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Helicobacter pylori antibody responses and evolution of precancerous gastric lesions in a Chinese population

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Helicobacter pylori-specific proteins are involved in gastric carcinogenesis. To investigate the seroprevalence of six _H. pylori_-specific antibodies in patients with different gastric histology, and the impact of seropositivities on the evolution of precancerous gastric lesions, a follow-up study was conducted in Linqu County, China. The seropositivities for CagA, VacA, GroEL, UreA, HcpC and gGT were assessed by recomLine analysis in 573 _H. pylori_-positive subjects and correlated with evolution of precancerous gastric lesions. We found that the score of _H. pylori_ recomLine test was significantly increased in subjects with chronic atrophic gastritis (CAG, $p < 0.0001$) or intestinal metaplasia (IM, $p = 0.0125$), and CagA was an independent predictor of advanced gastric lesions, adjusted odds ratios (ORs) were 2.54 (95% CI = 1.42–4.55) for IM and 2.38 (95% CI = 1.05–5.37) for dysplasia (DYS). Moreover, seropositivities for CagA and GroEL were identified as independent predictors for progression of gastric lesions in a longitudinal study, and ORs were 2.89 (95% CI = 1.27–6.59) and 2.20 (95% CI = 1.33–3.64), respectively. Furthermore, the risk of progression was more pronounced in subjects with more than three positive antigens ($p_{for\ trend} = 0.0003$). This population-based study revealed that seropositivities for CagA and GroEL might be potential markers to identify patients infected with high-risk _H. pylori_ strains, which are related to the development of GC in a Chinese high-risk population, and recomLine test might serve as a tool for risk stratification.

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium identified as the strongest known risk factor for gastric cancer (GC).[1] *H. pylori* infection can induce chronic gastritis, persisting for decades and eventually progressing to chronic atrophic gastritis (CAG), intestinal metaplasia (IM), dysplasia (DYS) and GC.[2,3] Our two randomized, placebo-controlled factorial-design intervention trials in Linqu County, a high-risk area of GC in Shandong Province, China, indicated that *H. pylori* eradication significantly reduces the risk of precancerous gastric lesions and subsequent GC,[4–6] suggesting that *H. pylori* eradication could be an effective strategy to prevent GC.

However, although about half of the world's population is infected with *H. pylori*, only a minority of infected subjects

Key words: *Helicobacter pylori*, protein, serology, gastric cancer, precancerous gastric lesions

Abbreviations: CAG: chronic atrophic gastritis; CagA: cytotoxin-associated antigen A; CI: confidence interval; DYS: dysplasia; GC: gastric cancer; gGT: gamma-glutamyl transpeptidase; GroEL: chaperonin GroEL; HcpC: *Helicobacter* cysteine-rich protein C; H. pylori: Helicobacter pylori; IM: intestinal metaplasia; Ind DYS: indefinite dysplasia; OR: odds ratio; SG: superficial gastritis; UreA: urease subunit A; VacA: vacuolating toxin

Additional Supporting Information may be found in the online version of this article

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What's new?

The bacteria *H. pylori* is the strongest known risk factor for gastric cancer, but only a small percentage of those infected ever develop cancer. To help predict who those will be, researchers have identified several markers that associate with gastric cancer. This study sought to expand on earlier data associating the presence of antibodies to these markers with the risk of developing gastric lesions and cancer in a high-risk population. Patients with antibodies for either CagA and GroEL were more likely to have gastric lesions progress to cancer, as were people who had antibodies for more than three markers. These tests could be useful in assessing risk among those with *H. pylori infections*.

eventually develops GC.[7] Genetic variations of host and *H. pylori* virulence factors influence gastric carcinogenesis.[8] Because of large variations in *H. pylori* strains and antibiotic resistance induction by *H. pylori* eradication,[9,10] the identification of risk markers to classify *H. pylori*-infected patients into high- and low-risk groups is highly desirable for personalized prevention.

