Research Article

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Serologic Profile of Antiparietal Cell Antibodies, Pepsinogens, and *H. pylori* and Risk of Upper Gastrointestinal Cancer: A Nested Case–Control Study in China

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Abstract

Background: Autoimmune gastritis is understudied and possibly associated with gastric noncardia adenocarcinoma (GNCA) and esophageal squamous cell carcinoma (ESCC) in Western populations when it presents as pernicious anemia.

Methods: A nested case–control study within a Chinese cohort included 100 ESCC, 200 gastric cardia adenocarcinoma (GCA), and 200 GNCA cases diagnosed between 1986 and 2001 and 400 controls. Serostatus of antiparietal cell antibodies (APCA), *Helicobacter pylori* antibodies, and pepsinogens were measured using commercial kits and serum collected at baseline. We used logistic regression to calculate odds ratios (OR) and 95% confidence interval (CI) for associations between serologic biomarkers and cancer risk adjusted for numerous potential confounders.

Results: There was an average interval of 8 years between baseline blood draw and cancer diagnosis. The baseline prevalence of APCA seropositivity was 10.0% and 14.5% in subjects who developed GCA and GNCA, respectively. APCA seropositivity was inversely associated with later development of GCA (OR = 0.42; 95% CI, 0.24–0.75), but not significantly associated with later development of GNCA (OR = 0.82; 95% CI, 0.50–1.36) or ESCC (OR = 1.05; 95% CI, 0.58–1.88). APCA seropositivity was significantly associated with low pepsinogen I/II ratios (OR = 3.69; 95% CI, 1.66–8.21), and individuals with low pepsinogen I/II ratios who were seronegative for APCA had the highest risk of both GCA and GNCA.

Conclusions: APCA seropositivity measured years prior to diagnosis was associated with prevalent atrophic gastritis but inversely associated with incident GCA in this Chinese population.

Impact: APCA may contribute to a growing list of serologic markers that can improve risk stratification for gastric cancer.

Introduction

Gastric cancer and esophageal cancer rank as the 3rd and 6th most common causes of cancer death globally, among which 45% and 49% occur in China (http://gco.iarc.fr/today). Esophagogastric junctional adenocarcinoma (EGJA) or gastric cardia adenocarcinoma (GCA), tumors located in the top 2 to 3 cm of the stomach, have increased in the recent decades, and more than half (52%) of these cases occur in China (1, 2). The reason for the high incidence of upper gastrointestinal (UGI) cancer in China is largely unknown, and there may be significant differences in risk factors between Eastern and Western populations. One notable example is *Helicobacter pylori* (*H. pylori*), a bacterium associated with an increased risk of GCA in Asian populations, but not in Western populations (3–5). The notably low rates of Barrett's esophagus and esophageal adenocarcinoma in Asia also indicate different origins and etiologies of UGI cancers between Eastern and Western populations (6).

Chronic gastritis and atrophic gastritis are associated with an increased risk of gastric cancer. There are two main types of chronic gastritis: type A or autoimmune gastritis, and type B or bacterial gastritis (7). Autoimmune gastritis is generally corpus-restricted and results from an autoimmune condition where antibodies are generated against gastric parietal cells, so called antiparietal cell antibodies (APCA), leading to decreased numbers of functioning parietal cells (5, 8). Autoimmune gastritis is often asymptomatic until it presents as pernicious anemia, an autoimmune disease that results from a deficiency in intrinsic factor excretion and subsequent absorption of vitamin B12 (7, 9). Bacterial gastritis is mainly caused by chronic *H. pylori* infection (7, 10). *H. pylori* has been widely studied and recognized as an important carcinogen for gastric noncardia adenocarcinoma

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(GNCA; ref. 11). Atrophic gastritis occurs as a result of chronic gastritis and is clinically recognized as a risk factor for gastric cancer, and may also be a risk factor for esophageal squamous cell cancer (ESCC; ref. 12). Serologic markers such as lower pepsinogen (PG) I or low pepsinogen I/pepsinogen II (PG I/II) ratio have been explored in different populations and used to identify atrophy and estimate risks of UGI cancers (13–15).

Autoimmune gastritis has not been widely studied for its association with cancer. With the notable decline in prevalence of *H. pylori*, particularly in Western countries, autoimmune gastritis may be increasing and might be underdiagnosed in some parts of the world (7). The prevalence of APCA was reported to be 19.5% in a German population ages 50 to 74 years (16), and about 85% to 90% in people with pernicious anemia (17), a disease associated with a higher risk of GNCA and ESCC but not associated with GCA in Western populations (18–21). However, very few studies have explored the associations between APCA, rather than clinically diagnosed pernicious anemia, and the risk of UGI cancer, so that the risks of UGI cancers apportioned to different types of gastritis are not well understood.

Linxian, a county in the Taihang Mountain range of north central China, has some of the highest mortality rates of esophageal squamous cell carcinoma (ESCC) and GCA in the world, and also has moderate mortality rates of GNCA, and the reasons for these rates are not well understood (22). We sought to investigate the associations between various serologic biomarkers of gastritis, including APCA, PG I and II, and *H. pylori* serostatus, and the risk of ESCC, GCA, and GNCA in this high-risk Chinese population.

