High-Negative Anti–*Helicobacter pylori* IgG Antibody Titers and Long-Term Risk of Gastric Cancer: Results from a Large-Scale Population-Based Cohort Study in Japan

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ABSTRACT

Background: Serologic testing of anti–*Helicobacter pylori* antibody, together with testing of pepsinogen I and II, is now widely used to stratify groups at high risk of gastric cancer in Japan. Those with a negative anti–*H. pylori* IgG titer, especially "high-negative" (3–<10 U/mL), are speculated to have higher risk of gastric cancer. We aimed to evaluate the association between a high-negative anti–*H. pylori* IgG titer and the long-term risk of gastric cancer in the Japan Public Health Center-based Prospective Study (JPHC Study) Cohort II.

Methods: The study population consisted of 19,106 Japanese men and women who were followed from 1993 to 2013. A Cox proportional hazards model was used to assess the risk of gastric cancer for plasma anti–*H. pylori* IgG titers, together with the severity of atrophic gastritis by pepsinogen I and II levels. A total of 595 cases of gastric cancer occurred during an average of 18 years of follow-up.

Results: Compared with those with a low-negative anti–*H. pylori* IgG titer (≤3 U/mL), subjects with a high-negative titer (3–<10 U/mL) showed a significantly elevated risk of gastric cancer [HR = 2.81; 95% confidence interval (CI) = 1.62–4.89]. Among those with a high-negative titer, risk increase was observed under moderate or severe atrophic gastritis (HR = 18.73; 95% CI = 8.83–39.70).

Conclusions: Our results suggest that those with a high-negative anti–*H. pylori* IgG titer and moderate and severe atrophic gastritis are at increased long-term risk of gastric cancer.

Impact: Development of moderate or severe atrophic gastritis in subjects with high-negative anti–*H. pylori* IgG titer is suggested to increase risk of gastric cancer.

Introduction

Despite Japan having among the highest rates of gastric cancer for the last several decades, rates over this period have nevertheless shown a constant and dramatic decline (International Agency for Research on Cancer; http://ci5.iarc.fr/CI5plus/). Infection with *Helicobacter pylori* (*H. pylori*) is the most important established cause of gastric cancer (1). The importance of reducing *H. pylori* infection to decreasing the incidence of gastric cancer at the population level in Japan has been unequivocal (2). *H. pylori* infection occurs during infancy, commonly by 5 years old (3), and prevalence has reflected the general hygiene environment, along with a reduction in salt and salted food intake (4). Salted food intake has been positively associated with the prevalence of *H. pylori* (5), and mucosal damage induced by salt and salted food may increase persistent infection with *H. pylori* (6). The reduction in the prevalence of *H. pylori* is also hypothesized to have resulted from the widespread use of antibiotics (7). In addition, a very recent meta-analysis suggests the potential of *H. pylori* eradication treatment in the prevention of gastric cancer (8).

H. pylori infection in Japan has dramatically declined by a birth cohort effect, from nearly 70% for those born before 1950 to around 5% for those born after 2000 (2, 9), mainly due to drastic improvements in the hygiene environment. This change will have clear generational effects on primary and secondary prevention strategies, likely requiring a risk-stratified approach to gastric cancer prevention, especially for the lower risk younger generation (2, 10). To date, however, no comprehensive and valid stratification approach for this low-risk population has yet been established.

Serologic testing of *H. pylori* antibody, either alone or in combination with pepsinogen (PG) I and II testing (11), is now commonly used to stratify high-risk groups for gastric cancer in Japan. In this testing, serum level of IgG antibody to *H. pylori* is commonly measured by enzyme immunoassay, wherein seropositivity for anti–*H. pylori* antibodies is defined as an IgG titer ≥10 U/mL. This high IgG titer group has been the target of eradication treatment. Several cohort studies have also used this conventional anti–*H. pylori* IgG titer cutoff to assess gastric cancer risk (12–15), and shown a clear increase in risk. In contrast, those with past and present *H. pylori* infection frequently fall into the category "negative (<10 U/mL)", especially in the high IgG titer range of the negative category (3–<10 U/mL; ref. 16), and are also speculated to have higher risk of gastric cancer (17). To date, however, no studies have epidemiologically investigated the predictive ability of this high-negative anti–*H. pylori* IgG titer on the long-term risk of gastric cancer using an observational design.