Cytotoxin-associated antigen A (CagA) and vacuolating toxin (VacA) of *H. pylori* were shown to be associated with the risk of GC.[11,12] The prevalence of seropositivity for CagA was significantly elevated in GC cases,[12] suggesting that serological responses toward this virulence factor might serve as a potential marker for GC development. Recently, additional *H. pylori* virulence factors were identified as new risk markers for GC, such as chaperonin GroEL and Helicobacter cysteine-rich protein C (HcpC).[13,14] An epidemiologic study analyzing antibody responses toward 15 individual *H. pylori* proteins revealed that seropositivities of CagA and GroEL were independent predictors of GC.[13] However, little of such data has been confirmed in population-based studies, specifically not in high-risk populations.

Since 1994, we have conducted two intervention trials to inhibit the progression of gastric lesions in Linqu County, including either anti-*H. pylori* treatment and/or supplementation with vitamin or garlic preparation, or anti-*H. pylori* treatment and/or COX-2 inhibitor (celecoxib) treatment,[4,5] respectively. All subjects underwent endoscopic examination before and after the trial, and were followed for up to 7 years. These long-term follow-up cohorts including untreated control subjects allow us to investigate a continuous stepwise evolution of premalignant gastric lesions during GC development, and open a unique opportunity to explore the association between *H. pylori* proteins and evolution of precancerous gastric lesions.

In our serological study, subjects from the placebo groups of the two intervention trials were included to investigate the relationship between seropositivities for CagA, VacA, GroEL, urease subunit A (UreA), gamma-glutamyl transpeptidase (gGT) and HcpC, respectively, with precancerous gastric lesion risk at baseline, and evolution of gastric lesions during the follow-up period, using a recently developed recomLine *H. pylori* test system.[15]

Methods

Study design and population

In 1994 and 2002, two independent randomized intervention trials in Linqu County were launched. All participants underwent endoscopic screening at baseline and repeated endoscopic examination at the end of the trial. The detailed information of the study population, endoscopic procedures and criteria of gastric pathology had been described elsewhere.[4,5] Briefly, for the first intervention trial, biopsies were taken from seven standard sites in the stomach: four from the antrum, one from the angulus and one each from the lesser and greater curvatures of the body. Each slide was reviewed by three senior pathologists based on the Chinese system,[4,16] and diagnosed as superficial gastritis (SG), mild/severe CAG, superficial IM, deep IM, mild/moderate DYS, severe DYS and GC. For the second intervention trial, biopsies were taken from five standard sites including two from the antrum, one from the angulus and two from the body, and diagnosed as SG, mild/severe CAG, superficial IM, deep IM, indefinite dysplasia (Ind DYS), low-grade DYS, high-grade DYS and GC, according to the criteria of Updated Sydney System[17] and Padovo International Classification.[18] Each subject was assigned a global diagnosis based on the most severe diagnosis among any of the biopsies. Trained field staff from Peking University Cancer Hospital interviewed the participants using a standard structured questionnaire including age, gender, cigarette smoking history, alcohol drinking, previous history of peptic ulcers, NSAID or antibiotic treatment and allergic reactions to antibiotics.

For our study, a total of 453 subjects with *H. pylori* infection from the placebo group of two intervention trials were included, and all subjects underwent blood and endoscopic examinations both before and after each trial. To standardize both intervention trials, the biopsies were classified into nine categories based on pathological diagnosis according to the Chinese system as follows: normal SG, mild/moderate CAG, severe CAG, superficial IM, deep IM, mild/moderate DYS, severe DYS and GC by the same three pathologists who conducted the endoscopic screening at baseline in 1994 and 2002. We further selected 120 *H. pylori*-positive subjects with SG from baseline endoscopic screening survey as a reference group.

For the first intervention trial (cohort 1), we determined *H. pylori* status by ELISA at baseline, and ^{13}C-urea breath test (^{13}C-UBT) after anti-*H. pylori* treatment and at the end of the trial. We used the serum collected at the trial end for our study. For the second intervention trial (cohort 2), *H. pylori* status was determined by ^{13}C-UBT at baseline in 2002. For those subjects, the serum collected in 2002 was used for

our study. The whole study was approved by the Institutional Review Board of Peking University Cancer Hospital and all subjects gave written informed consent.