Materials and Methods

Design and follow-up of the Linxian General Population Nutrition Intervention Trial study

We designed a nested case–control study within the Linxian General Population Nutrition Intervention Trial (NIT) study. The NIT recruitment commenced in 1985 in Linxian, China. After a baseline questionnaire interview, a total of 29,584 eligible residents ages 40 to 69 years were enrolled and randomly assigned to receive daily supplements of different combinations of 9 nutrients grouped into 4 factors, or placebo, for 5.25 years (March 1986–May 1991) using a one-half 2^4 fractional factorial design (23). Both in the trial period (1986–1991) and the posttrial follow-up (1991–2016), village health workers checked vital status and the occurrence of incident cancers and ascertained causes of deaths for all participants by monthly home visits, supplemented by quarterly crosschecks of the data in the Linxian Cancer and Death Registries. Case ascertainment is considered complete and loss to follow-up was minimal ($n = 381$, or 1.3%; ref. 24). Diagnostic materials (case records, pathology slides, and X-rays) for cancer cases were reviewed and the diagnoses of cancer confirmed by a panel of American and Chinese experts (1986–1996) or senior Chinese diagnosticians from Beijing (1996–2016).

Ethics approval was obtained from the Institutional Review Boards of the Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS), and the U.S. NCI.

Case and control selection

By March 2001, 1,958 cases of ESCC, 1,089 cases of GCA, and 363 cases of GNCA were diagnosed in the NIT cohort. Gastric tumors were defined as GCA if they were in the most proximal 3 cm of the stomach, and as GNCA if they were distal to this region (6, 25). We previously conducted a case–cohort study to measure *H. pylori* serostatus and serum pepsinogens and their associations with subsequent risk of UGI cancers (3). In that study, we selected all 363 GNCA cases, a random sample of 300 ESCC and 600 GCA, and a random subcohort of 1,050 subjects from the entire NIT cohort (3). This study was performed on a subset of these cases and controls, consisting of a random sample of 100 ESCC, 200 GCA, 200 GNCA, and 400 random selected cancer-free controls (Fig. 1).

Blood sample collection and laboratory analyses

In 1985, before the intervention, each participant had 10 mL of blood drawn. Immediately after sample collection, serum specimens were separated, aliquoted, and stored frozen at −70°C until they were prepared for analysis.

Parietal cell antibodies serologic analysis. Serum parietal cell antibodies (#143 96; Phadia AB) were measured using ELISA and were performed according to the manufacturer's instructions by experienced technicians who were blinded to the subject's case–control status. Sample concentrations were calculated by averaging duplicate optical density measures. Duplicate results were strongly correlated with a Pearson's correlation coefficient of 0.94 and highly reproducible with a κ coefficient of 0.91.

Duplicate control samples provided with the kits and included on each assayed plate showed a coefficient of variation (CV) of 4%. In addition, 2 quality control samples aliquoted from pooled serum from CHCAMS were distributed among the APCA assay plates. The CV was 9% for these pooled samples. Standard concentration curves for each plate were derived from the Manufacturer's serially diluted calibrators using Sigma Plot (Systat Software Inc.). The seropositive cut point was defined as $\geq$10 U/mL based on the manufacturer's suggestion.

Pepsinogen and* H. pylori *serologic analyses. The serologic analyses of pepsinogens and *H. pylori* were measured in the same serum as APCA and have been reported previously (3, 13). In brief, serum PGI and PGII were measured by ELISA (Biohit) in duplicate, and the average value was used (13). Duplicate results were strongly correlated; Pearson's correlation coefficient was 0.995 for PGI and 0.997 for PGII. The QC strategies were similar to those used in the APCA analysis. The CVs for the duplicate control samples provided with the kits were 6.5% and 2.7% for PGI and PGII, and the CVs for the duplicate aliquots of pooled CHCAMS serum were 5.5% and 6.7% for PGI and PGII, respectively (13). IgG antibodies to whole cell (WC) and CagA *H. pylori* antigens were measured using ELISA (26). The seropositive cut points were defined as optical density ratios $\geq$1.0 for *H. pylori* WC antibodies and $\geq$0.35 for CagA antibodies. Individuals who were negative for both antibodies were classified as *H. pylori* seronegative, whereas individuals who were seropositive for either *H. pylori* WC or CagA antibodies were classified as *H. pylori* seropositive. This classification system was selected because culture-based studies have shown that individuals who are negative for *H. pylori* WC antibodies but positive for CagA antibodies are true positives (27). Many previous studies have used this classification system and these individuals have been targeted as a group at particularly high risk of gastric cancer in some risk stratification methods (3, 27). Therefore, we classified individuals with $WC^-/CagA^-$ as seronegative group, and further reclassified seropositive individuals as carrying

