saju P, Murata-Kamiya N, Hayashi T, et al. Host SHP1 phosphatase antagonizes *Helicobacter pylori* CagA and can be down-regulated by Epstein–Barr virus. Nat Microbiol. 2016; 1:16026. [PubMed: 27572445]
8. De Vathaire F, Sancho-Garnier H, de-The H, et al. Prognostic value of EBV markers in the clinical management of nasopharyngeal carcinoma (NPC): a multicenter follow-up study. Int J Cancer. 1988; 42:176–181. [PubMed: 2841245]
9. Henle G, Henle W. Epstein–Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. Int J Cancer. 1976; 17:1–7. [PubMed: 175020]
10. Kim Y, Shin A, Gwack J, et al. Epstein–Barr virus antibody level and gastric cancer risk in Korea: a nested case-control study. Br J Cancer. 2009; 101:526–529. [PubMed: 19550421]
11. Koshiol J, Qiao YL, Mark SD, et al. Epstein–Barr virus serology and gastric cancer incidence and survival. Br J Cancer. 2007; 97:1567–1569. [PubMed: 17987041]

12. Levine PH, Stemmermann G, Lennette ET, Hildesheim A, Shibata D, Nomura A. Elevated antibody titers to Epstein–Barr virus prior to the diagnosis of Epstein–Barr-virus-associated gastric adenocarcinoma. Int J Cancer. 1995; 60:642–644. [PubMed: 7860138]
13. Shinkura R, Yamamoto N, Koriyama C, Shinmura Y, Eizuru Y, Tokunaga M. Epstein–Barr virus-specific antibodies in Epstein–Barr virus-positive and -negative gastric carcinoma cases in Japan. J Med Virol. 2000; 60:411–416. [PubMed: 10686024]
14. Michel A, Waterboer T, Kist M, Pawlita M. *Helicobacter pylori* multiplex serology. Helicobacter. 2009; 14:525–535. [PubMed: 19889070]
15. Teras LR, Rollison DE, Pawlita M, et al. Epstein–Barr virus and risk of non-Hodgkin lymphoma in the cancer prevention study-II and a meta-analysis of serologic studies. Int J Cancer. 2015; 136:108–116. [PubMed: 24831943]
16. Cai H, Ye F, Michel A, et al. *Helicobacter pylori* blood bio-marker for gastric cancer risk in East Asia. Int J Epidemiol. 2016; 45:774–781. [PubMed: 27170766]
17. Gao L, Michel A, Weck MN, Arndt V, Pawlita M, Brenner H. *Helicobacter pylori* infection and gastric cancer risk: evaluation of 15 *H. pylori* proteins determined by novel multiplex serology. Cancer Res. 2009; 69:6164–6170. [PubMed: 19602590]
18. Shakeri R, Malekzadeh R, Nasrollahzadeh D, et al. Multiplex *H. pylori* serology and risk of gastric cardia and noncardia adenocarcinomas. Cancer Res. 2015; 75:4876–4883. [PubMed: 26383162]
19. Gao L, Weck MN, Michel A, Pawlita M, Brenner H. Association between chronic atrophic gastritis and serum antibodies to 15 *Helicobacter pylori* proteins measured by multiplex serology. Cancer Res. 2009; 69:2973–2980. [PubMed: 19318564]
20. Camargo MC, Murphy G, Koriyama C, et al. Determinants of Epstein–Barr virus-positive gastric cancer: an international pooled analysis. Br J Cancer. 2011; 105:38–43. [PubMed: 21654677]
21. Shannon-Lowe C, Rowe M. Epstein–Barr virus infection of polarized epithelial cells via the basolateral surface by memory B cell-mediated transfer infection. PLoS Pathog. 2011; 7:e1001338. [PubMed: 21573183]
22. Ji MF, Wang DK, Yu YL, et al. Sustained elevation of Epstein-Barr virus antibody levels preceding clinical onset of nasopharyngeal carcinoma. Br J Cancer. 2007; 96:623–630. [PubMed: 17285127]
23. Li S, Deng Y, Li X, Chen QP, Liao XC, Qin X. Diagnostic value of Epstein-Barr virus capsid antigen-IgA in nasopharyngeal carcinoma: a meta-analysis. Chin Med J (Engl). 2010; 123:1201–1205. [PubMed: 20529563]
24. Amieva M, Peek RM Jr. Pathobiology of *Helicobacter pylori*-Induced Gastric Cancer. Gastroenterology. 2016; 150:64–78. [PubMed: 26385073]
25. Treece AL, Duncan DL, Tang W, et al. Gastric adenocarcinoma microRNA profiles in fixed tissue and in plasma reveal cancer-associated and Epstein–Barr virus-related expression patterns. Lab Investig J Tech Methods Pathol. 2016; 96:661–671.

