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Validation of a blood biomarker for identification of individuals at high risk for gastric cancer

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Abstract

Background: *Helicobacter pylori* is the leading cause of gastric cancer, yet the majority of infected individuals will not develop neoplasia. Previously we developed and replicated serologic *H. pylori* biomarkers for gastric cancer risk among prospective cohorts in East Asia, and now seek to validate the performance of these biomarkers in identifying individuals with premalignant lesions.

Methods: This cross-sectional study included 1,402 individuals from Linqu County screened by upper endoscopy. *H. pylori* protein-specific antibody levels were assessed using multiplex serology. Multivariable-adjusted logistic regression models were used to calculate odds ratios

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(ORs) and 95% confidence intervals (CIs) for prevalent intestinal metaplasia, indefinite dysplasia, or dysplasia, compared to superficial or mild atrophic gastritis.

Results: Compared to individuals sero-negative to Omp and HP0305, individuals sero-positive to both were seven times more likely to have precancerous lesions (OR, 7.43; 95% CI, 5.59–9.88). A classification model for precancerous lesions that includes age, smoking, and sero-positivity to *H. pylori*, Omp, and HP0305 resulted in an area under the curve (AUC) of 0.751 (95% CI, 0.725–0.777), which is significantly better than the same model including the established gastric cancer risk factor CagA (AUC, 0.718; 95% CI, 0.691 −0.746, $p_{difference}$=0.0002).

Conclusions: The present study of prevalent precancerous gastric lesions provides support for two new serum biomarkers of gastric cancer risk, Omp and HP 0305.

Impact: Our results support further research into the serological biomarkers Omp and HP0305 as possible improvements over the established virulence marker CagA for identifying individuals with precancerous lesions in East Asia.

Keywords

Helicobacter pylori; biomarker; gastric lesions; gastric cancer; East Asia

INTRODUCTION

Infection with the micro-aerophilic, spiral bacterium *Helicobacter pylori* is the leading cause of gastric cancer (GC), the fifth most common cancer worldwide (1), and is overall responsible for more total incident cancers each year than any other single infectious agent (2). While a vaccine against this bacterium has not yet been successfully developed, there exists effective eradication therapy in the form of two weeks of triple or quadruple therapy, involving treatment with two to three antibiotics plus a proton pump inhibitor and/or bismuth (3, 4). However, mass eradication is neither feasible nor recommended as half of the global population harbors this bacterium but the vast majority of these individuals will not develop neoplasia (5). Moreover, population-based *H. pylori* eradication could increase antibiotic resistance, and in addition some benefits have been observed with carriage of the bacteria, including reduced incidence of esophageal disease (6).

Thus, there remains a pressing need to identify those individuals at highest risk for GC for targeted cancer prevention through *H. pylori* eradication treatment, which has been shown to reduce risk for this malignancy (7). This is particularly important in the region of East Asia, where over half of all incident GCs occur in the world each year (1). In our efforts to achieve this aim, we developed a serologic *H. pylori* biomarker panel for GC risk in a cohort of urban men in Shanghai, China, using a fluorescent bead-based multiplex serology assay developed at the German Cancer Research Center (8). We then replicated this initial finding in a consortium of eight prospective cohorts in China, Japan, and Korea, among 1,608 incident non-cardia GCs and 1,958 matched controls. In this consortium, we found that sero-positivity to two, Omp and HP0305, of the initial six identified *H. pylori* proteins (Omp, HP0305, HyuA, HpaA, CagA, and VacA), were strongly and consistently associated with cancer risk among all cohorts, so that prior to cancer diagnosis, sero-positivity to both,

compared to sero-positivity to neither, was associated with an over four-fold increase in the odds of GC incidence (9).

In the present study, we sought to validate these *H. pylori* blood biomarkers for precancerous gastric lesions in an independent East Asian population, that of the high-risk population in Linqu County, Shandong Province, China. We assessed whether our previously identified risk markers could identify individuals with prevalent gastric precursor lesions, specifically those that are on the cascade of events leading to GC.