Here, we evaluated the association between high-negative anti–*H. pylori* IgG titer and long-term risk of gastric cancer based on a large-scale population-based cohort study in Japan, the Japan Public Health Center-based Study (JPHC Study) Cohort II.

Materials and Methods

Study population

JPHC Study Cohort II was started in 1993 to 1994 among registered Japanese residents aged 40 to 69 years at the time of baseline survey

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Note: JPHC Study Group members are listed at the following site: http://epi.ncc.go.jp/en/jphc/781/3838.html.

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drawn from six prefectural Public Health Centers (PHC) areas in Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka. Details of the study design have been described elsewhere (18). This study was approved by the Institutional Review Board of the National Cancer Center, Japan (approval number: 2001–021, 2004–059). Part of one PHC area was excluded due to its use of a different definition for study population. We initially defined a population-based cohort of 68,969 subjects after exclusion of ineligible subjects ($n = 109$). For the current study, we enrolled 20,643 subjects who responded to the questionnaire (response rate: 82%) and provided a blood sample with their health check-up data (31%). We then excluded 453 with a self-reported previous history of cancer at baseline. Of the remaining 20,190 subjects, we excluded 1,084 (5.4%) with missing information on the variables included in the analysis, finally leaving 19,106 subjects for use in the present analyses. This study was done without participant involvement in the study design or interpretation of the results.

Baseline survey

A baseline self-administered questionnaire survey on various lifestyle factors was conducted at the time of baseline (1993–1994). Some subjects (31%) voluntarily provided 10-mL samples of blood during their health check-up. Individual plasma samples were divided into three tubes holding 1.0 mL each, which were stored at −80°C.

Exposure measurement

Plasma levels of IgG antibodies to *H. pylori* (anti–*H. pylori* IgG titer) were measured by enzyme immunoassay (E plate "Eiken" H. pylori Antibody II; Eiken Kagaku) and grouped into 3 categories by anti–*H. pylori* IgG titer of ≤3 U/mL, >3 to <10 U/mL, and ≥10 U/mL. In addition, as a marker of atrophic gastritis, plasma levels of PG I and II were measured by latex agglutination (LZ test "Eiken" Pepsinogen I, II; Eiken Kagaku) and defined as "negative" (PG I >70 ng/mL or PG I/II >3.0) or "positive" (PG I ≤70 ng/mL and PG I/II ≤3.0), with positive further categorized as "mild" (PG I ≤70 ng/mL and PG I/II ≤3.0 and not moderate/severe), "moderate" (PG I ≤50 ng/mL and PG I/II ≤3.0 and not severe), or "severe" (PG I ≤30 ng/mL and PG I/II ≤2.0). We previously reported a validation study result from an ROC analysis in our current population elsewhere (19).

Follow-up and identification of gastric cancer

Subjects were followed from the baseline survey until December 2013. Residential status, including survival, was confirmed through the residential registry. Resident and death registration are required in Japan by law and the registries are believed to be complete. The occurrence of gastric cancer was determined by notification from hospitals in the study areas and data linkage with population-based cancer registries. Death certificates were used as a supplementary information source. The site of origin and histologic type were coded using the International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3; C16; ref. 20). In our cancer registry system, the proportion of cases having information from death certificates only was 3.4%, which was considered satisfactory for the current study. Through this procedure, a total of 595 newly diagnosed cases of gastric cancer were identified during follow-up. These gastric cancers were classified according to tumor location into proximal (C16.0–C16.1) and distal subsites (C16.2–C16.7). Histologic type was grouped into two major categories according to the degree of structural differentiation into differentiated and nondifferentiated types. In Japan, determination of histologic subtype is based on the Japanese classification of gastric carcinomas (Japanese Gastric Cancer Association; ref. 21). Accordingly, the differentiated type consists of papillary adenocarcinoma (*pap*), tubular adenocarcinoma, well-differentiated type (*tub1*) and moderately differentiated type (*tub2*), and mucinous adenocarcinoma (*muc*); and the nondifferentiated type consists of poorly differentiated adenocarcinoma, solid type (*por1*) and non-solid type (*por2*), and signet-ring cell carcinoma (*sig*). In accordance with a conversion table (22) for the Japanese classification and Lauren's classification for grouping, the differentiated type corresponds to the intestinal type by Lauren and the nondifferentiated type corresponds to the diffuse type. For cases falling into both histologic categories, the dominant type was applied. Other or unspecified histologic types were excluded from analysis by histologic subtype. Consequently, among the 595 cases of gastric cancer, 50 were classified as proximal and 383 as distal; and 240 as differentiated and 133 as nondifferentiated.