Serum sample collection

Up to 5 ml of whole blood from each fasting participant was collected at baseline and middle or end-point survey of intervention trial. Blood samples were allowed to stand for 30–40 min and serum separation was accomplished by centrifugation at 965*g* for 15 min. The supernatant serum was recovered and stored at −80°C until analysis.

recomLine *H. pylori* IgG analysis

The recomLine *H. pylori* IgG is a line immune assay (Mikrogen, Munich, Germany) that, in contrast to ELISA, allows the identification of specific antibody responses against distinct *H. pylori* antigens.[15] Highly purified recombinant *H. pylori* antigens (CagA, VacA, GroEL, UreA, HcpC and gGT) were individually immobilized on nitrocellulose membrane strips as described.[15] The test strips were incubated with the diluted serum sample (dilution 1:100 with wash buffer A) for 1 hr with gentle shaking. After washing the strips were incubated with anti-human immune globulin antibodies (IgG, dilution 1:100) for 45 min, which were coupled to horseradish peroxidase. Unbound conjugate antibodies were removed by three washing steps. Bound antibodies were detected by a peroxidase-based staining reaction leading to a dark band appearing on the strip at the corresponding antigen lane.

The test result was determined according to the total scores of the individual band. An individual was considered to be positive if the total score was equal or more than 2.0. The score of individual antigens was assigned as follows: 2 for CagA, VacA and GroEL and 1 for UreA, HcpC and gGT. Thus, the total score of the line assay ranges from 0 (all six antibodies negative) to 9 (all six antibodies positive).

^{13}C-urea breath test

All participants received ^{13}C-UBT to determine their *H. pylori* status. Details of the ^{13}C-UBT were described in the previous publications.[19,20] Briefly, participants fasted overnight before baseline samples of exhaled CO_2 were collected. Each participant was then requested to drink 20 ml of water with a pill of 80 mg ^{13}C-urea (min. 99 atom % ^{13}C). Exhaled CO_2 was collected in sampling tubes 30 min later. $^{13}CO_2$ values were determined using a gas isotopic ratio mass spectrometer, and any concentration of $^{13}CO_2$ at 30 min that exceeded the baseline concentration by more than four parts per 1,000 (>0.4%) was regarded as a positive result. The sensitivity and specificity of the test were 93.1 and 95.7%, respectively.[21]

Statistical analysis

The data are expressed as mean and standard deviation (SD) or as numbers together with percentages. ANOVA was used to compare the total score of *H. pylori* antibodies and prevalence of gastric lesions at baseline adjusting for age and sex. Patients with SG served as reference group. Bonferroni *t*-test was used to compare the difference of total score in the different groups. Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated by using logistic regression to evaluate the associations between individual *H. pylori* antibodies and baseline histology adjusting for age and sex.

For the follow-up data, to assess the evolution of gastric lesions, each subject was assigned a global severity score at baseline and the end of the trial. Each patient was classified into the progression (increase in severity), no change or regression (decrease in severity), respectively. ANOVA was used to examine the overall difference in age, whereas Pearson's χ^2 test was performed to test the differences between the three groups with respect to gender, smoking, drinking and baseline histology. Stepwise backward multiple logistic regression analysis was used to identify serum markers that were independently associated with gastric lesions. Two different analyses were performed with (*i*) combining no change and regression groups together in comparison to progression and (*ii*) combining no change and progression groups together in comparison to regression. All *p* values were two-sided and less than 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 20.0, IBM).

Results

Association between seropositivities for *H. pylori* antibodies and risk of gastric lesions

A total of 573 subjects with *H. pylori* positive by ^{13}C-UBT were included in our study, including 254 from cohort 1, 199 from cohort 2 and 120 from the baseline survey. At baseline, 126 (22.0%) were SG, 144 (25.1%) were CAG, 235 (41.0%) were IM and 68 (11.9%) were DYS. There was no significant difference in cigarette smoking or alcohol consumption among different groups. However, the mean age was significantly higher in subjects with IM or DYS than in SG ($p < 0.05$), and the mean ages were 44.63 ± 7.40 for SG, 45.15 ± 6.67 for CAG, 48.46 ± 7.34 for IM and 48.81 ± 7.70 for DYS, respectively. Gender distribution was significantly different, with more female than male participants in CAG or IM group than SG ($p < 0.001$, data not shown).