Table 1

Demographic characteristics by case–control status (*n*, %)

	All (*N* = 3244)	Cases (*N* = 1447)	Controls (*N* = 1797)
Sex *			
Female	1549 (47.75)	632 (43.68)	917 (51.03)
Male	1695 (52.25)	815 (56.32)	880 (48.97)
Age *			
≤ 45 Years	367 (11.31)	131 (9.05)	236 (13.13)
45–55	931 (28.70)	421 (29.09)	510 (28.38)
56–65	1119 (34.49)	541 (37.39)	578 (32.16)
> 65	827 (25.49)	354 (24.46)	473 (26.32)
Smoke *			
Never	1903 (58.68)	783 (54.11)	1120 (62.36)
Former	400 (12.33)	185 (12.79)	215 (11.97)
Current	940 (28.99)	479 (33.10)	461 (25.67)
H. pylori *			
Negative	450 (13.87)	109 (7.53)	341 (18.98)
Positive	2794 (86.13)	1338 (92.47)	1456 (81.02)
Omp and HP0305 status *			
Omp− and HP0305−	550 (16.95)	119 (8.22)	431 (23.98)
Omp + or HP0305+	863 (26.60)	356 (24.60)	507 (28.21)
Omp + and HP0305+	1831 (56.45)	972 (67.17)	859 (47.80)
VCA			
< 25th percentile	821 (25.31)	371 (25.64)	450 (25.04)
25–75th percentile	1612 (49.69)	714 (49.34)	898 (49.97)
> 75th percentile	811 (25.00)	362 (25.02)	449 (24.99)
EBNA			
< 25th percentile	842 (25.96)	393 (27.16)	449 (24.99)
25–75th percentile	1604 (49.45)	705 (48.72)	899 (50.02)
> 75th percentile	798 (24.60)	349 (24.12)	449 (24.99)
EA			
< 25th percentile	821 (25.31)	372 (25.71)	449 (24.99)
25–75th percentile	1609 (49.60)	711 (49.14)	898 (49.97)
> 75th percentile	814 (25.09)	364 (25.16)	450 (25.04)
ZEBRA			
< 25th percentile	855 (26.36)	406 (28.06)	449 (24.99)
25–75th percentile	1579 (48.67)	681 (47.06)	898 (49.97)
> 75th percentile	810 (24.97)	360 (24.88)	450 (25.04)

* $p \leq 0.0001$

Table 2

Distribution of individual EBV antigen antibody titers with demographic characteristics (*n*, %)

	VCA				EBNA				EA				ZEBRA			
	< 25th	**25th–75th**	**>75th**	***P****	**< 25th**	**25th–75th**	**>75th**	***P****	**< 25th**	**25th– 75th**	**>75th**	***P****	**< 25th**	**25th– 75th**	**>75th**	***P****
Sex				0.003				0.06				< 0.0001				< 0.0001
Female	354 (43.12)	786 (48.76)	409 (50.43)		407 (48.34)	794 (49.50)	348 (43.61)		307 (37.39)	803 (49.91)	439 (53.93)		291	815	443	
Male	467 (56.88)	826 (51.24)	402 (49.57)		435 (51.66)	810 (50.50)	450 (56.39)		514 (62.61)	806 (50.09)	375 (46.07)		564 (65.96)	764 (48.39)	367 (45.31)	
Age				< 0.0001				0.24				< 0.0001				< 0.0001
≤ 45 Years	126 (15.35)	172 (10.67)	69 (8.50)		103 (12.23)	169 (10.54)	95 (11.90)		107 (13.03)	197 (12.24)	63 (7.74)		118 (13.80)	180 (11.40)	69 (8.52)	
45–55	250 (30.45)	464 (28.78)	217 (26.76)		249 (29.57)	441 (27.49)	241 (30.20)		254 (30.94)	465 (28.90)	212 (26.04)		253 (29.59)	470 (29.77)	208 (25.68)	
56–65	282 (34.35)	565 (35.05)	272 (33.54)		313 (37.17)	554 (34.54)	252 (31.58)		269 (32.76)	568 (35.30)	282 (34.64)		286 (33.45)	526 (33.31)	307 (37.90)	
> 65	163 (19.85)	411 (25.50)	253 (31.20)		177 (21.03)	440 (27.43)	210 (26.32)		191 (23.26)	379 (23.56)	257 (31.58)		198 (23.16)	403 (25.52)	226 (27.90)	
Smoke				0.15				< 0.01				0.03				0.01
Never	460 (56.03)	973 (60.36)	470 (58.02)		517 (61.40)	954 (59.51)	432 (54.14)		448 (54.57)	968 (60.20)	487 (59.83)		450 (52.69)	963 (60.99)	490 (60.49)	
Former	98 (11.94)	187 (11.60)	115 (14.20)		85 (10.10)	188 (11.73)	127 (15.91)		108 (13.15)	195 (12.13)	97 (11.92)		115 (13.47)	206 (13.05)	79 (9.75)	
Current	263 (32.03)	452 (28.04)	225 (27.78)		240 (28.50)	461 (28.76)	239 (29.95)		265 (32.28)	445 (27.67)	230 (28.26)		289 (33.84)	410 (25.97)	241 (29.75)	
H. pylori				0.12				0.95				0.03				0.03
Negative	118 (14.37)	237 (14.70)	95 (11.71)		110 (13.06)	237 (14.78)	103 (12.91)		133 (16.20)	215 (13.36)	102 (12.53)		131 (15.32)	224 (14.19)	95 (11.73)	
Positive	703 (85.63)	1375 (85.30)	716 (88.29)		732 (86.94)	1367 (85.22)	695 (87.09)		688 (83.80)	1394 (86.64)	712 (87.47)		724 (84.68)	1355 (85.81)	715 (88.27)	
Omp and HP0305 status				0.58				0.65				0.68				0.18
Omp− and HP0305−	144 (17.54)	272 (16.87)	134 (16.52)		133 (15.80)	278 (17.33)	139 (17.42)		141 (17.17)	268 (16.66)	141 (17.32)		139 (16.26)	277 (17.54)	134 (16.54)	
Omp + or HP0305+	216 (26.31)	434 (26.92)	213 (26.26)		213 (25.30)	448 (27.93)	202 (25.31)		230 (28.01)	420 (26.10)	213 (26.17)		245 (28.65)	418 (26.47)	200 (24.69)	
Omp + and HP0305+	461 (56.15)	906 (56.21)	464 (57.22)		496 (58.90)	878 (54.74)	457 (57.27)		450 (54.81)	921 (57.24)	460 (56.51)		471 (55.09)	884 (55.98)	476 (58.77)	