Statistical analysis

Person-years of follow-up for each individual were calculated from the starting point to the date of gastric cancer diagnosis, date of emigration from the study area, date of death, or end of follow-up, whichever came first. For those who withdrew or were lost to follow-up, the date of withdrawal and the last confirmed date of presence, respectively, were used as the date of censor.

The relative risk of occurrence of gastric cancer by category of anti–*H. pylori* IgG titer [≤3 U/mL (reference), >3–<10 U/mL, ≥10 U/mL] was described using HRs and 95% confidence intervals (CI). HR was also estimated by presence [negative (reference), positive] and severity (mild, moderate, severe) of atrophic gastritis by plasma levels of PG I and II, either in parallel or in combination with anti–*H. pylori* IgG titer categories. A Cox proportional hazards model was used to control for potential confounding factors, such as sex, age at baseline (5-year age categories), study area (6 PHC areas), smoking status (never or former, current), family history of gastric cancer (no, yes), and consumption of highly salted food (no, yes). These variables, obtained from the questionnaire, were based on associations identified in previous studies (12). Along with the main analysis, we also conducted stratified analyses by sex, subsite (proximal, distal), and histologic type (differentiated type, non-differentiated type). Sex, age, and area were treated as strata to allow for a different baseline hazard for each stratum. Testing of the proportional hazards assumption by Schoenfeld and scaled Schoenfeld residuals found no violation of proportionality.

All statistical analyses were performed using Stata 15 (Stata Corp LLC).

Results

During 344,441 person-years of follow-up (average follow-up period: 18.0 years) for 19,106 subjects (6,934 men and 12,172 women), a total of 595 subjects (370 men and 225 women) were identified with newly diagnosed gastric cancer and included in the analyses. Baseline characteristics of the study subjects according to anti–*H. pylori* IgG titer are shown in **Table 1**. The proportion of current smokers increased in those with anti–*H. pylori* IgG titer >3U/mL in both sexes. In contrast, the proportion of current smokers was higher among those with more severe atrophic gastritis in men, whereas no such tendency was observed in women. The proportion of those with a family history of gastric cancer, consumption of highly salted food, and atrophic gastritis increased with both increased anti–*H. pylori* IgG titer category and severity of atrophic gastritis.

HR of gastric cancer according to anti–*H. pylori* IgG titer in combination with the presence and severity of atrophic gastritis is shown in **Table 2**. Compared with those with a low-negative anti–*H.*

Table 1. Baseline characteristics of study subjects according to anti–*H. pylori* IgG titer and severity of atrophic gastritis (JPHC Cohort II; $n = 19{,}106$).