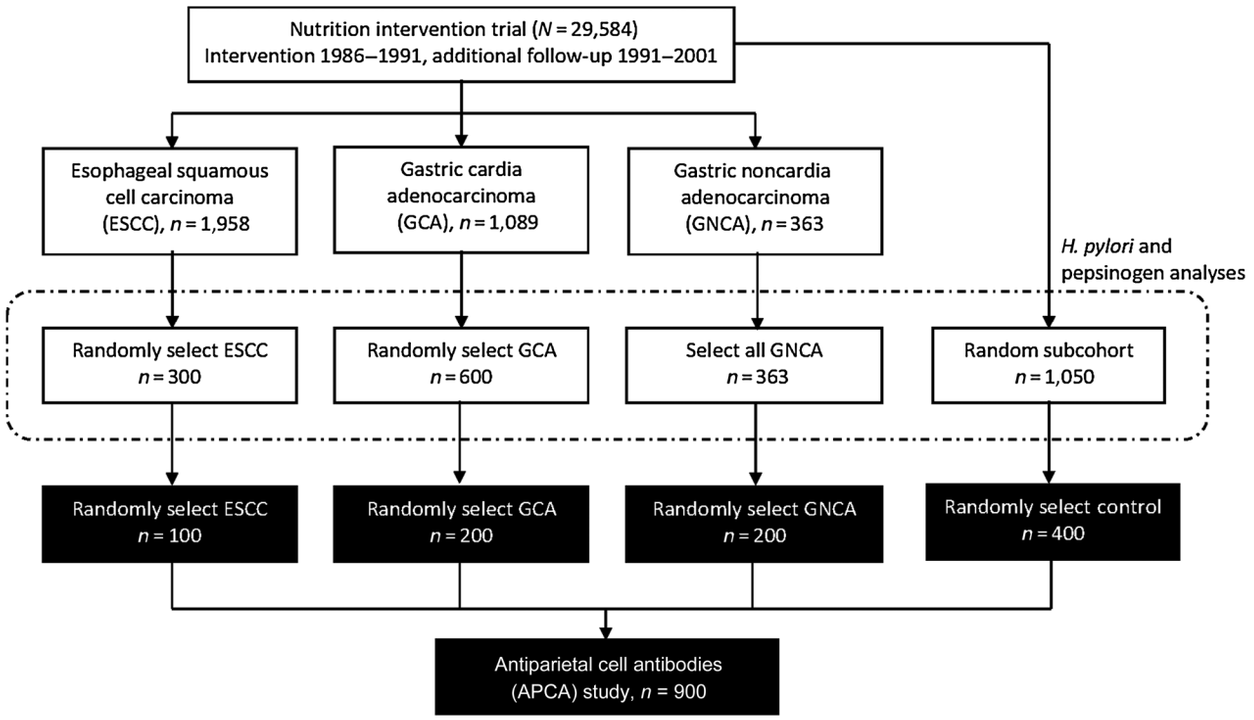

Figure 1.

Flowchart of the antiparietal cell antibodies study, which shows the selection method of ESCC, GCA, and GNCA cases and controls from the NIT cohort for the antiparietal cell antibodies study.

CagA-negative strains (WC^+, $CagA^-$) CagA-positive strains (WC^+ or WC^-, $CagA^+$), which is consistent with previous use of the Hp serology data in this cohort.

Statistical analyses

We used logistic regression models to calculate the odds ratios (OR) and 95% confidence intervals (95% CI) for the association between APCA and risk of UGI cancers. Potential confounders, including age, sex, history of smoking (ever smoked regularly for 6 months or longer), alcohol consumption (any consumption in the past 12 months), family history of UGI cancer, body mass index (BMI; kg/m^2), *H. pylori* CagA serostatus, and serum PGI/II ratio, were tabulated by case and control groups, and were included in multivariable-adjusted models.

Serum APCA was evaluated as a dichotomous variable (positive $\geq$10 and negative <10 U/mL, according to the manufacturer's instructions). Consistent with our previous analyses on PGI/II ratios and gastric cancer (13), we used a cutoff of 4 to define normal (>4) and low ($\leq$4) PGI/II ratio groups, and assumed subjects with low PGI/II ratios have atrophic gastritis. We then compared the risks of UGI cancers among those with normal and low PGI/II ratios by seropositivity to APCA. *H. pylori* status was also stratified into 3 subgroups according to *H. pylori* WC and CagA antibody serostatus as: *H. pylori* $WC^-/CagA^-$, *H. pylori* $WC^+/CagA^-$, and *H. pylori* WC^+ or $WC^-/CagA^+$. Accordingly, we ran models stratified by sex, baseline age group (<55, $\geq$55 years), 3 *H. pylori* status subgroups, and normal/low PGI/II ratios to further assess the associations. We used likelihood ratio tests to assess the potential interactive effects of sex, age group, *H. pylori* infection status, and group of normal/low PGI/II ratios on the association between seropositivity of APCA and risk of different subtypes of UGI cancers.