MATERIALS AND METHODS

Study population

In 2002, an intervention trial was established in Linqu County, Shandong Province, China, to compare the effect of *H. pylori* treatment and selective COX-2 inhibitors on precancerous gastric lesions. At baseline, study subjects completed a standard structured questionnaire; provided a blood sample; and were screened by upper endoscopy. Details of these methods have been published previously (10); briefly, 3,161 residents aged 35–64 from 12 randomly selected villages in Linqu were assessed for eligibility, and 2,813 (89%) individuals agreed to participate in the initial screening. Four experienced gastroenterologists conducted the endoscopies, and five biopsy samples were taken from the standard sites in the stomach according to the Updated Sydney System (11). A global diagnosis was then made for each participant based on the biopsy specimen with the most severe diagnosis. A panel of three pathologists then reviewed each slide and graded as normal, superficial gastritis (SG), chronic atrophic gastritis (CAG), intestinal metaplasia (IM), indefinite dysplasia (Ind DYS), dysplasia (DYS,), and cancer, following the criteria of the Updated Sydney System (11) and the Padovo International Classification (12). At baseline, a 5-mL sample of blood was also collected from each study participant, allowed to clot for 30 to 40 minutes at room temperature, and then centrifuged at 965 g for 15 minutes. Serum was then aliquoted into vials and frozen immediately at –20°C and stored in a –70°C freezer.

For the present study, a total of 1,402 individuals screened by upper endoscopy at baseline were included. Because there were so few participants with normal gastric mucosa, 512 participants with SG (138) or mild CAG (374) were randomly selected as the control group. Furthermore, all participants with IM (n=412) and DYS (n=145) were included, and 333 participants with Ind DYS were randomly selected as the precancerous gastric lesions group. A written informed consent was obtained from each participant and the study was approved by the Institutional Review Board of Peking University Cancer Hospital.

H. pylori multiplex serology

Serum samples from all study participants were assayed for antibodies to 13 *H. pylori* recombinantly expressed fusion proteins (UreA, Catalase, GroEL, NapA, CagA, HP0231, VacA, HpaA, Cad, HyuA, Omp, HcpC, and HP0305) (13, 14). As previously described, *H. pylori* multiplex serology is based on a glutathione *S*-transferase capture immunosorbent assay combined with fluorescent bead technology (Luminex) to simultaneously detect human IgA, IgM, and IgG antibodies to selected *H. pylori* proteins. Calculation of antigen-

specific cutoff points (mean of the median reporter fluorescence intensity [MFI] plus three times SD, excluding positive outliers) was done using 17 *H. pylori*-negative sera previously classified for *H. pylori* status run within the same experiment. Defining *H. pylori* sero-positivity as reactivity with ≥4 proteins has shown good agreement (k = 0.70) with commercial serologic assay, resulting in 89% sensitivity and 82% specificity (13).

Pepsinogen assay

Pepsinogen I and II levels in serum were determined by Pepsinogen I and II ELISA assay kits (Eagle Biosciences, Nashua, NH, USA) according to manufacturer's instructions. Briefly, 25 μl of serum for measurement of Pepsinogen I and 50 μl of serum for Pepsinogen II, respectively, were applied in duplicates to a Streptavidin coated microplate. After incubation with the respective capture and tracer antibody HRP substrate was added for signal detection. The reaction was stopped with stop solution and the absorbance was measured at 450 nm in a microplate reader. Provided assay standards were run on each plate to obtain a plate specific standard curve for determination of the concentration (ng/ml) of Pepsinogen I and II in each sample. Two control samples with a given Pepsinogen I and II concentration were provided by the manufacturer and applied on each plate to ensure reliability of the assay result.

Statistical analysis

Initially, we sought to validate the analyses previously performed among the prospective cohort studies from East Asia included in the *H. pylori* Biomarker Cohort Consortium (HpBCC) (9). Accordingly, we first assessed the individual associations of sero-positivity to each of the 13 *H. pylori* antigens included in the multiplex serology panel, using logistic regression to produce ORs and 95% CIs for each type of prevalent precancerous gastric lesion (IM, Ind DYS, and DYS) after adjusting for age (continuously) and smoking status (current vs. not current), factors that were associated both with *H. pylori* status and disease outcome in this population. However, when comparing ORs for *H. pylori* overall and for each individual antigen, there were no statistically significant differences in the results by type of precancerous lesion, so we combined them all into one outcome. The Bonferroni correction was applied to recognize p-values at ≤0.0038 (0.05/13 markers).