		Anti–*H. pylori* IgG titer			Severity of atrophic gastritis			
	Number of subjects *n* (%)	Low-negative ≤3 U/mL *n* (%)	High-negative >3 and <10 U/mL *n* (%)	Positive ≥10 U/mL *n* (%)	None *n* (%)	Mild *n* (%)	Moderate *n* (%)	Severe *n* (%)
Total	19,106 (100.0)	4,244 (22.2)	1,784 (9.3)	13,078 (68.5)	5,429 (28.4)	5,638 (29.5)	7,440 (38.9)	599 (3.1)
Men	*6,934*	*1,339 (19.3)*	*669 (9.7)*	*4,926 (71.0)*	*1,748 (25.2)*	*2,204 (31.8)*	*2,722 (39.3)*	*260 (3.7)*
Women	*12,172*	*2,905 (23.9)*	*1,115 (9.1)*	*8,152 (67.0)*	*3,681 (30.2)*	*3,434 (28.2)*	*4,718 (38.8)*	*339 (2.8)*
Age (years ± SD)	56.7 (±8.3)	55.2 (±8.7)	57.5 (±8.0)	57.0 (±8.1)	55.7 (±8.4)	56.6 (±8.0)	57.4 (±8.0)	60.9 (±6.7)
Smoking status								
Never and former	15,634 (81.8)	3,585 (84.5)	1,372 (76.9)	10,677 (81.6)	9,074 (82.0)	2,344 (85.7)	2,648 (82.0)	1,568 (75.6)
Current	3,472 (18.2)	659 (15.5)	412 (23.1)	2,401 (18.4)	1,993 (18.0)	390 (14.3)	583 (18.0)	506 (24.4)
Men								
Never and former	*3,976 (57.3)*	*800 (18.9)*	*318 (17.8)*	*2,858 (21.9)*	*2,281 (57.7)*	*521 (62.0)*	*663 (57.3)*	*511 (51.9)*
Current	*2,958 (42.7)*	*539 (12.7)*	*351 (19.7)*	*2,068 (15.8)*	*1,671 (42.3)*	*319 (38.0)*	*494 (42.7)*	*474 (48.1)*
Women								
Never and former	*11,658 (57.3)*	*2,785 (65.6)*	*1,054 (59.1)*	*7,819 (59.8)*	*6,793 (95.5)*	*1,823 (96.3)*	*1,985 (95.7)*	*1,057 (97.1)*
Current	*514 (42.7)*	*120 (2.8)*	*61 (3.4)*	*333 (2.5)*	*322 (4.5)*	*71 (3.7)*	*89 (4.3)*	*32 (2.9)*
Family history (parents or siblings)								
No	17,836 (93.4)	4,003 (94.3)	1,674 (93.8)	12,159 (93.0)	10,390 (93.9)	2,554 (93.4)	2,994 (92.7)	1,898 (91.5)
Yes	1,270 (6.6)	241 (5.7)	110 (6.2)	919 (7.0)	677 (6.1)	180 (6.6)	237 (7.3)	176 (8.5)
Consumption of highly salted food (fish roe, fish gut)								
No	8,460 (44.3)	2,029 (47.8)	832 (46.6)	5,599 (42.8)	5,072 (45.8)	1,182 (43.2)	1,372 (42.5)	834 (40.2)
Yes	10,646 (55.7)	2,215 (52.2)	952 (53.4)	7,479 (57.2)	5,995 (54.2)	1,552 (56.8)	1,859 (57.5)	1240 (59.8)
Atrophic gastritis								
Absent	11,067 (57.9)	4,020 (94.7)	1,409 (79.0)	5,638 (43.1)				
Mild	2,734 (14.3)	11 (0.3)	53 (3.0)	2,670 (20.4)				
Moderate	3,231 (16.9)	92 (2.2)	146 (8.2)	2,994 (22.9)				
Severe	2,074 (10.9)	121 (2.9)	176 (9.9)	1,777 (13.6)				
Atrophic gastritis								
Low-negative ≤3 U/mL	4,244 (22.2)				4,020 (36.3)	11 (0.4)	92 (2.9)	121 (5.8)
High-negative >3 and <10 U/mL	1,784 (9.3)				1,409 (12.7)	53 (1.9)	146 (4.5)	176 (8.5)
Positive ≥10 U/mL	13,078 (68.4)				5,638 (51.0)	2,670 (97.7)	2,993 (92.6)	1,777 (85.7)

Note: Atrophic gastritis: Negative (PG I >70 ng/mL or PG I/II >3.0), positive (PG I ≤70 ng/mL and PG I/II ≤3.0), mild (PG I ≤70 ng/mL and PG I/II ≤3.0 and not moderate/severe), moderate (PG I ≤50 ng/mL and PG I/II ≤3.0 and not severe), severe (PG I ≤30 ng/mL and PG I/II ≤2.0). Sex-specific values are in italic.

pylori IgG titer, we observed a significantly elevated risk of gastric cancer for those with a high-negative anti–*H. pylori* IgG titer (>3 and <10 U/mL) at baseline (HR = 2.81; CI = 1.62–4.89), with HR being lower than those with a positive anti–*H. pylori* IgG titer (≥10 U/mL; HR = 6.51; CI = 4.20–10.09). The presence of atrophic gastritis also elevated the risk of gastric cancer (HR = 3.06; CI = 2.55–3.68) and the risk similarly increased by severity of atrophic gastritis, namely mild (HR = 2.45; CI = 1.91–3.14), moderate (HR = 3.32; CI = 2.68–4.12), and severe (HR = 3.38; CI = 2.68–4.24).