Prevalence of antibody responses at baseline in *H. pylori*-infected individuals with different gastric pathology

All subjects were determined *H. pylori* positive by recomLine analysis (total score was equal or more than 2.0). The seroprevalences of CagA, VacA, GroEL, UreA, HcpC and gGT were 83.9, 38.9, 66.1, 17.8, 59.7 and 43.3%, respectively.

We first evaluated the association between the total score of *H. pylori* antibodies and risk of gastric lesions. Compared to subjects with SG (mean ± SD, 4.20 ± 1.798), the mean of total score was significantly increased in subjects with CAG (5.40 ± 1.738, $p < 0.0001$) or IM (4.79 ± 1.672, $p =$

Table 1. Association between individual *H. pylori* antibodies and baseline histology

	SG *n* (%)	CAG *n* (%)	OR (95% CI)[1]	IM *n* (%)	OR (95% CI)	DYS *n* (%)	OR (95% CI)	*p* trend
CagA								**0.045**
0	36 (28.57)	9 (6.25)	1	33 (14.04)	1	14 (20.59)	1	
1	90 (71.43)	135 (93.75)	**6.73 (2.96,15.28)**	202 (85.96)	**2.57 (1.45,4.55)**	54 (79.41)	**2.29 (1.05,5.01)**	
VacA								0.220
0	94 (74.6)	65 (45.14)	1	147 (62.55)	1	44 (64.71)	1	
1	32 (25.4)	79 (54.86)	**3.23 (1.90,5.51)**	88 (37.45)	1.54 (0.93,2.55)	24 (35.29)	1.54 (0.78,3.04)	
GroEL								0.325
0	55 (43.65)	32 (22.22)	1	81 (34.47)	1	26 (38.24)	1	
1	71 (56.35)	112 (77.78)	**2.91 (1.68,5.05)**	154 (65.53)	1.44 (0.90,2.28)	42 (61.76)	1.09 (0.58,2.04)	
UreA								0.309
0	104 (82.54)	122 (84.72)	1	189 (80.43)	1	56 (82.35)	1	
1	22 (17.46)	22 (15.28)	0.85 (0.44,1.66)	46 (19.57)	1.16 (0.65,2.09)	12 (17.65)	1.06 (0.48,2.37)	
HcpC								0.321
0	51 (40.48)	55 (38.19)	1	95 (40.43)	1	30 (44.12)	1	
1	75 (59.52)	89 (61.81)	1.15 (0.69,1.91)	140 (59.57)	1.07 (0.67,1.69)	38 (55.88)	0.91 (0.49,1.69)	
gGT								**<0.001**
0	52 (41.27)	89 (61.81)	1	139 (59.15)	1	45 (66.18)	1	
1	74 (58.73)	55 (38.19)	**0.41 (0.25,0.69)**	96 (40.85)	**0.49 (0.31,0.77)**	23 (33.82)	**0.35 (0.18,0.66)**	
Total	126	144		235		68		

[1]Logistic regression analysis, adjusted for age and sex.
Abbreviations: CAG: chronic atrophic gastritis; CagA: cytotoxin-associated antigen A; CI: confidence interval; DYS: dysplasia; gGT: gamma-glutamyl transpeptidase; GroEL: chaperoninGroEL; HcpC: Helicobacter cysteine-rich protein C; IM: intestinal metaplasia; OR: odds ratio; SG: superficial gastritis; UreA: urease subunit A; VacA: vacuolating toxin.

0.0125), whereas no significant difference was found for DYS (4.52 ± 1.577, $p = 0.999$) after adjusting for age and gender.

We further assessed the association between the seropositivity for each of the six antigens and risk of gastric lesions. Compared with SG, a significantly increased risk of CAG (OR = 6.73, 95% CI = 2.96–15.28), IM (OR = 2.57, 95% CI = 1.45–4.55) or DYS (OR = 2.29, 95% CI = 1.05–5.01) was found in subjects with anti-CagA antibodies (Table 1). An increased OR for CAG was also observed in subjects with VacA or GroEL immune responses. However, a decreased risk of CAG (OR = 0.41, 95% CI = 0.25–0.69), IM (OR = 0.49, 95% CI = 0.31–0.77) or DYS (OR = 0.35, 95% CI = 0.18–0.66) was found in subjects with antibodies recognizing gGT. Stepwise backward selection identified seropositivity for CagA as an independent predictor of advanced gastric lesions (Table 2), whereas gGT antibodies were inversely associated with increased risk of advanced gastric lesions.

