HHS Public Access

Author manuscript

Aliment Pharmacol Ther. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Aliment Pharmacol Ther. 2018 February ; 47(4): 494–503. doi:10.1111/apt.14471.

Serum pepsinogen I and anti-*Helicobacter pylori* IgG antibodies as predictors of gastric cancer risk in Finnish males

Minkyo Song[1], **M. Constanza Camargo**[1], **Stephanie J. Weinstein**[1], **Gwen Murphy**[1], **Neal D. Freedman**[1], **Jill Koshiol**[1], **Rachael Z. Stolzenberg-Solomon**[1], **Christian C. Abnet**[1], **Satu Männistö**[2], **Demetrius Albanes**[1], and **Charles S. Rabkin**[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA [2]Department of Public Health Solutions, National Institute for Health and Welfare, Helsinki, Finland

SUMMARY

Background—Serum pepsinogen (SPG) and anti-*Helicobacter pylori* serology have been used for gastric risk stratification in Asia.

Aim—To assess utility of these markers in a Western population.

Methods—SPGI measurements were available for 21,895 Finnish male smokers in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. We used Cox proportional hazards models adjusted for potential confounders to estimate gastric cancer hazard ratios (HR) and 95% confidence intervals (95% CI) for low SPGI (<25μg/l). In a subset (n=3,555) with anti-*H. pylori* serology, these markers jointly defined the following: Group A (*H. pylori*[−], SPGI[normal]; reference group), Group B (*H. pylori*[+], SPGI[normal]), Group C (*H. pylori*[+], SPGI[low]) and Group D (*H. pylori*[−], SPGI[low]). Odds ratios (ORs) and 95% CI were calculated using multivariate logistic regression.

Results—There were 329 gastric cancers diagnosed an average of 13.9 years after baseline. Prediagnostic low SPGI was significantly associated with increased gastric cancer risk (HR 2.68, 95% CI 1.99–3.61). Among subjects with both SPGI and *H. pylori* serology, groups B, C and D had increased gastric cancer ORs (95% CI) of 1.79 (1.21–2.64), 3.85 (2.36–6.28) and 6.35 (2.20–18.34) respectively. CagA seropositives had significantly higher ORs than CagA seronegatives within group B ($P_{heterogeneity}$=0.01). For groups B and C, repeat SPGI level at 3 years did not further stratify gastric cancer risk.

Correspondence to: Name: Minkyo Song, Address: Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, 6E204, Bethesda, MD 20892-9776, Phone: 240-276-7985, Fax: 240-276-7806, minkyo.song@nih.gov.

AUTHORSHIP

Guarantor of the article: Minkyo Song

Author contributions: MS, MCC and CSR designed the study. SJW, GM, NDF, JK, RZSS, CCA, SM and DA collected the data. MS performed the statistical analysis. MS drafted the manuscript. MS, MCC, SJW, GM, NDF, JK, RZSS, CCA, SM, DA and CSR interpreted the results. MS, MCC and CSR edited and revised the manuscript. All authors approved the final version of the manuscript.

Conclusions—Low SPGI was associated with increased gastric cancer risk in our large Finnish cohort. A single measurement of SPGI along with *H. pylori* whole cell and CagA serology provides potentially useful prediction of gastric cancer risk.

Keywords

GASTRIC CANCER; EPIDEMIOLOGY; *HELICOBACTER PYLORI*; SCREENING

INTRODUCTION

Chronic infection with *Helicobacter pylori* (*H. pylori*) is well-known to be a strong risk factor for gastric cancer [1–3]. *H. pylori* colonization of the gastric mucosa induces inflammation that causes chronic gastritis and mucosal atrophy that may eventually lead to gastric cancer [4, 5]. Serologic response to infection by *H. pylori* can be assessed by measuring anti-*H. pylori* immunoglobulin G (IgG) antibodies using assays based on whole cell sonicate or one or more individual bacterial antigens [6].

Altered levels of serum pepsinogens (SPG), which are mainly produced by the chief cells of the fundic glands of the stomach, reflect the atrophic status (i.e., gland loss) of gastric mucosa [7, 8]. SPG levels not only reveal the past infection status or current atrophy of the stomach, respectively, but have also been shown to be predictive of gastric cancer risk[9, 10]. However, previous studies in Western populations are limited, failing to adjust for potential confounders [11–13] and/or to quantitate risk [14–16], mostly because of small sample sizes. Even in Asian populations, there are only a few prospective studies investigating the main effect of SPGI with gastric cancer risk [17, 18].

Anti-*H. pylori* antibodies may undergo seroreversion with time and/or progression of disease, and may be undetectable later in the course of disease [19, 20]. SPG levels are normal among *H. pylori*- infected individuals without atrophic gastritis [21] as well as in some cases of gastric cancer, particularly with diffuse-type histology [17, 22]. Thus, the combination of the two markers has been suggested to overcome the limits of each, and this has been applied in Japan as a screening tool for gastric cancer, an approach known as the “ABC(D) method” [21, 23]. A recent meta-analysis of East Asian studies reported a gastric cancer meta-HR as high as 13 times in the highest risk group [24]. Previous studies in Western populations that examined the joint association of serum pepsinogen and anti-*H. pylori* seropositivity with gastric cancer risk have been limited in sample size (less than 100 cases) [11, 13, 25], did not provide overall risk estimates for the combined effects [26–28], and/or were not adjusted for possible confounders [12].

Gastric cancer is a heterogenous disease, with important epidemiologic differences in among subtypes. For instance, with regards to anatomical subsites, *H. pylori* is a major risk factor for noncardia but not for cardia gastric cancer [29]. Divergent incidence trends have been reported for these subtypes in different populations [30, 31]. While intestinal-type gastric cancer is often related to environmental factors such as *H. pylori* or diet, diffuse-type cancer is more closely associated with genetic predisposition [32]. Furthermore, some studies report stronger associations with higher anti-*H. pylori* antibody titer and/or infection with cytotoxin-associated gene A (CagA) virulence factor-positive strains [33, 34].