In sensitivity analyses, we excluded people who were diagnosed with cancer within the first 2 years to assess potential reverse causation; we additionally adjusted for the 4 intervention factors in the NIT: A (retinol/zinc), B (riboflavin/niacin), C (vitamin C/molybdenum), and D (selenium/vitamin E/β-carotene; Supplementary Table S1); and we performed a lag analysis by stratifying time from serum collection to cancer diagnosis (<5 years, 5–10 years, or >10 years; Supplementary Table S2). None of these analyses materially changed the resultant risk estimates, so we did not include them in the paper. Analyses were performed using SAS, version 9.3, software (SAS Institute, Inc.). An α level of less than 0.05 was considered statistically significant and all tests were two sided.

Results

Table 1 shows the distributions of demographic and potential risk factors by cancer case type and control groups. There was an average interval of 8 years between baseline blood draw and cancer diagnosis. Compared with healthy controls, participants who developed UGI cancer were more likely to be older, to be male, to be tobacco smokers, to have a lower BMI, to show serologic positivity for *H. pylori* antibodies, and to have a lower PG I/II ratio. Table 2 shows the associations between various risk factors and seropositivity of APCA and different subtypes of UGI cancers. In general, age, sex, *H. pylori* CagA seropositivity, and PG I/II ratio were associated with UGI cancers. Having a family

Table 1. Selected characteristics of cancer cases and controls from the Linxian General Population NIT study, China (1986–2001)

Variable	ESCC	GCA	GNCA	UGI cancer	Control	*P* value
Number	100	200	200	500	400	
Age, mean (SD)	52.8 (8.3)	54.5 (8.1)	56.1 (7.8)	54.8 (8.1)	51.1 (8.7)	<0.0001
Sex, male, *n* (%)	46 (46.0)	117 (58.5)	133 (66.5)	296 (59.2)	171 (42.8)	<0.0001
Tobacco smoking[a], *n* (%)	34 (34.0)	74 (37.2)	95 (47.5)	203 (40.7)	125 (31.3)	0.004
Alcohol drinking[b], *n* (%)	25 (25.0)	39 (19.6)	45 (22.5)	109 (21.8)	96 (24.1)	0.4
Family history of UGI cancer, *n* (%)	36 (36.0)	76 (38.2)	63 (31.5)	175 (35.1)	116 (29.1)	0.06
BMI, mean (SD)	21.7 (2.4)	21.8 (2.3)	21.4 (2.2)	21.6 (2.3)	22.0 (2.5)	0.01
H. pylori seropositivity, *n* (%)	77 (77.0)	153 (76.5)	145 (72.5)	375 (75.0)	275 (68.8)	0.04
H. pylori CagA seropositivity, *n* (%)	58 (58.0)	149 (74.5)	119 (59.5)	326 (65.2)	223 (55.8)	0.004
PG I/II ratio[c], mean (SD)	9.9 (5.9)	8.2 (5.2)	9.4 (10.2)	9.0 (7.7)	11.5 (10.6)	<0.0001

[a]Tobacco smoking was defined as ever smoking cigarettes for 6 or more months.
[b]Alcohol drinking was defined as any alcoholic beverage consumption in the last 12 months.
[c]PG I/II ratio, pepsinogen I/pepsinogen II ratio.

history of UGI cancer was associated with risk of GCA, and BMI was inversely associated with risk of GNCA. People with low (≤4) PG I/II ratios (25.8%) and the alcohol drinkers (19.0%) had significantly higher prevalence of APCA than other people. Accordingly, alcohol drinking (OR = 1.79; 95% CI, 1.13–2.83) and a low PG I/II ratio (OR = 2.24; 95% CI, 1.41–3.57) were associated with the seropositivity of APCA. Thus, we included age, sex, alcohol drinking, BMI, family history of UGI cancer, *H. pylori* CagA sero-positivity, and ratio of PG I/II in the multivariate logistic regression models and additionally adjusted for tobacco smoking, which was an important risk factor for most cancers.

The distributions of concentrations of serum APCA in different cancer subtypes and controls were shown in Supplementary Table S3. Less than a quarter of the subjects was seropositive for APCA. The prevalence of APCA seropositivity was 17.3% in the control group and lower in participants who developed GCA (10.0%) or GNCA (14.5%). We present the associations between APCA seropositivity and risks of developing different UGI cancers using the established cutoff of an APCA concentration >10 U/mL, overall and stratified by sex and age (Table 3) and stratified by *H. pylori* whole cell and CagA antibody serostatus or pepsinogen levels (Table 4). We observed a significant inverse association between serologic positivity for APCA and future risk of GCA (OR = 0.42; 95% CI, 0.24–0.75). The association between APCA seropositivity and later GNCA was also inverse, but not statistically significant (OR = 0.82; 95% CI, 0.50–1.36). There was no association between APCA seropositivity and risk of ESCC (OR = 1.05; 95% CI, 0.58–1.88). There was no interaction between APCA seropositivity and sex, age group, *H. pylori* infection status, or PGI/II ratios ($P > 0.05$). Accordingly, risk estimates were generally similar across different strata by sex, age group, 3 *H. pylori* whole cell, and CagA serostatus subgroups, and normal or low PGI/II ratios groups.