Because gGT antibodies were inversely associated with advanced gastric lesions, we were interested to re-evaluate the association between the score for the other five antigens and risk of gastric lesions. We found that the trend was similar after deletion of gGT. Compared with SG (mean ± SD, 3.75 ± 1.690), the mean of score was significantly increased in subjects with CAG (5.04 ± 1.598, $p < 0.0001$) or IM (4.42 ± 1.537, $p = 0.0003$). No significant difference was found for DYS (4.23 ± 1.475, $p = 0.109$) after adjusting for age and gender.

Association between seropositivities for *H. pylori* antibodies and evolution of gastric lesions

To evaluate the associations between seropositivities for *H. pylori* antibodies and evolution of gastric lesions, a total of 453 subjects from the placebo groups of two intervention trials were included in our study. From baseline to the end of each trial (the mean follow-up was 5.7 years, ranged 4–7 years), 95 subjects had a decreased histopathologic severity score (indicating regression), 121 subjects had increased severity score (indicating progression) and 237 subjects remained at the same level (indicating no change in histology). The baseline characteristics of study participants are summarized in Supporting Information Table 1. The distributions of age, gender, smoking and drinking status were similar in different groups; however, the percentages of baseline pathology in regression, progression and no change groups were significantly different ($p < 0.001$).

We initially compared the difference of the total score in the different groups. As shown in Figure 1, there was a statistically significant difference between the three groups ($p < 0.001$).

Table 2. Association between individual *H. pylori* antibodies and baseline histology by backward stepwise multivariate logistic regression[1]

	SG *n* (%)	CAG *n* (%)	OR (95% CI)[2]	IM *n* (%)	OR (95% CI)	DYS *n* (%)	OR (95% CI)
CagA	90 (71.43)	133 (93.66)	**4.46 (1.94,10.26)**	202 (85.96)	**2.54 (1.42,4.55)**	54 (79.41)	**2.38 (1.05,5.37)**
VacA	32 (25.4)	79 (54.86)	**2.99 (1.68,5.33)**	88 (37.45)	–	24 (35.29)	–
GroEL	71 (56.35)	112 (77.78)	**2.45 (1.33,4.52)**	154 (65.53)	–	42 (61.76)	–
gGT	74 (58.73)	55 (38.19)	**0.37 (0.21,0.65)**	96 (40.85)	**0.49 (0.31,0.79)**	23 (33.82)	**0.35 (0.18,0.66)**

[1]Age, sex and status of CagA, VacA, GroEL, UreA, HcpC and gGT were included in the initial model.
[2]Adjusted for age and sex.
Abbreviations: CAG: chronic atrophic gastritis; CagA: cytotoxin-associated antigen A; CI: confidence interval; DYS: dysplasia; gGT: gamma-glutamyl transpeptidase; GroEL: chaperoninGroEL; IM: intestinal metaplasia; OR: odds ratio; SG: superficial gastritis; VacA: vacuolating toxin.