The high mortality rate of gastric cancer is mostly a consequence of late detection, stemming from the lack of specific symptoms of the disease [35, 36]. Gastric cancer when found early may be curable by endoscopic or minimally invasive surgery [37]. In countries of high gastric incidence where general population screening by endoscopy is not routinely conducted, triaging high risk individuals for definitive evaluation by endoscopy would be useful. However, the utility of non-invasive risk stratification by blood tests has not been evaluated outside of a few high-income Asian countries. Therefore, the aims of this study are to evaluate the association of low serum pepsinogen I (SPGI) with gastric cancer risk overall and by subtypes and to assess the combination of *H. pylori* serology and SPGI as a joint predictor of gastric cancer risk, in a prospective cohort study conducted in a Western population.

METHODS

Study Population

The current analysis represents an extension of prior reports [14, 15, 38], with inclusion of additional cancer cases and consideration of repeated SPGI measurements. Subjects were from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, a randomized, double-blinded, placebo-controlled, 2 × 2 factorial trial of daily supplementation of alpha-tocopherol (50 mg) and/or beta-carotene (20 mg) for the primary prevention of lung cancer [39, 40]. A total of 29,133 Caucasian male smokers aged 50–69 years were originally recruited between 1985 and 1988 in southwestern Finland. At baseline, study participants completed questionnaires on demographic characteristics, self-reported medical history, life-style factors and dietary history. Fasting blood samples were collected at baseline and after 3 years' intervention, stored in serum aliquots at −70 °C until testing. The study was approved by the Institutional Review Boards of both the National Cancer Institute, Bethesda, Maryland, USA and the National Public Health Institute, Helsinki, Finland. All participants provided written informed consent.

SPGI was measured at baseline and follow-up for ATBC participants who continued in the study for more than 3–5 years (n=21,895, 75% of the original cohort). Low SPGI in either blood sample triggered referral for upper gastrointestinal endoscopy, as previously reported [14, 15]. In the current study, baseline measurements were used for determining the association between SPGI and incidence of gastric cancer.

Anti-*H. pylori* antibody status determined in prior nested-case control analyses of pancreas, biliary tract, esophagus, lung, colorectal, and gastric cancers [38, 41–48] was used to evaluate the joint effect of SPGI and anti-*H. pylori* antibodies on gastric cancer risk for a total of 3,555 subjects with both measures.

Identification and Classification of Cancer Cases

The intervention phase ended in April, 1993, but subjects have been passively followed-up using the Finnish Cancer Registry which has nearly 100% case coverage of the ATBC cohort [49]. Diagnoses of gastric cancer were classified according to the International Classification of Diseases, Ninth Revision (ICD-9) as cardia (ICD-9 code 151.0) or noncardia (ICD-9

codes 151.1–151.9). Two histological subtypes according to Lauren classification, intestinal- and diffuse-types, were separately assessed [50]. The median time to gastric cancer diagnosis was 13.9 (Standard Deviation, SD, 6.8) years.

Laboratory Analysis

SPGI analyses were performed by radioimmunoassay in two laboratories. Serum samples were assayed at the laboratory of I. M. Samloff, Sepulveda, California, USA during 1989–1991 [51]. After that facility was damaged by an earthquake, the remaining samples were assayed at the laboratory of M. Härkönen, Helsinki, Finland from 1992–1993[14, 15]. The SPGI measurements from the two laboratories were standardized and transformed for compatibility, with low SPGI defined as 25 μg/L or less, as previously reported [14, 15, 38].

Anti-*H. pylori* IgG antibodies were measured by whole cell enzyme-linked immunosorbent assays (ELISA) [52] or multiplex bead-based assays [53, 54], as previously described [38, 41–48]. The two methods were standardized to create an indicator variable for seropositivity and the continuous values dichotomized into low *vs.* high titer among seropositives, as previously reported [55]. Antibodies to the *H. pylori* virulence factor CagA antigen were also measured by ELISA or bead-based assays and classified for statistical analysis as present *vs.* absent, as previously reported [55]. Furthermore, positivity for anti-CagA antibodies was considered indicative of anti-*H. pylori* seropositivity regardless of other test results, since anti-CagA antibodies can remain positive relatively longer than anti-*H. pylori* antibodies [56]. Antibody titer and anti-CagA seropositivity were evaluated for further refinement of anti-*H. pylori* associations with gastric cancer risk.

Statistical Analyses

Gastric cancer hazard ratios (HRs) and 95% confidence intervals (95% CI) associated with low SPGI were estimated using Cox proportional hazards models. For each participant, follow-up time was calculated from the date of randomization until the diagnosis of cancer, death or December 31, 2014. In the subset of subjects (n=3,555) with both baseline SPGI and *H. pylori* serology information available, odds ratios (ORs) and 95% CI were estimated using logistic regression models for the following 4 categories: Group A (*H. pylori*[−], SPGI[normal]; reference group), Group B (*H. pylori*[+], SPGI[normal]), Group C (*H. pylori*[+], SPGI[low]) and Group D (*H. pylori*[−], SPGI[low]). Subgroup analyses were performed to evaluate variation by anti-*H. pylori* antibody titer and anti-CagA seropositivity within anti-*H. pylori* seropositive groups. We calculated the ratio of the ORs and p-heterogeneity for subtypes of gastric cancer in case-case comparisons [57]. The associations with histologic subtypes were assessed in both the Cox proportional hazards model and in the logistic models.

The effects of changes between baseline and 3-year follow-up SPGI within each ABCD group were analyzed using logistic regression. The 3-year follow-up measurements were available for 3,462 subjects, approximately 97.4% of the participants with both baseline SPGI and *H. pylori* serology information. Lag analyses were conducted to estimate marker associations with incident gastric cancer occurring less than 5 years, 5–10 years, and more than 10 years after enrollment. Sensitivity analyses were performed by: 1) excluding

overlapping and unspecified subsites for noncardia cancer, 2) limiting to only cancer-free controls and 3) using a previously defined gastric cancer nested case-control set [45]. To address the concern for potential bias in case ascertainment due to pepsinogen measurement, two sensitivity analyses were performed. First, a log-rank test assessed whether gastric cancer incidence overall differed between the participants with *vs.* without SPGI. Second, a chi-square test assessed difference in early (IA, IB) *vs.* late (II–IV) stage distributions of these incident cancers.