Using a previously identified low PG I/II ratio (≤4) as a marker for atrophic gastritis (13), we found a significantly higher risk of atrophic gastritis (OR = 3.69; 95% CI, 1.66–8.21) for the controls who were seropositive for APCA (Supplementary Table S4). The prevalence of APCA seropositivity was highest (40.6%) in controls with low PG I/II ratios (≤4), 15.2% in controls without normal PG I/II ratios (>4), and lowest (10.0%) in participants who developed GCA during follow-up. Across all 3 cancer sites, *H. pylori* CagA seropositivity was higher in subjects with a low PG I/II ratio or those who were seropositive for APCA (Supplementary Table S5).

We then compared the risks of UGI cancers stratified by PG I/II ratios groups and APCA seropositivity (Table 5). Overall, we found no association between APCA or PG I/II ratio and risk of ESCC. Low PG I/II ratio was associated with a significant increase

Table 2. OR (95% CI) for the associations between various risk factors and seropositivity for antiparietal cell antibodies and different subtypes of UGI cancers in the Linxian General Population NIT study

	OR (95% CI)				Seropositivity for antiparietal cell antibodies (>10 units)	
Variable	ESCC	GCA	GNCA	UGI cancer	*n* (%)	OR (95% CI)
Age	1.02 (0.99–1.05)	**1.05 (1.03–1.07)**	**1.07 (1.04–1.09)**	**1.05 (1.03–1.07)**	137 (15.2)	1.01 (0.99–1.03)
<55 years	1.00	1.00	1.00	1.00	78 (15.7)	1.00
≥55 years	1.30 (0.83–2.05)	**1.81 (1.24–2.63)**	**2.47 (1.69–3.59)**	**1.89 (1.42–2.51)**	59 (14.6)	0.94 (0.65–1.38)
Sex						
Female	1.00	1.00	1.00	1.00	73 (16.9)	1.00
Male	1.16 (0.57–2.38)	**3.19 (1.87–5.43)**	**2.84 (1.64–4.90)**	**2.37 (1.56–3.60)**	64 (13.7)	0.77 (0.45–1.32)
Tobacco smoking[a], yes	0.75 (0.34–1.65)	0.66 (0.38–1.14)	0.94 (0.54–1.63)	0.79 (0.51–1.22)	44 (13.4)	0.85 (0.48–1.51)
Alcohol drinking[b], yes	1.21 (0.68–2.14)	0.80 (0.49–1.29)	0.74 (0.47–1.19)	0.81 (0.56–1.16)	39 (19.0)	**1.79 (1.13–2.83)**
Family history of UGI cancer, yes	1.38 (0.85–2.23)	**1.57 (1.06–2.32)**	1.06 (0.71–1.59)	1.34 (0.99–1.81)	48 (16.5)	1.20 (0.81–1.76)
BMI	0.96 (0.88–1.06)	0.99 (0.92–1.07)	**0.92 (0.85–1.00)**	0.96 (0.90–1.02)	137 (15.2)	1.00 (0.93–1.08)
H. pylori CagA seropositivity	1.03 (0.63–1.68)	**2.48 (1.59–3.87)**	1.28 (0.86–1.91)	**1.58 (1.16–2.14)**	92 (16.8)	1.24 (0.82–1.89)
PG I/II ratio[c]	0.98 (0.95–1.01)	**0.95 (0.92–0.99)**	0.99 (0.97–1.01)	**0.98 (0.96–1.00)**	137 (15.2)	0.98 (0.95–1.01)
Normal PG I/II ratio	1.00	1.00	1.00	1.00	105 (13.5)	1.00
Low PG I/II ratio	1.87 (0.94–3.73)	**1.81 (1.03–3.20)**	**3.45 (2.03–5.89)**	**2.32 (1.48–3.63)**	32 (25.8)	**2.24 (1.41–3.57)**

NOTE: Numbers in bold mean that the *P* value was less than 0.05.
[a]Tobacco smoking was defined as ever smoking cigarettes for 6 or more months.
[b]Alcohol drinking was defined as any alcoholic beverage consumption in the last 12 months.
[c]PG I/II ratio, pepsinogen I/pepsinogen II ratio, ratio of PG I/II was defined as low for PGI/II ratio ≤4 and normal for PGI/II ratio >4.

Table 3. OR (95% CI) for the associations between seropositivity for antiparietal cell antibodies and esophageal and gastric cancers in the Linxian General Population NIT study, stratified by sex and age subgroups