*Cochran Mantel–Haenszel Chi

Table 3

Association between EBV antigen antibody titers and gastric cancer incidence (OR, 95%CI)

	VCA (Percentile)				EBNA (Percentile)				EA (Percentile)				ZEBRA (Percentile)			
	< 25th	**25–75th**	**>75th**	***P* trend**	**< 25th**	**25–75th**	**> 75th**	***P* trend**	**< 25th**	**25–75th**	**> 75th**	***P* trend**	**< 25th**	**25–75th**	**> 75th**	***P* trend**
All[a]	1	0.96 (0.81, 1.14)	0.95 (0.78, 1.17)	0.65	1	0.91 (0.76, 1.08)	0.88 (0.72, 1.08)	0.22	1	0.96 (0.80, 1.14)	0.97 (0.79, 1.19)	0.79	1	0.86 (0.72, 1.02)	0.87 (0.71, 1.07)	0.19
H pylori[b]																
Negative	1	1.10 (0.66, 1.84)	0.62 (0.31, 1.23)	0.23	1	1.00 (0.60, 1.69)	0.60 (0.31, 1.17)	0.15	1	0.86 (0.52, 1.43)	0.77 (0.42, 1.42)	0.39	1	1.02 (0.61, 1.70)	0.91 (0.48, 1.73)	0.79
Positive	1	0.96 (0.80, 1.15)	0.99 (0.80, 1.23)	0.95	1	0.89 (0.74, 1.06)	0.89 (0.72, 1.10)	0.28	1	0.97 (0.81, 1.17)	0.98 (0.80, 1.22)	0.88	1	0.84 (0.70, 1.01)	0.86 (0.70, 1.07)	0.17
H. pylori CagA[b]																
Negative	1	0.85 (0.52, 1.38)	0.68 (0.38, 1.22)	0.2	1	0.65 (0.40, 1.07)	0.76 (0.42, 1.37)	0.32	1	0.88 (0.54, 1.44)	0.92 (0.52, 1.64)	0.77	1	0.87 (0.54, 1.40)	0.83 (0.46, 1.48)	0.51
Positive	1	0.99 (0.82, 1.18)	1.03 (0.83, 1.27)	0.81	1	0.94 (0.78, 1.13)	0.88 (0.71, 1.09)	0.24	1	0.99 (0.82, 1.19)	0.99 (0.80, 1.23)	0.93	1	0.85 (0.71, 1.03)	0.89 (0.72, 1.10)	0.28
Omp and HP0305 status[b]																
Omp− and HP0305−	1	0.78 (0.49, 1.24)	0.31 (0.16, 0.61)	< 0.01	1	0.93 (0.57, 1.52)	0.69 (0.38, 1.25)	0.22	1	1.06 (0.64, 1.75)	0.93 (0.52, 1.66)	0.8	1	0.81 (0.49, 1.34)	1.09 (0.62, 1.93)	0.76
Omp + or HP0305+	1	0.93 (0.67, 1.30)	1.08 (0.73, 1.59)	0.71	1	0.70 (0.50, 0.97)	0.78 (0.53, 1.16)	0.21	1	0.90 (0.65, 1.25)	0.87 (0.59, 1.27)	0.45	1	0.78 (0.57, 1.08)	0.80 (0.55, 1.18)	0.23
Omp + and HP0305+	1	1.03 (0.82, 1.29)	1.09 (0.84, 1.42)	0.52	1	1.02 (0.81, 1.27)	0.96 (0.75, 1.25)	0.79	1	0.96 (0.76, 1.21)	1.04(0.80, 1.36)	0.75	1	0.91 (0.72, 1.15)	0.87 (0.67, 1.13)	0.29

OR odds ratio; *CI* confidence interval

[a]Adjusted for age, sex, smoke and *H. pylori* status (combined Omp and HP0305)

[b]Adjusted for age, sex, and smoke