For all analyses, except for the log-rank model, minimally adjusted models included age at randomization and type of assigned intervention in order to account for the experimental study design. Based on known or suspected associations with gastric cancer risk, additional covariates for the full models included Body Mass Index (BMI; kg/m^2, continuous), pack years of smoking (continuous), alcohol drinking (g/day, continuous), highest level of education (categorical), fruit intake (g/day), vegetable intake (g/day). The fully adjusted models excluded approximately 5% of subjects who did not have information on dietary factors.

Statistical analyses were conducted using SAS 9.3 (SAS Institute Inc, Cary, NC). All P values were two-sided, and were considered significant for $P<0.05$.

RESULTS

The baseline characteristics of the total cohort and gastric cancer cases, and subjects with both *H. pylori* serology and pepsinogen measurements in subset analysis are presented in Table 1. Within the cohort analysis, cancer cases had more years of smoking and lower consumption of fruit and vegetables. Low SPGI was more common among gastric cancer cases than controls. In the subset analysis, gastric cancer cases consumed less vegetables and showed a higher prevalence of low SPGI. All other variables showed no difference between the cases and controls.

Table 2 shows the association of low baseline SPGI with subsequent gastric cancer risk. In the fully adjusted Cox model (model 2), low SGPI at baseline conferred 2.7-fold higher risk for gastric cancer (95% CI 1.99–3.61) as compared to normal SPGI. Analysis by anatomical subsite showed HRs of 2.95 (95% CI 2.11–4.12) for noncardia and 2.01 (95% CI 1.05–3.83) for cardia gastric cancers. HRs were significantly elevated for intestinal-type gastric cancer (HR 2.57, 95% CI 1.57–4.21), but not for diffuse-type gastric cancer (HR 0.92, 95% CI 0.33–2.55).

In the subset with both SPGI and *H. pylori* serology information available, Groups B, C, D had significantly elevated risks for total gastric cancer as compared to group A, with ORs (95% CI) of 1.79 (1.21–2.64), 3.85 (2.36–6.28), and 6.35 (2.20–18.34), respectively, P_{trend} <0.0001 (Table 3). ORs (95% CI) restricted to noncardia gastric cancer were higher for groups B, C and D (P_{trend} <0.0001), 3.59 (2.02–6.39), 7.49 (3.84–14.61), and 16.55 (5.26–52.04) respectively. Associations with cardia gastric cancer were not statistically significant for any group, nor were P_{trends} statistically significant.

H. pylori titer was not associated with risk of noncardia gastric cancer either within group B, the normal SPGI and *H. pylori* seropositive group ($P_{heterogeneity}$=0.23), or within group C, the low SPGI and anti-*H. pylori* seropositive group ($P_{heterogeneity}$=0.52) (Table 4). On the other hand, the CagA seropositive group showed significantly higher OR (4.34, 95% CI 2.41–7.79) than the CagA seronegative group OR (2.45, 95% CI 1.23–4.88) within group B ($P_{heterogeneity}$ =0.01). There was no significant difference by CagA status in group C ($P_{heterogeneity}$ =0.06).

The trend across groups was statistically significant in intestinal-type gastric cancer (P_{trend} <0.0001) but not in diffuse-type gastric cancer in multivariate analysis (P_{trend} =0.25) (Table 5).

Gastric cancer risks associated with 3-year change of SPGI are shown in Figure 1. Within groups B and C, gastric cancer ORs did not significantly differ between individuals with normal *vs*. low SPGI at follow-up.

In a lag analysis of prediagnostic SPGI and subsequent gastric cancer risk, HR was highest within 5 years after baseline (4.73, 95% CI 1.88–11.88). The estimate is accentuated in noncardia and intestinal-type gastric cancers with HR (95% CI) of 5.97 (2.15–16.56) and 5.63 (1.30–24.32), respectively (Supporting Information Table 1).

Similarly, in the combined analysis of SPGI and anti-*H. pylori* seropositivity, group D, which is mainly the association of low SPGI (anti-*H. pylori* seronegative group), had the highest OR when restricted to cancers diagnosed < 5 years after enrollment (OR 16.93, 95% CI 2.85–100.43). The OR decreased to 5.98 (95% CI 1.24–28.93) in lag 5–10 years and 3.16 (95% CI 0.39–25.67) in lag > 10 years (Supporting Information Figure 1). On the other hand, when SPGI was normal the association of anti-*H. pylori* seropositivity was lowest in lag < 5 years with OR 0.64 (95% CI 0.22–1.91) and increased to OR 1.58 (95% CI 0.86–2.90) in lag 5–10 years and OR 2.43 (95supp% CI 1.38–4.30) in lag > 10 years.

Three separate sensitivity analyses restricting the noncardia cases excluding overlapping and unspecified site (Supporting Information Table 2), using only non-cancer controls from subset of the cohort with both SPGI and *H. pylori* serology information available from previous nested case-control sets (Supporting Information Figure 2), and testing within one nested case-control set for gastric cancer risk (Supporting Information Figure 3) did not change the risk estimates or the trend of risk.

Based on the log-rank test, there was no significant difference in gastric cancer incidence between those with SPGI testing and those without (p=0.38). Furthermore, the fraction of early gastric cancer did not differ between these two groups (p=0.19).

DISCUSSION

We report the largest study of the association of SPGI and *H. pylori* serology with gastric cancer risk in a Western population. Our analyses were based on combined marker categories similar to the Japanese “ABC(D)” method which defines low pepsinogen as PGI ≤ 70ng/ml and PGI/PG II ≤ 3.

Despite the difference in definition of low pepsinogen, the risk estimates for noncardia gastric cancer derived from our study are similar to estimates for overall gastric cancer from previous studies in Asia, where cardia cancer is relatively uncommon [58–62] (Supporting Information Table 3).

There are only limited studies that further stratify *H. pylori* seropositivity within ABCD groups in relation to gastric cancer risk. In our study, titer of anti-*H. pylori* antibodies was not associated with level of risk among participants with normal SPGI status (i.e., group B). Among individuals with low SPGI status (i.e., group C), the non-significantly higher OR we found for low anti-*H. pylori* antibody titer may reflect longer exposure to *H. pylori* infection and more severe disease progression leading to diminished antibody production [63]. Previous reports in Asian populations have been inconsistent, associating increased gastric cancer risk with high antibody titer in some studies [59, 64] and with low titer in others [33, 65]. To the best of our knowledge, this study is the first to investigate *H. pylori* antibody titer in relation to SPGI status in a Western population.