			Seropositivity for antiparietal cell antibodies (>10 units)	
Group	**Cancer type**	**Case no.**	***n* (%)**	**OR (95% CI)**
All[a]	Control	400	69 (17.25)	—
	UGI cancer	500	68 (13.60)	0.71 (0.48–1.05)
	ESCC	100	19 (19.00)	1.05 (0.58–1.88)
	GCA	200	20 (10.00)	**0.42 (0.24–0.75)**
	GNCA	200	29 (14.50)	0.82 (0.50–1.36)
Females[b]	Control	229	44 (19.21)	—
	UGI cancer	204	29 (14.22)	0.59 (0.34–1.02)
	ESCC	54	13 (24.07)	1.23 (0.59–2.55)
	GCA	83	6 (7.23)	**0.22 (0.08–0.59)**
	GNCA	67	10 (14.93)	0.60 (0.27–1.34)
Males[b]	Control	171	25 (14.62)	—
	UGI cancer	296	39 (13.18)	0.90 (0.50–1.60)
	ESCC	46	6 (13.04)	0.85 (0.31–2.30)
	GCA	117	14 (11.97)	0.71 (0.33–1.52)
	GNCA	133	19 (14.29)	1.08 (0.53–2.17)
<55 years[c]	Control	258	43 (16.67)	—
	UGI cancer	239	35 (14.64)	0.75 (0.45–1.25)
	ESCC	58	11 (18.97)	1.00 (0.46–2.18)
	GCA	103	12 (11.65)	**0.43 (0.20–0.93)**
	GNCA	78	12 (15.38)	0.90 (0.44–1.87)
≥55 years[c]	Control	142	26 (18.31)	—
	UGI cancer	261	33 (12.64)	0.62 (0.35–1.12)
	ESCC	42	8 (19.05)	1.17 (0.47–2.95)
	GCA	97	8 (8.25)	**0.34 (0.14–0.86)**
	GNCA	122	17 (13.93)	0.66 (0.33–1.35)

NOTE: Numbers in bold mean that the *P* value was less than 0.05. We found no evidence for a statistically significant interaction between APCA seropositivity and sex or age group ($P > 0.05$).
[a]Adjusted for age, sex, tobacco smoking, alcohol drinking, BMI, family history of UGI cancer, *H. pylori* CagA seropositivity, and ratio of PG I/II.
[b]Adjusted for age, tobacco smoking, alcohol drinking, BMI, family history of UGI cancer, *H. pylori* CagA seropositivity, and ratio of PG I/II.
[c]Adjusted for sex, tobacco smoking, alcohol drinking, BMI, family history of UGI cancer, *H. pylori* CagA seropositivity, and ratio of PG I/II.

Table 4. OR (95% CI) for the associations between seropositivity for antiparietal cell antibodies and esophageal and gastric cancers in the Linxian General Population NIT study, stratified by *H. pylori* infection status and pepsinogen levels

			Seropositivity for antiparietal cell antibodies (>10 units)	
Group	**Cancer type**	**Case no.**	***n* (%)**	**OR (95% CI)**
H. *pylori* $WC^-/CagA^-$[a]	Control	101	14 (13.86)	—
	UGI cancer	87	10 (11.49)	0.86 (0.33–2.23)
	ESCC	15	2 (13.33)	0.83 (0.14–4.90)
	GCA	31	3 (9.68)	0.60 (0.13–2.72)
	GNCA	41	5 (12.20)	1.08 (0.31–3.75)
H. *pylori* $WC^+/CagA^-$[a]	Control	76	12 (15.79)	—
	UGI cancer	87	9 (10.34)	0.63 (0.23–1.69)
	ESCC	27	5 (18.52)	1.35 (0.37–4.86)
	GCA	20	0	—
	GNCA	40	4 (10.00)	0.44 (0.12–1.59)
H. *pylori* WC^+ or $WC^-/CagA^+$[a]	Control	223	43 (19.28)	—
	UGI cancer	326	49 (15.03)	0.73 (0.45–1.18)
	ESCC	58	12 (20.69)	1.08 (0.51–2.27)
	GCA	149	17 (11.41)	**0.48 (0.25–0.93)**
	GNCA	119	20 (16.81)	0.99 (0.52–1.87)
Normal PG I/II ratio[b]	Control	368	56 (15.22)	—
	UGI cancer	408	49 (12.01)	0.70 (0.45–1.08)
	ESCC	86	14 (16.28)	1.00 (0.52–1.91)
	GCA	169	15 (8.88)	**0.45 (0.24–0.85)**
	GNCA	153	20 (13.07)	0.81 (0.46–1.45)
Low PG I/II ratio[b]	Control	32	13 (40.63)	—
	UGI cancer	92	19 (20.65)	0.72 (0.24–2.19)
	ESCC	14	5 (35.71)	4.88 (0.23–105.06)
	GCA	31	5 (16.13)	0.42 (0.08–2.29)
	GNCA	47	9 (19.15)	0.64 (0.16–2.52)

NOTE: Numbers in bold mean that the *P* value was less than 0.05. We found no evidence for a statistically significant interaction between APCA seropositivity and *H. pylori* WC/CagA status or pepsinogen levels ($P > 0.05$).
[a]Adjusted for age, sex, tobacco smoking, alcohol drinking, BMI, family history of UGI cancer, and ratio of PG I/II.
[b]Adjusted for age, sex, tobacco smoking, alcohol drinking, BMI, family history of UGI cancer, *H. pylori* CagA seropositivity, and ratio of PG I/II.

in risk of both GCA and GNCA. APCA seropositivity was significantly inversely associated with risk of GCA and nonsignificantly inversely associated with risk of GNCA. Evaluating the co-occurrence of these markers, relative to subjects with a normal PGI/II ratio who were seronegative for APCA (the expected combination in "normal" subjects), subjects with a normal PG I/II ratio who were seropositive for APCA had a significantly lower risk of GCA (OR = 0.47; 95% CI, 0.25–0.89) and those with a low PG I/II ratio who were seronegative for APCA had a significantly increased risk of GCA (OR = 2.06; 95% CI, 1.04–4.07). Similar associations were found for GNCA. Subjects with a low PG I/II ratio who were seronegative for APCA had the highest risk of GNCA (OR = 4.13; 95% CI, 2.16–7.88), although the inverse association for subjects who had a normal PG I/II ratio and were seropositive for APCA was not statistically significant (OR = 0.81; 95% CI, 0.45–1.46; Table 5).