We looked at gastric cancer risk in combination with severity of atrophic gastritis. Compared with those with low-negative anti–*H. pylori* IgG titer and negative or mild atrophic gastritis at baseline as reference, those with high-negative (>3 and <10 U/mL) anti–*H. pylori* IgG titer showed no significant increase in gastric cancer risk when atrophic gastritis remained negative or mild (HR = 2.24; CI = 0.93–5.40). However, elevated HRs were observed in those with a high-negative (>3 and <10 U/mL) anti–*H. pylori* IgG titer when atrophic gastritis was moderate or severe (HR = 18.73; CI = 8.83–39.70).

The results were not substantially different when we conducted further stratified analyses by subsite (proximal and distal) and histologic type (differentiated and nondifferentiated type; **Table 3**).

Discussion

In this study, we evaluated the association between high-negative anti–*H. pylori* IgG titer and the long-term risk of gastric cancer based on a large-scale population-based cohort in Japan, the JPHC Study Cohort II, using anti–*H. pylori* IgG titer along with atrophic gastritis biomarkers. On long-term follow-up of 18 years, the results revealed an increased risk of gastric cancer for those with high-negative anti–*H. pylori* IgG titers. However, we also found that this increase in risk was generally dependent on the severity of atrophic gastritis, suggesting that the severity of atrophic gastritis, resulting from persistent *H. pylori* infection, is a crucial factor in the long-term risk of gastric cancer.

It is worth mentioning that when atrophic gastritis was moderate or severe, the risk of gastric cancer was similarly increased regardless of anti–*H. pylori* IgG titer category. A previous study suggested a precancerous course, in which extremely severe gastric atrophy leads to a spontaneous decrease in *H. pylori*, at about which point gastric atrophy reaches an irreversible stage and then invariably proceeds to gastric cancer (23).

Our results raise a number of interesting points. First, they suggest the existence of certain factors which exacerbate, directly or indirectly, the severity of atrophic gastritis. One possibility is an effect on

Table 2. Anti-*H. pylori* IgG titer and risk of gastric cancer in combination with atrophic gastritis status (19,106 subjects: 6,934 men, 12,172 women; 18 years of follow-up).

Category	Number of participants	Person-years	Number of cases	HR (95%CI)	Number of cases	HR (95%CI)	Number of cases	HR (95%CI)
	19,106	344,441	Both sexes ($n = 595$)		Men ($n = 370$)		Women ($n = 225$)	
H. pylori								
Negative	6,028	109,304	53	1.00	28	1.00	25	1.00
Positive	13,078	235,136	542	3.96 (2.98–5.27)	342	4.35 (2.96–6.42)	200	3.53 (2.33–5.36)
Low-negative	4,244	77,583	21	1.00	7	1.00	14	1.00
High-negative	1,784	31,721	32	2.81 (1.62–4.89)	21	4.67 (1.98–11.01)	11	1.82 (0.83–4.02)
Positive	13,078	235,136	542	6.51 (4.20–10.09)	342	10.64 (5.02–22.53)	200	4.41 (2.56–7.59)
P_{trend} *(categorical)*				*<0.001*		*<0.001*		*<0.001*
Anti-H. pylori-IgG titer (increase per 1 U/mL)				*1.01 (1.00–1.01)*		*1.01 (1.00–1.01)*		*1.01 (1.00–1.01)*
Anti-H. pylori-IgG titer (increase per 10 U/mL)				*1.07 (1.04–1.10)*		*1.06 (1.02–1.10)*		*1.09 (1.05–1.13)*
AG								
Negative	11,067	201,857	164	1.00	91	1.00	73	1.00
Positive	8,039	142,584	431	3.06 (2.55–3.68)	279	3.48 (2.73–4.43)	152	2.56 (1.93–3.39)
Mild	2,734	49,639	103	2.45 (1.91–3.14)	53	2.52 (1.79–3.55)	50	2.34 (1.63–3.36)
Moderate	3,231	57,590	176	3.32 (2.68–4.12)	119	4.05 (3.07–5.33)	57	2.44 (1.73–3.46)
Severe	2,074	35,354	152	3.38 (2.68–4.24)	107	3.63 (2.72–4.86)	45	3.11 (2.13–4.55)
P_{trend} *(categorical)*				*<0.001*		*<0.001*		*<0.001*
H. pylori detail and AG (negative/mild, moderate/severe)								
H. pylori low-negative and AG negative/mild	4,031	73,989	10	1.00	2	1.00	8	1.00
H. pylori high-negative and AG negative/mild	1,462	26,474	10	2.24 (0.93–5.40)	6	5.99 (1.21–29.75)	4	1.29 (0.39–4.30)
H. pylori positive and AG negative/mild	8,308	151,044	247	10.08 (5.35–18.99)	83	24.83 (6.14–100.40)	61	6.53 (3.18–13.40)
H. pylori low-negative and AG moderate/severe	213	3,604	11	17.31 (7.33–40.89)	5	37.76 (7.30–195.44)	6	12.76 (4.41–36.93)
H. pylori high-negative and AG moderate/severe	322	5,247	22	18.73 (8.83–39.70)	15	45.90 (10.46–201.30)	7	13.24 (4.78–36.68)
H. pylori positive and AG moderate/severe	4,770	84,093	295	17.75 (9.42–33.42)	259	52.17 (12.92–210.58)	139	8.65 (4.19–17.89)
$P_{interaction}$				*<0.001*		*<0.001*		*<0.001*