Mucosal atrophy represents the intermediate outcome in the causal pathway from *H. pylori* infection to gastric cancer. Antibodies to CagA have been associated with higher risk of gastric cancer among *H. pylori* seropositive populations [34], presumably because CagA positive *H. pylori* strains cause more severe mucosal damage than negative strains. We found anti-CagA seropositivity was associated with gastric cancer risk among individuals with normal pepsinogen (group B), but not with low pepsinogen (group C). Thus, our data implies that CagA-positive infection confers no additional risk once mucosal damage has occurred.

Another point to note is the assessment of the usefulness of repeated measurement of SPGI in predicting gastric cancer risk (Figure 1). Our analysis shows that a 3-year follow-up of SPGI does not differentiate risk within baseline categories of B and C. The current analysis cannot provide evidence for other follow-up intervals but, in our lag analysis for prediagnostic SPGI, the association of SPGI with gastric cancer was highest in the first five years prior to diagnosis (Supporting Information Table 1). Therefore, we cautiously conclude that repeated measurement may not be necessary for SPGI and *H. pylori* serology assessed at baseline. For individuals with normal levels of SPGI, measurement of anti-CagA antibodies may provide better discrimination of gastric cancer risk.

The current Cox regression analysis associated low SPGI with gastric cancer risk, in line with our previous nested case-control analysis of cases diagnosed through April 2006 [38]. We found a stronger association within shorter time intervals with the highest HR observed for cancers occurring within 5 years after baseline (Supporting Information Table 1). Our findings replicate previous studies (Supporting Information Table 4), not only regarding the significant association of low SPGI with gastric cancer overall, but also higher risk estimates for noncardia subsites and intestinal-type histology.

The major strength of our study is the use of prediagnostic samples analyzed for SPGI and anti-*H. pylori* seropositivity at baseline, with a long follow-up period. Another strength is that we have the largest number of cases (n=329) ever studied in a Western population,

allowing us for a more comprehensive analysis across group categories and anatomical and histological subtypes, as well as accounting for potential confounder effects. While this study has a large number of gastric cancer cases, sub-group analyses are likely to be underpowered.

Although both men (as compared to women) and smokers (as compared to non-smokers) have higher risks of gastric cancer, our findings based on male smokers may have limited generalizability. Nonetheless, the risk estimates were similar to the previous studies. Another possible limitation of our study is that the SPGI measurement selected high-risk individuals for further screening with endoscopy, but we did not find evidence of excess cancer diagnoses among the screened fraction of the cohort compared to those who were unscreened. Moreover, the analysis incorporating *H. pylori* serology information was based on availability of data from prior nested case-control datasets for various cancers, but the results were unchanged by restriction to the matched gastric cancer case-control set. We also did not gather information about possible *H. pylori* eradication therapy post-enrollment.

In conclusion, we found that the joint consideration of SPGI and anti-*H. pylori* whole cell and CagA seropositivity is a potentially useful predictor for the development of gastric cancer, especially noncardia gastric cancer, in a large population study of a Western population. This noninvasive and relatively inexpensive method could be used to identify high risk individuals for definitive evaluation by endoscopy and/or to advise on *H. pylori* eradication. Risk stratification could potentially be improved by incorporating other factors, such as an individual's genetic and epigenetic information [66]. Further studies of cost-effectiveness and of risk-specific screening intervals are warranted for development of personalized screening guidelines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the Intramural Research Program, Division of Cancer Epidemiology and Genetics, US National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was supported by funding provided by the Intramural Research Program of the National Cancer Institute and US Public Health Service contracts HHSN261201500005C.

References

1. Schistosomes liver flukes and Helicobacter pylori. IARC Monogr Eval Carcinog Risks Hum; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans; Lyon. 7–14 June 1994; 1994. p. 1-241.
2. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med. 2001; 345(11):784–9. [PubMed: 11556297]
3. Danesh J. Helicobacter pylori infection and gastric cancer: systematic review of the epidemiological studies. Aliment Pharmacol Ther. 1999; 13(7):851–6. [PubMed: 10383517]
4. Kuipers EJ, Uyterlinde AM, Pena AS, et al. Long-term sequelae of Helicobacter pylori gastritis. Lancet. 1995; 345(8964):1525–8. [PubMed: 7791437]
5. Correa P, Piazuelo MB. Helicobacter pylori Infection and Gastric Adenocarcinoma. US Gastroenterol Hepatol Rev. 2011; 7(1):59–64. [PubMed: 21857882]