Discussion

We investigated the associations between serum APCA positivity and risk of 3 types of UGI cancer. We found that the prevalence of APCA seropositivity was 15.2% in subjects with normal PG I/II ratios (PG I/II ratios >4), 40.6% in subjects with low PG I/II ratios (PG I/II ratios ≤4), 10.0% in subjects who went on to develop GCA, and 14.5% in subjects who developed GNCA. Assuming subjects with low PGI/II ratios have atrophic gastritis, the APCA seropositivity was significantly associated with prevalent atrophic gastritis (OR = 3.69; 95% CI, 1.66–8.21), but was inversely associated with later development of GCA (OR = 0.42; 95% CI, 0.24–0.75) in this Chinese population. The association between APCA seropositivity and later GNCA was also inverse, but it was not statistically significant (OR = 0.82; 95% CI, 0.50–1.36). There was no evidence of an association between APCA seropositivity and risk of ESCC.

The prevalence of APCA seropositivity observed in this study are comparable to those reported in a German population that found that 19.5% of the general population and 46% of subjects with low PG I/II ratios were APCA seropositive (16). To our knowledge, few previous studies have explored the association between APCA serostatus and risk of UGI cancers, and none have evaluated this association prospectively. This study is the first to find that subjects who later developed GCA and GNCA had a lower baseline prevalence of APCA seropositivity than the general population or people with low PG I/II ratios (patients with serologically defined atrophic gastritis), and thus, that baseline APCA seropositivity was inversely associated with future risk of developing these gastric adenocarcinomas, although the association was not statistically significant for GNCA. This result was unexpected, and the mechanism for the association is not clear. In autoimmune gastritis, APCA antibodies progressively destroy the

Table 5. OR (95% CI) for the associations between serologic profiles of antiparietal cell antibodies and pepsinogen I/II ratio and UGI cancers in the Linxian General Population NIT study

Disease	Serologic profile[a]	Case no.	OR[b]	95% CI		*P* value	P_{global}
ESCC	Normal PG I/II ratio[c]	86	1.00				
	Low PG I/II ratio[c]	14	1.80	0.89	3.65	0.1	
	$APCA^{-}$[d]	81	1.00				
	$APCA^{+}$[d]	19	1.05	0.58	1.88	0.9	
	Normal PG I/II ratio, $APCA^{-}$	72	1.00				
	Normal PG I/II ratio, $APCA^{+}$	14	1.05	0.55	2.01	0.9	
	Low PG I/II ratio, $APCA^{+}$	5	1.55	0.52	4.69	0.4	
	Low PG I/II ratio, $APCA^{-}$	9	2.00	0.84	4.75	0.1	0.1
GCA	Normal PG I/II ratio[c]	169	1.00				
	Low PG I/II ratio[c]	31	**1.90**	**1.05**	**3.43**	**0.03**	
	$APCA^{-}$[d]	180	1.00				
	$APCA^{+}$[d]	20	**0.42**	**0.24**	**0.75**	**0.003**	
	Normal PG I/II ratio, $APCA^{-}$	154	1.00				
	Normal PG I/II ratio, $APCA^{+}$	15	**0.47**	**0.25**	**0.89**	**0.02**	
	Low PG I/II ratio, $APCA^{+}$	5	0.69	0.23	2.10	0.5	
	Low PG I/II ratio, $APCA^{-}$	26	**2.06**	**1.04**	**4.07**	**0.04**	0.3
GNCA	Normal PG I/II ratio[c]	153	1.00				
	Low PG I/II ratio[c]	47	**3.48**	**2.02**	**6.02**	**<0.0001**	
	$APCA^{-}$[d]	171	1.00				
	$APCA^{+}$[d]	29	0.82	0.50	1.36	0.4	
	Normal PG I/II ratio, $APCA^{-}$	133	1.00				
	Normal PG I/II ratio, $APCA^{+}$	20	0.81	0.45	1.46	0.5	
	Low PG I/II ratio, $APCA^{+}$	9	1.77	0.69	4.55	0.2	
	Low PG I/II ratio, $APCA^{-}$	38	**4.13**	**2.16**	**7.88**	**<0.0001**	<0.0001
UGI	Normal PG I/II ratio[c]	408	1.00				
	Low PG I/II ratio[c]	92	**2.32**	**1.46**	**3.67**	**0.0004**	
	$APCA^{-}$[d]	432	1.00				
	$APCA^{+}$[d]	68	0.71	0.48	1.05	**0.08**	
	Normal PG I/II ratio, $APCA^{-}$	359	1.00				
	Normal PG I/II ratio, $APCA^{+}$	49	0.70	0.46	1.08	0.1	
	Low PG I/II ratio, $APCA^{+}$	19	1.26	0.59	2.69	0.5	
	Low PG I/II ratio, $APCA^{-}$	73	**2.58**	**1.47**	**4.52**	**0.0009**	0.005

NOTE: Numbers in bold mean that the *P* value was less than 0.05.