Note: *H. pylori*: Negative (IgG titer <10 U/mL), positive (IgG titer ≥10 U/mL), low-negative (IgG titer ≤3 U/mL), high-negative (3 U/mL <IgG titer <10 U/mL). Atrophic gastritis: Negative (PG I >70 ng/mL or PG I/II >3.0), positive (PG I ≤70 ng/mL and PG I/II ≤3.0), mild (PG I ≤70 ng/mL and PG I/II ≤3.0 and not moderate/severe), moderate (PG I ≤50 ng/mL and PG I/II ≤3.0 and not severe), severe (PG I ≤30 ng/mL and PG I/II ≤2.0). HR: Estimated by a Cox proportional hazards model, including sex (strata), age at baseline (strata, 5-year age categories), study area (strata, 6 PHC areas), smoking status (never or former, current), family history of gastric cancer (no, yes), consumption of highly salted food (no, yes). Point estimate and *P* values for trend are in italic.
Abbreviation: AG, atrophic gastritis.

virulence factors, such as CagA, which is thought to play an important role in gastric carcinogenesis, and to have genetic variation, which contributes to the geographical variation in gastric carcinogenesis (24). Our previous report using a nested case–control study design, however, found that those with a positive anti-*H. pylori* IgG titer were at an approximately 10-fold increased risk of gastric cancer, regardless of CagA status (25). Because of a lack of information, we were unable to consider CagA status in the current study, which is one of its limitations.

Second, it is possible that other factors promote the severity of atrophic gastritis after *H. pylori* infection. Common traditional Japanese food practices, including high consumption of rice and salted foods, may play a role in the development of atrophic gastritis after *H. pylori* infection (26), as well as in a decrease in risk by behavioral change, such as decreased intake of salty food (27, 28). Although smoking is an established risk factor for gastric cancer (29), its association with atrophic gastritis remains controversial (30–33). Some studies reported that gastric atrophy grade in *H. pylori*–positive

Table 3. Anti–*H. pylori* IgG titer and risk of gastric cancer in combination with atrophic gastritis status by subsite and histologic type (19,106 subjects: 6,934 men, 12,172 women; 18 years of follow-up).