6. Kosunen TU, Seppala K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of Helicobacter pylori. Lancet. 1992; 339(8798):893–5. [PubMed: 1348298]
7. Samloff IM. Immunologic studies of human group I pepsinogens. J Immunol. 1971; 106(4):962–8. [PubMed: 4396533]
8. Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterology. 1982; 83(1 Pt 2):204–9. [PubMed: 7084603]
9. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. Gastroenterology. 1998; 114(6):1169–79. [PubMed: 9609753]
10. Huang YK, Yu JC, Kang WM, et al. Significance of Serum Pepsinogens as a Biomarker for Gastric Cancer and Atrophic Gastritis Screening: A Systematic Review and Meta-Analysis. PLoS One. 2015; 10(11):e0142080. [PubMed: 26556485]
11. Aromaa A, Kosunen TU, Knekt P, et al. Circulating anti-Helicobacter pylori immunoglobulin A antibodies and low serum pepsinogen I level are associated with increased risk of gastric cancer. Am J Epidemiol. 1996; 144(2):142–9. [PubMed: 8678045]
12. Knekt P, Teppo L, Aromaa A, Rissanen H, Kosunen TU. Helicobacter pylori IgA and IgG antibodies, serum pepsinogen I and the risk of gastric cancer: changes in the risk with extended follow-up period. Int J Cancer. 2006; 119(3):702–5. [PubMed: 16496404]
13. Vohlonen I, Pukkala E, Malila N, et al. Risk of gastric cancer in Helicobacter pylori infection in a 15-year follow-up. Scand J Gastroenterol. 2016; 51(10):1159–64. [PubMed: 27338132]
14. Varis K, Sipponen P, Laxen F, et al. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Helsinki Gastritis Study Group. Scand J Gastroenterol. 2000; 35(9):950–6. [PubMed: 11063155]
15. Varis K, Taylor PR, Sipponen P, et al. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effect of supplementation with alpha-tocopherol and beta-carotene. The Helsinki Gastritis Study Group. Scand J Gastroenterol. 1998; 33(3):294–300. [PubMed: 9548624]
16. Lomba-Viana R, Dinis-Ribeiro M, Fonseca F, Vieira AS, Bento MJ, Lomba-Viana H. Serum pepsinogen test for early detection of gastric cancer in a European country. Eur J Gastroenterol Hepatol. 2012; 24(1):37–41. [PubMed: 21989121]
17. Oishi Y, Kiyohara Y, Kubo M, et al. The serum pepsinogen test as a predictor of gastric cancer: the Hisayama study. Am J Epidemiol. 2006; 163(7):629–37. [PubMed: 16443800]
18. Ren JS, Kamangar F, Qiao YL, et al. Serum pepsinogens and risk of gastric and oesophageal cancers in the General Population Nutrition Intervention Trial cohort. Gut. 2009; 58(5):636–42. [PubMed: 19136509]
19. Kuipers EJ, Pena AS, van Kamp G, et al. Seroconversion for Helicobacter pylori. Lancet. 1993; 342(8867):328–31. [PubMed: 8101585]
20. Weck MN, Gao L, Brenner H. Helicobacter pylori infection and chronic atrophic gastritis: associations according to severity of disease. Epidemiology. 2009; 20(4):569–74. [PubMed: 19404195]
21. Miki K. Gastric cancer screening by combined assay for serum anti-Helicobacter pylori IgG antibody and serum pepsinogen levels - “ABC method”. Proc Jpn Acad Ser B Phys Biol Sci. 2011; 87(7):405–14.
22. Correa P. Serum pepsinogens in gastric cancer screening. Dig Dis Sci. 2010; 55(8):2123–5. [PubMed: 20428942]
23. Sasazuki S. The ABC Method and Gastric Cancer: Evidence From Prospective Studies. J Epidemiol. 2016; 26(12):611–612. [PubMed: 27546709]
24. Terasawa T, Nishida H, Kato K, et al. Prediction of gastric cancer development by serum pepsinogen test and Helicobacter pylori seropositivity in Eastern Asians: a systematic review and meta-analysis. PLoS One. 2014; 9(10):e109783. [PubMed: 25314140]
25. Chen XZ, Schottker B, Castro FA, et al. Association of helicobacter pylori infection and chronic atrophic gastritis with risk of colonic, pancreatic and gastric cancer: A ten-year follow-up of the ESTHER cohort study. Oncotarget. 2016; 7(13):17182–93. [PubMed: 26958813]

26. Hansen S, Vollset SE, Derakhshan MH, et al. Two distinct aetiologies of cardia cancer; evidence from premorbid serological markers of gastric atrophy and Helicobacter pylori status. Gut. 2007; 56(7):918–25. [PubMed: 17317788]
27. Kurilovich S, Belkovets A, Reshetnikov O, et al. Stomach-specific Biomarkers (GastroPanel) Can Predict the Development of Gastric Cancer in a Caucasian Population: A Longitudinal Nested Case-Control Study in Siberia. Anticancer Res. 2016; 36(1):247–53. [PubMed: 26722050]
28. Tu H, Sun L, Dong X, et al. A Serological Biopsy Using Five Stomach-Specific Circulating Biomarkers for Gastric Cancer Risk Assessment: A Multi-Phase Study. Am J Gastroenterol. 2017; 112(5):704–715. [PubMed: 28323271]
29. Cavaleiro-Pinto M, Peleteiro B, Lunet N, Barros H. Helicobacter pylori infection and gastric cardia cancer: systematic review and meta-analysis. Cancer Causes Control. 2011; 22(3):375–87. [PubMed: 21184266]
30. Camargo MC, Anderson WF, King JB, et al. Divergent trends for gastric cancer incidence by anatomical subsite in US adults. Gut. 2011; 60(12):1644–9. [PubMed: 21613644]
31. Song M, Kang D, Yang JJ, et al. Age and sex interactions in gastric cancer incidence and mortality trends in Korea. Gastric Cancer. 2015; 18(3):580–9. [PubMed: 25091081]
32. Hu B, El Hajj N, Sittler S, Lammert N, Barnes R, Meloni-Ehrig A. Gastric cancer: Classification, histology and application of molecular pathology. J Gastrointest Oncol. 2012; 3(3):251–61. [PubMed: 22943016]
33. Tatemichi M, Sasazuki S, Inoue M, Tsugane S, Group JS. Clinical significance of IgG antibody titer against Helicobacter pylori. Helicobacter. 2009; 14(3):231–6. [PubMed: 19702853]
34. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology. 2003; 125(6):1636–44. [PubMed: 14724815]
35. Orditura M, Galizia G, Sforza V, et al. Treatment of gastric cancer. World J Gastroenterol. 2014; 20(7):1635–49. [PubMed: 24587643]
36. Rosati G, Ferrara D, Manzione L. New perspectives in the treatment of advanced or metastatic gastric cancer. World J Gastroenterol. 2009; 15(22):2689–92. [PubMed: 19522017]
37. Min YW, Min BH, Lee JH, Kim JJ. Endoscopic treatment for early gastric cancer. World J Gastroenterol. 2014; 20(16):4566–73. [PubMed: 24782609]
38. Murphy G, Kamangar F, Dawsey SM, et al. The relationship between serum ghrelin and the risk of gastric and esophagogastric junctional adenocarcinomas. J Natl Cancer Inst. 2011; 103(14):1123–9. [PubMed: 21693726]
39. The ATBC Cancer Prevention Study Group. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods participant characteristics, and compliance. Ann Epidemiol. 1994; 4(1):1–10. [PubMed: 8205268]
40. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 1994; 330(15):1029–35. [PubMed: 8127329]
41. Stolzenberg-Solomon RZ, Blaser MJ, Limburg PJ, et al. Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. J Natl Cancer Inst. 2001; 93(12):937–41. [PubMed: 11416115]
42. Limburg PJ, Stolzenberg-Solomon RZ, Colbert LH, et al. Helicobacter pylori seropositivity and colorectal cancer risk: a prospective study of male smokers. Cancer Epidemiol Biomarkers Prev. 2002; 11(10 Pt 1):1095–9. [PubMed: 12376513]
43. Abnet CC, Kamangar F, Dawsey SM, et al. Tooth loss is associated with increased risk of gastric non-cardia adenocarcinoma in a cohort of Finnish smokers. Scand J Gastroenterol. 2005; 40(6): 681–7. [PubMed: 16036528]
44. Cook MB, Dawsey SM, Diaw L, et al. Serum pepsinogens and Helicobacter pylori in relation to the risk of esophageal squamous cell carcinoma in the alpha-tocopherol, beta-carotene cancer prevention study. Cancer Epidemiol Biomarkers Prev. 2010; 19(8):1966–75. [PubMed: 20647397]
45. Cook MB, Kamangar F, Weinstein SJ, et al. Iron in relation to gastric cancer in the Alpha-tocopherol, Beta-carotene Cancer Prevention Study. Cancer Epidemiol Biomarkers Prev. 2012; 21(11):2033–42. [PubMed: 23001240]