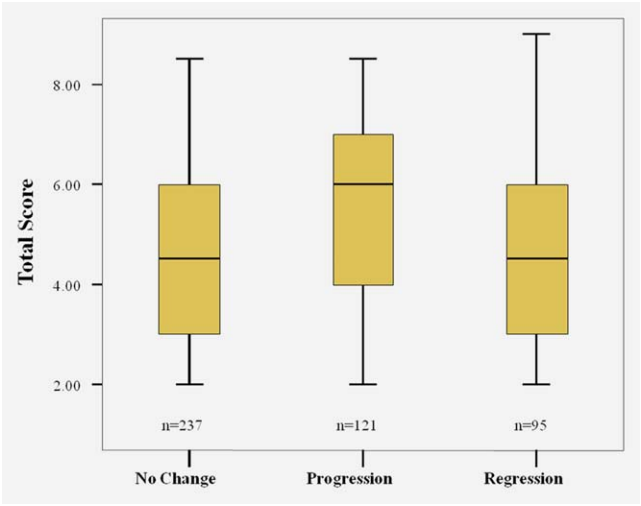

Figure 1. Distribution of the total score in the three groups. Box plots of total score in the progression, no change and regression groups. ANOVA was used to compare the difference among the three groups. Compared with the no change group, the mean of total score was higher in the progression group ($p < 0.001$), whereas no significant difference was found in regression group ($p = 0.999$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Compared to the no change group, the mean of total score was higher in the progression group (5.52 ± 1.512 *versus* 4.73 ± 1.719, $p < 0.001$), whereas no significant difference was found in the regression group (4.70 ± 1.742 *versus* 4.73 ± 1.719, $p = 0.999$). This data indicated that patients with a higher score, reflecting a more pronounced immune response, are prone to develop more severe gastric lesions.

We further explored the association between the seropositivity for each of the six antigens and evolution of gastric lesions. As shown in Table 3, an increased risk of progression was found for CagA (OR = 2.85, 95% CI = 1.24–6.53), VacA (OR = 1.58, 95% CI = 1.03–2.41), GroEL (OR = 2.25, 95% CI = 1.36–3.71) or gGT (OR = 1.55, 95% CI = 1.02–2.37). However, a significant association with regression was only suggested for absence of CagA antibodies (OR = 0.53, 95% CI = 0.28–0.99). Further stepwise backward selection identified seropositivities for CagA (OR = 2.89, 95% CI = 1.27–6.59) and GroEL (OR = 2.20, 95% CI = 1.33–3.64) as independent predictors for progression of gastric lesions (Table 4).

We were also interested to evaluate the number of positive *H. pylori* serum responses and evolution of gastric lesions. As shown in Table 5, compared to subjects with ≤2 serum responses, we found that risk of the progression of gastric lesions was more pronounced in subjects with three to four or five to six serum responses; the ORs were 2.54 (95% CI = 1.45–4.43) for three to four antigens and 3.19 (95% CI = 1.59–6.42) for five to six antigens, respectively. Further trend test showed a statistically significant trend for the number of positive antigens and evolution of gastric lesions ($p_{\text{for trend}} = 0.0003$).

Discussion

In our population-based study, six specific serological responses for *H. pylori* proteins were identified by recomLine analysis. We found a significant association between seropositivities for *H. pylori* antibodies and risk of gastric lesions. The risk of progression of gastric lesions increased with the number of positive *H. pylori* immune responses. Moreover, seropositivities for CagA and GroEL were identified as independent predictors for gastric lesion progression, suggesting these serum markers might serve as potential predictors for high-risk *H. pylori* strain infections, which are related to the development of GC. To the best of our knowledge, our long-term follow-up study is the first one to explore the association between a spectrum of *H. pylori* proteins and evolution of gastric lesions.

On the basis of our cross-sectional study at baseline, we found that the total score of *H. pylori* antibodies was markedly increased in subjects with CAG and IM, particularly for CAG, supporting our previous findings that *H. pylori* may play an important role in the inflammation process and early stage of gastric lesions.[4] Interestingly, no significant association with DYS was found, consistent with the hypothesis and our previous studies that with the progression of gastric lesions toward GC, *H. pylori* can be lost in the stomach mucosa owing to the changed internal environment, associated with declining antibody positivity (86.8% for CAG

Table 3. Association between *H. pylori* individual antibodies and evolution of gastric lesions