Category	Number of participants	Person-years	Number of cases	HR (95%CI)	Number of cases	HR (95%CI)	Number of cases	HR (95%CI)	Number of cases	HR (95%CI)
	19,106	344,441	Proximal ($n = 50$)		Distal ($n = 383$)		Differentiated type ($n = 240$)		Nondifferentiated type ($n = 133$)	
H. pylori										
Negative	6,028	109,304	5	1.00	31	1.00	17	1.00	7	1.00
Positive	13,078	235,136	45	3.39 (1.34–8.58)	352	4.38 (3.03–6.33)	223	4.78 (2.91–7.84)	126	7.55 (3.52–16.20)
Low-negative	4,244	77,583	0	—	16	1.00	8	1.00	0	
High-negative	1,784	31,721	5	—	15	1.70 (0.84–3.45)	9	1.97 (0.76–5.13)	7	—
Positive	13,078	235,136	45	—	352	5.48 (3.31–9.06)	223	6.48 (3.19–13.15)	126	—
P_{trend} *(categorical)*				*0.005*		*<0.001*		*<0.001*		*<0.001*
Anti–H. pylori-IgG titer (increase per 1 U/mL)				*1.00 (0.99–1.01)*		*1.01 (1.00–1.01)*		*1.00 (1.00–1.01)*		*1.01 (1.01–1.02)*
Anti–H. pylori-IgG titer (increase per 10 U/mL)				*1.03 (0.94–1.13)*		*1.07 (1.03–1.10)*		*1.05 (1.01–1.09)*		*1.11 (1.05–1.17)*
AG										
Negative	11,067	201,857	12	1.00	110	1.00	60	1.00	44	1.00
Positive	8,039	142,584	38	3.60 (1.86–6.95)	272	2.84 (2.27–3.56)	180	3.27 (2.43–4.39)	89	2.55 (1.76–3.68)
Negative	11,067	201,857	12	1.00	111	1.00	60	1.00	44	1.00
Mild	2,734	49,639	10	3.31 (1.43–7.71)	59	2.08 (1.52–2.86)	32	2.01 (1.31–3.10)	36	3.21 (2.05–5.00)
Moderate	3,231	57,590	14	3.58 (1.64–7.78)	119	3.30 (2.54–4.29)	79	3.91 (2.79–5.49)	32	2.32 (1.46–3.67)
Severe	2,074	35,354	14	3.90 (1.76–8.63)	94	3.05 (2.29–4.06)	69	3.72 (2.60–5.32)	21	2.08 (1.22–3.56)
P_{trend} *(categorical)*				*<0.001*		*<0.001*		*<0.001*		*<0.001*
H. pylori detail and AG (negative/mild, moderate/severe)										
H. pylori low-negative and AG negative/mild	4,031	73,989	0	1.00	7	1.00	4	1.00	0	1.00
H. pylori high-negative and AG negative/mild	1,462	26,474	2	—	5	1.59 (0.50–5.00)	2	1.08 (0.20–5.91)	2	—
H. pylori positive and AG negative/mild	8,308	151,044	20	—	158	9.14 (4.28–19.51)	86	8.30 (3.04–22.67)	78	—
H. pylori low-negative and AG moderate/severe	213	3,604	0	—	9	20.41 (7.57–55.04)	4	14.84 (3.69–59.67)	0	—
H. pylori high-negative and AG moderate/severe	322	5,247	3	—	10	11.99 (4.54–31.65)	7	13.97 (4.06–48.07)	5	—
H. pylori positive and AG moderate/severe	4,770	84,093	25	—	194	16.48 (7.72–35.15)	137	18.38 (6.77–49.90)	45	—
$P_{interaction}$						*<0.01*		*<0.01*		

Note: *H. pylori*: Negative (IgG titer <10 U/mL), positive (IgG titer ≥10 U/mL), low-negative (IgG titer ≤3 U/mL), high-negative (3 U/mL <IgG titer <10 U/mL). Atrophic gastritis: Negative (PG I >70 ng/mL or PG I/II >3.0), positive (PG I ≤70 ng/mL and PG I/II ≤3.0), mild (PG I ≤70 ng/mL and PG I/II ≤3.0 and not moderate/severe), moderate (PG I ≤50 ng/mL and PG I/II ≤3.0 and not severe), severe (PG I ≤30 ng/mL and PG I/II ≤2.0). HR: Estimated by a Cox proportional hazards model, including sex (strata), age at baseline (strata, 5-year age categories), study area (strata, 6 PHC areas), smoking status (never or former, current), family history of gastric cancer (no, yes), consumption of highly salted food (no, yes), *H. pylori* status, and atrophic gastritis status. Point estimate and *P* values for trend are in italic.
Abbreviation: AG, atrophic gastritis.

subjects was higher among those who smoked (30, 32), whereas others saw no clear association (33). In our data, the proportion of current smokers was higher only in men with severe atrophy. Further studies are needed to clarify the mechanism by which smoking influences gastric carcinogenesis, either through the promotion of gastric atrophy or via an independent pathway.