46. Koshiol J, Flores R, Lam TK, et al. Helicobacter pylori seropositivity and risk of lung cancer. PLoS One. 2012; 7(2):e32106. [PubMed: 22384154]
47. Yu G, Murphy G, Michel A, et al. Seropositivity to Helicobacter pylori and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2013; 22(12):2416–9. [PubMed: 24089457]
48. Murphy G, Michel A, Taylor PR, et al. Association of seropositivity to Helicobacter species and biliary tract cancer in the ATBC study. Hepatology. 2014; 60(6):1963–71. [PubMed: 24797247]
49. Korhonen P, Malila N, Pukkala E, Teppo L, Albanes D, Virtamo J. The Finnish Cancer Registry as follow-up source of a large trial cohort--accuracy and delay. Acta Oncol. 2002; 41(4):381–8. [PubMed: 12234031]
50. Lauren P. The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. An Attempt at a Histo-Clinical Classification. Acta Pathol Microbiol Scand. 1965; 64:31–49. [PubMed: 14320675]
51. Samloff IM. Pepsinogens I and II: purification from gastric mucosa and radioimmunoassay in serum. Gastroenterology. 1982; 82(1):26–33. [PubMed: 7053333]
52. Blaser MJ, Perez-Perez GI, Kleanthous H, et al. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. 1995; 55(10):2111–5. [PubMed: 7743510]
53. Michel A, Waterboer T, Kist M, Pawlita M. Helicobacter pylori multiplex serology. Helicobacter. 2009; 14(6):525–35. [PubMed: 19889070]
54. 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(7):2973–80. [PubMed: 19318564]
55. Sung H, Camargo MC, Yu K, et al. Association of 4p14 TLR locus with antibodies to Helicobacter pylori. Genes Immun. 2015; 16(8):567–70. [PubMed: 26312625]
56. Shiota S, Matsunari O, Watada M, Yamaoka Y. Serum Helicobacter pylori CagA antibody as a biomarker for gastric cancer in east-Asian countries. Future Microbiol. 2010; 5(12):1885–93. [PubMed: 21155667]
57. Wang M, Spiegelman D, Kuchiba A, et al. Statistical methods for studying disease subtype heterogeneity. Stat Med. 2016; 35(5):782–800. [PubMed: 26619806]
58. Charvat H, Sasazuki S, Inoue M, et al. Prediction of the 10-year probability of gastric cancer occurrence in the Japanese population: the JPHC study cohort II. Int J Cancer. 2016; 138(2):320–31. [PubMed: 26219435]
59. Yoshida T, Kato J, Inoue I, et al. Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and Helicobacter pylori antibody titer. Int J Cancer. 2014; 134(6):1445–57. [PubMed: 24009139]
60. Ikeda F, Shikata K, Hata J, et al. Combination of Helicobacter pylori Antibody and Serum Pepsinogen as a Good Predictive Tool of Gastric Cancer Incidence: 20-Year Prospective Data From the Hisayama Study. J Epidemiol. 2016; 26(12):629–636. [PubMed: 27265836]
61. Mizuno S, Miki I, Ishida T, et al. Prescreening of a high-risk group for gastric cancer by serologically determined Helicobacter pylori infection and atrophic gastritis. Dig Dis Sci. 2010; 55(11):3132–7. [PubMed: 20204698]
62. Sasazuki S, Inoue M, Iwasaki M, et al. Effect of Helicobacter pylori infection combined with CagA and pepsinogen status on gastric cancer development among Japanese men and women: a nested case-control study. Cancer Epidemiol Biomarkers Prev. 2006; 15(7):1341–7. [PubMed: 16835334]
63. Kishikawa H, Kimura K, Takarabe S, Kaida S, Nishida J. Helicobacter pylori Antibody Titer and Gastric Cancer Screening. Dis Markers. 2015; 2015:156719. [PubMed: 26494936]
64. Watanabe M, Kato J, Inoue I, et al. Development of gastric cancer in nonatrophic stomach with highly active inflammation identified by serum levels of pepsinogen and Helicobacter pylori antibody together with endoscopic rugal hyperplastic gastritis. Int J Cancer. 2012; 131(11):2632–42. [PubMed: 22383377]
65. Suzuki G, Cullings H, Fujiwara S, et al. Low-positive antibody titer against Helicobacter pylori cytotoxin-associated gene A (CagA) may predict future gastric cancer better than simple seropositivity against H. pylori CagA or against H. pylori. Cancer Epidemiol Biomarkers Prev. 2007; 16(6):1224–8. [PubMed: 17548689]

66. Shah S, Bonder MJ, Marioni RE, et al. Improving Phenotypic Prediction by Combining Genetic and Epigenetic Associations. Am J Hum Genet. 2015; 97(1):75–85. [PubMed: 26119815]