	Progression			Regression		
	No (*n*, %)	Yes (*n*, %)	OR (95% CI)[1]	No (*n*, %)	Yes (*n*, %)	OR (95% CI)
CagA						
0	50 (15.06)	7 (5.79)	1	40 (11.17)	17 (17.89)	1
1	282 (84.94)	114 (94.21)	**2.85 (1.24,6.53)**	318 (88.83)	78 (82.11)	**0.53 (0.28,0.99)**
VacA						
0	202 (60.84)	60 (49.59)	1	207 (57.82)	55 (57.89)	1
1	130 (39.16)	61 (50.41)	**1.58 (1.03,2.41)**	151 (42.18)	40 (42.11)	0.97 (0.61,1.54)
GroEL						
0	117 (35.24)	24 (19.83)	1	106 (29.61)	35 (36.84)	1
1	215 (64.76)	97 (80.17)	**2.25 (1.36,3.71)**	252 (70.39)	60 (63.16)	0.72 (0.45,1.15)
UreA						
0	274 (82.53)	99 (81.82)	1	297 (82.96)	76 (80)	1
1	58 (17.47)	22 (18.18)	1.11 (0.64,1.92)	61 (17.04)	19 (20)	1.26 (0.71,2.26)
HcpC						
0	138 (41.57)	44 (36.36)	1	142 (39.66)	40 (42.11)	1
1	194 (58.43)	77 (63.64)	1.24 (0.80,1.90)	216 (60.34)	55 (57.89)	0.90 (0.57,1.43)
gGT						
0	210 (63.25)	64 (52.89)	1	211 (58.94)	63 (66.32)	1
1	122 (36.75)	57 (47.11)	**1.55 (1.02,2.37)**	147 (41.06)	32 (33.68)	0.73 (0.46,1.18)

[1]Logistic regression analysis, adjusted for age and sex.
Abbreviations: CagA: cytotoxin-associated antigen A; CI: confidence interval; gGT: gamma-glutamyl transpeptidase; GroEL: chaperoninGroEL; HcpC: Helicobacter cysteine-rich protein C; OR: odds ratio; UreA: urease subunit A; VacA: vacuolating toxin.

Table 4. Association between *H. pylori* individual antibodies and evolution of gastric lesions by backward stepwise multivariate logistic regression[1]

	Progression		
	No (*n*, %)	Yes (*n*, %)	OR (95% CI)[2]
CagA	282 (84.94)	114 (94.21)	**2.89 (1.27,6.59)**
GroEL	215 (64.76)	97 (80.17)	**2.20 (1.33,3.64)**

[1]Age, sex and status of CagA, VacA, GroEL, UreA, HcpC and gGT were included in the initial model.
[2]Adjusted for age and sex.
Abbreviations: CagA: cytotoxin-associated antigen A; CI: confidence interval; GroEL: chaperoninGroEL; OR: odds ratio.

versus 78.0% for DYS[22] or 83.5% for CAG *versus* 75.2% for DYS[23]) and titers. Indeed, when comparing baseline serum IgG titer by ELISA between CAG and DYS groups of cohort 1, we found that the mean IgG titer was lower in the DYS group than in the CAG group (2.79 ± 1.64 for CAG *versus* 2.43 ± 1.80 for DYS, $p = 0.0128$). This is in line with our observation that the recomLine score in the DYS group was lower than in CAG and IM groups, indicating that loss of *H. pylori* strains may occur at this stage of disease.

Our results on CagA are consistent with previous studies showing that seropositivity for CagA was associated with the risk of GC development,[12] confirming that CagA is an oncogenic virulence. In our study, only CagA antibody response was identified as independent predictor for risk of advanced gastric lesions as well as progression of gastric lesions, suggesting that CagA might have a stronger antigenicity compared to the other antigens studied. Several studies have suggested that CagA-positive strains are dominating in the Chinese population.[24,25] In our study, seropositivity of CagA was 83.9%, similar to our previous studies in this high-risk area by ELISA (77.5% among adult and 88.5% among children).[26,27] In future, it should be interesting to compare serologic responses to different CagA variants (derived from western or Asian stains) by such recomLine assay. However, as the immune response to such antigens is polyclonal, it might not be possible to differentiate the immune reaction toward single epitopes when using recombinant proteins.

Apart from CagA, we were interested to identify other *H. pylori* proteins or different combinations to generate a multiplex *H. pylori* marker. We found that seropositivities for VacA and GroEL were also associated with the risk of CAG in the univariate analysis, although no significant differences were found by the multivariate analysis. We further observed that seropositivities for CagA and GroEL were significantly associated with the progression of gastric lesions. Our findings are consistent with a previous study that CagA and GroEL were independent predictors of GC,[13] and provide further evidence that these two markers may be risk markers for the progression of *H. pylori*-related gastric lesions.