Third, a negative anti–*H. pylori* IgG titer may result from any of several possible factors, other than misclassification by chance, such as a reduction in IgG antibody production by unknown factors, seroreversion by progression of atrophic gastritis and eradication treatment of *H. pylori*. If the severity of atrophic gastritis is an important risk factor in the long-term progression to gastric cancer, the question of whether *H. pylori* eradication prevents progression to gastric cancer is critical. As of now, *H. pylori* eradication does not guarantee the elimination of gastric cancer risk, as preneoplastic lesions may have already developed (34). The concept "point of no return" has been emphasized, in which the benefit of *H. pylori* eradication treatment diminishes after the appearance of precancerous regions with the molecular alteration (35). In the current study, we did not consider *H. pylori* eradication episodes, due to the lack of such follow-up information. This could have influenced our results. However, the Japanese health insurance scheme approved *H. pylori* eradication therapy for patients with chronic gastritis in February 2013, after which the number of prescriptions for *H. pylori* eradication therapy substantially increased (36). Although we cannot deny the possibility that our subjects were exposed to the same medication before this timing, our study follow-up period continued until 2013, and any influence of this therapy on the results is therefore likely to be negligible. Meanwhile, we should be aware of the possibility that results might differ if a baseline population were enrolled after the establishment of eradication treatment.

The major strength of our study is its prospective design. First, data for two core biomarkers, plasma anti–*H. pylori* IgG titer and pepsinogens, and information on lifestyle by questionnaire were collected before the subsequent diagnosis of gastric cancer, thereby avoiding the exposure recall bias inherent to case–control studies. Second, the population came from a large sample of the general Japanese population. Third, the high response rate and low loss to follow-up (0.1%) reduced possible selection bias. Fourth, the 18 years of follow-up provided not only a sufficient number of cases for analysis but also a sufficient period to reveal the long-term effects of *H. pylori* infection, especially in those with relatively low titers, the main focus of this study. In this regard, note that effects have not been observed in studies with less than 10 years of follow-up.

However, several limitations are also worth mentioning. First, the study population was mainly derived from non-metropolitan areas, which slightly limits our representativeness and generalizability. Second, bias could have been introduced by the fact that all subjects volunteered to have their blood taken. The subjects of this study were restricted to 31% of the total study subjects who had complete questionnaire responses and health checkup data, including blood samples. In our previous validation studies, more women than men tended to participate in health checkup surveys provided by local governments, and participants often differed from nonparticipants in socioeconomic status, having a more favorable lifestyle profile (37, 38); this may have influenced the association between anti–*H. pylori* IgG titer and risk of gastric cancer. Finally, the association may have been confounded by additional unmeasured or unknown risk factors.

Allowing for these methodologic issues, our results, based on a large-scale population-based cohort study with long-term follow-up, suggest that people with high-negative anti–*H. pylori* IgG titers are at increased long-term risk of gastric cancer, mostly among those with moderate and severe atrophic gastritis. Increased risk of gastric cancer in those with high-negative anti–*H. pylori* IgG titers is suggested to be an outcome of the development of moderate or severe atrophic gastritis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Inoue, S. Tsugane
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Inoue, N. Sawada, T. Shimazu, T. Yamaji, M. Iwasaki, S. Tsugane
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Inoue, A. Goto, T. Shimazu, T. Yamaji, S. Tsugane
Writing, review, and/or revision of the manuscript: M. Inoue, N. Sawada, A. Goto, T. Shimazu, T. Yamaji, M. Iwasaki, S. Tsugane
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Inoue, S. Tsugane
Study supervision: S. Tsugane

Data Availability Statement

All materials, data, and protocols described in the manuscript will be made available upon request, if the request is made within 6 years of publication. We cannot publicly provide individual data due to participant privacy, in accordance with ethical guidelines in Japan. Additionally, the informed consent we obtained does not include a provision for publicly sharing data. Qualifying researchers may apply to access a minimal dataset by contacting Dr. Shoichiro Tsugane, Principal Investigator, Epidemiology and Prevention Group, Center for Public Health Sciences, National Cancer Center, Japan, at stsugane@ncc.go.jp or the office of the JPHC Study Group at jphcadmin@ml.res.ncc.go.jp. More information about how to access JPHC data and/or biospecimens can be found here: https://epi.ncc.go.jp/en/jphc/805/index.html.

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