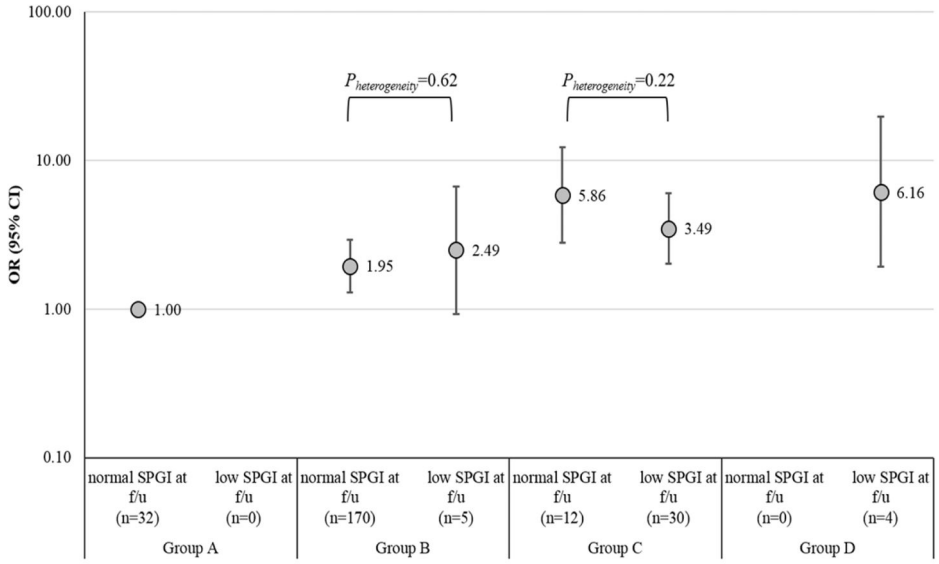

Figure 1.

Association of prediagnostic and 3-year follow-up measurement of serum pepsinogen I and subsequent gastric cancer risk in the ATBC Study

Abbreviations: ATBC – Alpha-Tocopherol, Beta-Carotene Cancer Prevention, f/u – follow-up, OR – odds ratio, CI – confidence interval, SPGI - Serum pepsinogen I,

Group A: *H. pylori* (−) and normal SPGI, Group B: *H. pylori* (+) and normal SPGI, Group C: *H. pylori* (+) and low SPGI, Group D: *H. pylori* (−) and low SPGI

Model adjusted for age at randomization, type of intervention, pack years of smoking, alcohol drinking, education, fruit intake, vegetable intake

* Numbers in parentheses indicate gastric cancer cases in each group at follow-up

Cases diagnosed before follow-up measurements (≤3 years) were excluded from the analysis (n=5)

Table 1

Baseline characteristics for ATBC study participants who did or did not develop gastric cancer in the overall study cohort and in the subgroup with both *H. pylori* serology and serum pepsinogen I measurements

	Cohort analysis (n=21,895)				Subset analysis (n=3,555)			
	entire cohort		gastric cancer		controls		gastric cancer	
N	21,895		329		3,291		264	
Age, y (SD)	57.0	(5.0)	57.3	(4.9)	58.2	(4.9)	58.0	(4.9)
Body mass index, kg/m^2 (SD)	26.3	(3.7)	26.5	(3.7)	26.2	(3.7)	26.5	(3.7)
Pack years of smoking (SD)	36.3	(18.0)	37.6	(19.0)	36.8	(17.9)	38.7	(19.9)
Alcohol intake, g (SD)	17.3	(20.3)	17.3	(20.5)	17.3	(21.1)	18.1	(21.7)
Daily fruit intake, g (SD)	221.4	(196.5)	205.0	(161.8)	217.9	(185.9)	201.3	(157.6)
Daily vegetable intake, g (SD)	297.7	(113.8)	289.2	(106.3)	291.5	(111.2)	284.9	(103.8)
Some high school, college, or technical school (%)	14,772	67.5	223	67.8	2,222	67.5	177	67.1
Low SPGI (< 25μg/l), n (%)	1,791	8.2	58	17.6	298	9.1	51	19.3
H. pylori seropositivity, n (%)					2,536	77.1	224	84.9

Abbreviations: ATBC - Alpha-Tocopherol, Beta-Carotene Cancer Prevention, SD - Standard deviation, SPGI – serum pepsinogen I

Aliment Pharmacol Ther. Author manuscript; available in PMC 2019 February 01.

Table 2

Association of prediagnostic low serum pepsinogen I and subsequent gastric cancer risk in the ATBC Study

			model 1		model 2	
	number of cases	**person years**	**HR**	**95% CI**	**HR**	**95% CI**
Gastric cancer total	329	5,724	2.53	1.89–3.37	2.68	1.99–3.61
Anatomical subsite						
Noncardia	245	4,274	2.76	1.99–3.83	2.95	2.11–4.12
Cardia	84	1,449	1.93	1.04–3.59	2.01	1.05–3.83
Lauren classification						
Intestinal	115	1,759	2.33	1.43–3.81	2.57	1.57–4.21
Diffuse	63	1,101	1.04	0.41–2.61	0.92	0.33–2.55
Others	151	2,864	3.49	2.35–5.17	3.74	2.49–5.61

Abbreviations: ATBC - Alpha-Tocopherol, Beta-Carotene Cancer Prevention, HR – hazard ratio, CI – confidence interval

model 1: adjusted for age at randomization and type of intervention

model 2: model 1 + body mass index, pack years of smoking, alcohol drinking, education, fruit intake, and vegetable intake

Table 3

Combination of anti-*H. pylori* seropositivity and pepsinogen I on risk of gastric cancer in ATBC Study

	control (n=3,291)		case (n=264)		model 1		model 2	
	N	%	N	%	OR	95% CI	OR	95% CI
Total Gastric Cancer (n=264)								
Group A	736	22.4	35	13.3	ref		ref	
Group B	2,257	68.6	178	67.4	1.70	1.17–2.48	1.79	1.21–2.64
Group C	279	8.5	46	17.4	3.71	2.32–5.93	3.85	2.36–6.28
Group D	19	0.6	5	1.9	5.94	2.09–16.92	6.35	2.20–18.34
p-trend					<0.0001		<0.0001	
Anatomical subsite								
Noncardia (n=197)								
Group A	736	22.4	14	7.1	ref		ref	
Group B	2,257	68.6	143	72.6	3.49	2.00–6.09	3.59	2.02–6.39
Group C	279	8.5	35	17.8	7.40	3.89–14.07	7.49	3.84–14.61
Group D	19	0.6	5	2.5	15.62	5.07–48.12	16.55	5.26–52.04
p-trend					<0.0001		<0.0001	
Cardia (n=67)								
Group A	736	22.4	21	31.3	ref		ref	
Group B	2,257	68.6	35	52.2	0.53	0.31–0.92	0.58	0.33–1.03
Group C	279	8.5	11	16.4	1.30	0.61–2.77	1.40	0.63–3.11
Group D	19	0.6	0	0.0	-		-	
p-trend					0.65		0.80	