Table 5. Association between number of *H. pylori* antibodies and evolution of gastric lesions

No. of positive antibodies	Progression			Regression		
	No (*n*, %)	Yes (*n*, %)	OR (95% CI)[1]	No (*n*, %)	Yes (*n*, %)	OR (95% CI)
≤2	111 (33.43)	19 (15.7)	1	100 (27.93)	30 (31.58)	1
3–4	175 (52.71)	78 (64.46)	**2.54 (1.45,4.43)**	198 (55.31)	55 (57.89)	0.93 (0.56,1.54)
5–6	46 (13.86)	24 (19.83)	**3.19 (1.59,6.42)**	60 (16.76)	10 (10.53)	0.54 (0.25,1.19)
$p_{for\ trend}$	–	–	**0.003**	–	–	0.094

[1]Logistic regression analysis, adjusted for age and sex.
Abbreviations: CI: confidence interval; OR: odds ratio.

GroEL is a protein that belongs to the chaperone family which is required for the proper folding of many proteins in the bacteria.[28] It was reported that GroEL was associated with the adhesion of *H. pylori* to gastric epithelial cells and induction of inflammatory responses.[29,30] GroEL is a new virulence factor of *H. pylori* and widely expressed in most, if not all, *H. pylori* strains.[13,31] The findings for GroEL seropositivity in our study are of specific interest because it may be helpful to identify high-risk *H. pylori* strain in the Chinese population. Our study showed that GroEL was an independent predictor for progression of gastric lesions. As cross reactivity between *H. pylori* and human GroEL has been described,[32,33] such cross-reacting antibodies might contribute to the inflammatory response and thus a more severe gastric pathology.

GGT was identified as a new virulence factor of *H. pylori* associated with immune evasion, bacterial colonization and cell apoptosis,[34,35] while the relationship with GC and its precursors was unclear. Interestingly, we found that seropositivity for gGT was inversely associated with the presence of preneoplastic gastric lesions. It is tempting to speculate that here, antibodies against gGT might interfere with the above mentioned gGT effects, thereby ameliorating the inflammation epithelial damage. Further studies with a larger sample size are needed to confirm these findings.

In addition, we observed that the progression risk was more pronounced in subjects with more than three antibody responses. This result further confirmed our findings and suggested that a high test score with more than three positive antigens may indicate the progression of gastric lesions and thus facilitate treatment decision of *H. pylori*-related gastric lesions. It is interesting to note that some patients with advanced gastric lesions showed lower serum responses compared to patients with other gastric pathology. One may speculate that in these patients, infection was lost during progression of gastric lesions, and thereby seropositivities declined.

Using ^{13}C-UBT as a gold standard in our study, the specific *H. pylori* multiplex line assay revealed a very high sensitivity and specificity, which could further be adopted in large epidemiological studies, and be particularly helpful in validation of borderline measurement and identification of individuals being at high risk for GC in the population.

The strength of our study was shown in certain aspects. First, the long-term follow-up, high completeness of endoscopy data, blinded assessment of pathology and *H. pylori* proteins and randomized factorial design of intervention trial allowed us to evaluate the association between the distinct immune responses toward *H. pylori* proteins and evolution of precancerous gastric lesions. Second, a spectrum of gastric lesions allows us to evaluate the effect of *H. pylori* proteins on the risk of advanced gastric lesions. Furthermore, our population-based study has less selection bias and thus allows more reliable conclusions.

Our study also has some limitations. Because very few subjects were diagnosed with normal gastric mucosa in this population, we used subjects with SG as controls from an independent study performed in parallel. The fact that controls with SG may dilute the disparity between cases and controls, which could cause an underestimation of the results. In addition, the exact mechanism of *H. pylori* proteins such as GroEL and gGT on the gastric carcinogenesis needs to be further clarified.

Conclusion

Through this population-based study and recomLine test for detection of multiple serum responses toward *H. pylori* proteins, we found that seropositivities for CagA and GroEL were independent risk markers for progression of gastric lesions, and more than three positive antibodies may be helpful to stratify subjects with *H. pylori* as high and low risk of GC.

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