[a]APCA seropositivity was defined as concentration of antiparietal cell antibodies ≥10 units; ratio of PG I/II was defined as low for PGI/II ratio ≤4 and normal for PGI/II ratio >4.

[b]Adjusted for age, sex, tobacco smoking, alcohol drinking, BMI, family history of UGI cancer, and *H. pylori* CagA seropositivity.

[c]Additionally adjusted for APCA seropositivity.

[d]Additionally adjusted for PG I/II ratio.

parietal cells, leading to reduced or absent acid production (hypochlorhydria or achlorhydria) and an increased gastric pH. It is possible that this acid suppression protects the gastric tissue, especially in the cardia, from acid-related mucosal damage, which reduces the chance of carcinogenesis, analogous to the finding that acid suppression protects Western populations from esophagogastric adenocarcinomas (including adenocarcinomas arising in both the lower esophagus and the gastric cardia; refs. 28, 29). It is also possible that the presence of APCA antibodies may be indicative of a more robust, and potentially protective, immune response, or a cross-reactivity with overexpressed normal antigens on preneoplastic and neoplastic cells (30, 31). Of note, Barrett esophagus and esophageal adenocarcinoma are very rare or absent in this rural Chinese population, so essentially all gastric cardia cancers in this study population originated in the stomach (6). But taken together, our results are unexpected, and indicate that further work is needed to replicate these associations and infer a possible mechanism underlying our observations.

We also found that low PG I/II ratio was a marker for a higher risk of GCA and GNCA, which was expected. Thus, the simultaneous presence of a (higher risk) low PG I/II ratio and (lower risk) APCA seropositivity canceled each other out to yield a null association for both GCA and GNCA, and the simultaneous presence of a (higher risk) low PG I/II ratio and (higher risk) APCA seronegativity combined to convey the highest risk of both GCA and GNCA. This finding was consistent with the results of a Japanese cross-sectional study which also found the highest risk of GNCA in subjects with a low PG I/II ratio who were seronegative for APCA (15). This earlier study also evaluated gastric biopsies of their patients and found higher APCA titers in patients with histologically moderate gastric atrophy and lower APCA titers in patients whose atrophy was histologically more severe, possibly reflecting a more severe loss of parietal cell antigenic stimulus and consistent with these latter patients, with more severe atrophy, having a greater risk of future cancer. These findings may be analogous to the reduction in *H. pylori* whole cell antibody titers as gastric atrophy becomes more severe, and the observation that the greatest risk of GNCA is found in patients with a low PGI/II ratio who are *H. pylori* CagA antibody positive (showing a history of *H. pylori* exposure) and *H. pylori* whole cell antibody negative (26). However, neither the Japanese study nor the present study could provide evidence for a causative linkage between autoimmune gastritis and the progression of gastric atrophy to incident cancer, and we cannot rule out the role of *H. pylori* in carcinogenesis, due to the lack of information on history of autoimmune gastritis. Future longitudinal studies are required to investigate the progression of autoimmune gastritis as well as the role of APCA as a serologic marker for the risk stratification of gastric cancer.

Our study had several strengths. It was the first to investigate the associations between APCA and 3 types of UGI cancer and the first prospective study of APCA and gastric cancer in an Asian population. With an average of 8 years of follow-up between baseline blood draw and cancer diagnosis, and extensive information on potential confounders, we were able to evaluate the associations prospectively and conduct analyses adjusted for and stratified by major UGI cancer risk factors. This study also had a number of limitations, including the modest sample size which may have limited out ability to detect a significant inverse association between APCA and GNCA risk. We also lacked data on vitamin B12 concentrations in circulation or pernicious anemia diagnoses, which may have interactions with the associations between APCA and gastric cancer. Moreover, one previous study found that a small fraction of people who were seropositive for APCA did not develop autoimmune gastritis or pernicious anemia, which raises questions about APCA as a definitive marker for autoimmune gastritis (17). In addition, in our cohort we defined GCA as tumors located in the most proximal 3 cm of the stomach, but there are no clear landmarks that allow inerrant classification of cardia versus noncardia gastric cancer so it is possible that we included some proximal corpus/fundus tumors as GCAs.

In conclusion, we found that serum positivity for APCA was statistically significantly associated with low PG I/II ratios, which was a serologic marker for prevalent atrophic gastritis, but inversely associated with later development of GCA in a high-risk Chinese population. Individuals with low PG I/II ratios who were also seronegative for APCA had the highest risk of both GCA and GNCA. APCA may contribute to a growing list of serologic markers that improve risk stratification for UGI cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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