Abbreviations: ATBC - Alpha-Tocopherol, Beta-Carotene Cancer Prevention, OR – odds ratio, CI – confidence interval, SPGI- Serum pepsinogen I,

Group A: *H. pylori* (−) and normal SPGI, Group B: *H. pylori* (+) and normal SPGI, Group C: *H. pylori* (+) and low SPGI, Group D: *H. pylori* (−) and low SPGI

model 1: adjusted for age at randomization and type of intervention

model 2: model 1 + body mass index, pack years of smoking, alcohol drinking, education, fruit intake, and vegetable intake

Table 4

Combination of anti-*H. pylori* titer and CagA seropositivity with pepsinogen I on risk of **noncardia gastric cancer** in ATBC Study

	control (n=3,291)		case (n=197)		model 1		model 2	
	N	%	N	%	OR	95% CI	OR	95% CI
Anti-*H. pylori antibody* titer in all noncardia cancer (n=197)								
Group A	736	22.4	14	7.1	ref		ref	
Group B + low titer *H. pylori*[b]	1,571	47.7	92	46.7	3.21	1.82–5.68	3.34	1.85–6.03
Group B + high titer *H. pylori*	686	20.8	51	25.9	4.15	2.27–7.58	4.17	2.23–7.79
Group C + low titer *H. pylori*[b]	186	5.7	25	12.7	7.96	4.03–15.73	8.09	4.00–16.37
Group C + high titer *H. pylori*	93	2.8	10	5.1	6.34	2.72–14.78	6.31	2.59–15.35
Group D	19	0.6	5	1.9	15.66	5.08–48.25	16.57	5.27–52.13
p-trend					<0.0001		<0.0001	
Anti-CagA seropositivity in all noncardia cancer (n=186)[a]								
Group A	736	24.1	14	7.5	ref		ref	
Group B + CagA (−)[c]	590	19.4	25	13.4	2.37	1.22–4.60	2.45	1.23–4.88
Group B + CagA (+)	1,451	47.6	110	59.1	4.18	2.38–7.36	4.34	2.41–7.79
Group C + CagA (−)[c]	46	1.5	11	5.9	14.14	6.04–33.11	13.33	5.47–32.47
Group C + CagA (+)	207	6.8	21	11.3	6.03	2.99–12.15	6.28	3.04–12.98
Group D	19	0.6	5	2.7	15.71	5.09–48.42	16.73	5.31–52.68
p-trend					<0.0001		<0.0001	

Abbreviations: ATBC - Alpha-Tocopherol, Beta-Carotene Cancer Prevention, OR – odds ratio, CI – confidence interval, SPGI- Serum pepsinogen I,

Group A: *H. pylori* (−) and normal SPGI, Group B: *H. pylori* (+) and normal SPGI, Group C: *H. pylori* (+) and low SPGI, Group D: *H. pylori* (−) and low SPGI

model 1: adjusted for age at randomization and type of intervention

model 2: model 1 + body mass index, pack years of smoking, alcohol drinking, education, fruit intake, and vegetable intake

[a] excluded with missing CagA status (11 cases & 242 controls) or *H. pylori* negative/CagA positive cases (157 controls)

[b] Within group B high titer vs. low titer *H. pylori* ($P_{heterogeneity}$ = 0.23), group C high titer vs. low titer *H. pylori* ($P_{heterogeneity}$ =0.52) in model 2

[c] Within group B CagA (+) vs. CagA (−) ($P_{heterogeneity}$ = 0.01), group C CagA (+) vs. CagA (−) ($P_{heterogeneity}$ =0.06) in model 2

Table 5

Combination of anti-*H. pylori* seropositivity and pepsinogen I on risk of **noncardia gastric cancer** by Lauren classification in ATBC Study

	control (n=3,291)		case (n=197)		model 1		model 2	
	N	%	N	%	OR	95% CI	OR	95% CI
Intestinal Type Gastric Cancer (n=69)								
Group A	736	22.4	5	7.3	ref		ref	
Group B	2,257	68.6	51	73.9	3.47	1.37–8.73	4.23	1.52–11.80
Group C	279	8.5	12	17.4	7.02	2.42–20.39	8.65	2.72–27.50
Group D	19	0.6	1	1.5	8.86	0.98–80.45	11.95	1.25–114.23
p-trend					<0.0001		<0.0001	
Diffuse Type Gastric Cancer (n=49)								
Group A	736	22.4	5	10.2	ref		ref	
Group B	2,257	68.6	40	81.6	2.69	1.05–6.86	2.57	1.00–6.59
Group C	279	8.5	4	8.2	2.27	0.60–8.64	1.74	0.41–7.46
Group D	19	0.6	0	0.0	-		-	
p-trend					0.15		0.25	
Other Type Gastric Cancer (n=79)								
Group A	736	22.4	4	5.1	ref		ref	
Group B	2,257	68.6	52	65.8	4.53	1.63–12.58	4.25	1.52–11.84
Group C	279	8.5	19	24.1	14.69	4.90–44.01	13.92	4.61–42.09
Group D	19	0.6	4	5.1	46.21	10.60–201.47	43.81	9.89–194.11
p-trend					<0.0001		<0.0001	

Abbreviations: ATBC - Alpha-Tocopherol, Beta-Carotene Cancer Prevention, OR – odds ratio, CI – confidence interval, SPGI- Serum pepsinogen I

Group A: *H. pylori* (−) and normal SPGI, Group B: *H. pylori* (+) and normal SPGI, Group C: *H. pylori* (+) and low SPGI, Group D: *H. pylori* (−) and low SPGI

model 1: adjusted for age at randomization and type of intervention

model 2: model 1 + body mass index, pack years of smoking, alcohol drinking, education, fruit intake, vegetable intake

Aliment Pharmacol Ther. Author manuscript; available in PMC 2019 February 01.