We then examined the association of combined Omp and HP0305 sero-positivity with odds of prevalent precancerous lesion, as that was the strongest finding in the HpBCC. As before, we created three categories: sero-negativity to both (reference); sero-positivity to only one; and sero-positivity to both. To compare these markers with the established virulence factor for GC risk, CagA, we also examined the association of prevalent precancerous lesion with dual *H. pylori*-positive (seropositive to ≥4 proteins) and CagA-positive status. Finally, we repeated the panel of six antigens found in the Shanghai Men's Health Study (8) and replicated in the HpBCC for association with risk. For all, we used logistic regression adjusting for age and smoking.

We also examined the data for potential differences in the association by sex and smoking status through stratified analyses, and the use of a multiplicative interaction term to assess effect modification, but no differences were found.

To create a classification model for prevalent precancerous lesion, we considered two populations: all study participants, and only those who were *H. pylori* positive. The rationale is that our primary motivation was to determine a biomarker for high-risk of GC, so that those individuals could be targeted for *H. pylori* eradication, and thus limiting the population to *H. pylori*-positive individuals would make sense in this instance. However, beyond eradication therapy for *H. pylori*-positive individuals, identification of high-risk individuals in the population at large (regardless of *H. pylori* status) could also be beneficial in terms of discovering precursor lesions earlier, and in changing screening schedules so to diagnose GC at earlier stages. Thus, we performed receiver operating characteristic (ROC) curve analyses among both populations to calculate the area under the curve (AUC) and to compare models with and without *H. pylori* antibody biomarkers.

As a secondary analysis, we performed ROC curve analyses among the subset of participants for whom a valid pepsinogen result was obtained, to determine if this measure of gastric atrophy strengthens the model. Among these 546 individuals (226 controls and 320 cases), the pepsinogen I:II ratio was independently associated with prevalence of precancerous lesion (OR for individuals with a ratio <4, compared to those ≥4 = 2.37; 95% CI = 1.15 to 4.90). However, inclusion of the pepsinogen I:II ratio did not change the main results with Omp and HP0305, and did not significantly improve the AUC. Moreover, the pepsinogen I:II ratio was highly inversely associated with Omp and HP0305 status (which are themselves highly correlated, Pearson correlation coefficient of 0.52, $p<0.0001$), with Pearson correlation coefficients of −0.34 and −0.24, respectively (both with p-values of <0.0001). Thus, as this measure was available on less than half of our study population, was highly correlated with our serologic antigen biomarkers, and did not improve the classification ability beyond our validated risk markers, we did not include it in our final models.

RESULTS

Individuals in Linqu County with prevalent precancerous lesions (IM, Ind DYS, or DYS), as compared to controls (those with SG or mild CAG), were more likely to be of older age and a current smoker (Table 1).

Overall, 54% of controls were identified as *H. pylori* sero-positive as compared to 83% of individuals with prevalent precancerous lesions, leading to an age- and smoking status-adjusted odds ratio of 4.51 (95% CI, 3.50–5.81; $p<0.0001$). Controls were also less likely than the cases to be sero-positive to each of the 10 *H. pylori* proteins previously identified as potential risk biomarkers in the HpBCC, the consortium of prospective studies in East Asia that this study sought to validate (all p values of <0.0001), as well as an eleventh antigen, HP0231 ($p=0.008$) (Table 2).

The strongest association among the individual antigens was for Omp, sero-positivity to which was associated with an over 5-fold odds for the prevalence of precancerous lesions (OR, 5.37; 95% CI, 4.20–6.89). These results did not significantly differ when separating out cases by individual diagnosis (IM, Ind DYS, or DYS) (Supplemental Table 1).

In replicating the panel of six antigens (Omp, HP0305, HyuA, HpaA, CagA, and VacA) originally created in the preliminary work for this study among the participants of the Shanghai Men's Health Study (8), sero-positivity to 4–5 or all 6 of these specific *H. pylori* antigens, compared to sero-positivity to three or fewer, resulted in a significant over 4-fold increase in the odds of prevalent precancerous lesion (OR, 4.69; 95% CI, 3.63–6.07; and OR, 4.41; 95% CI, 2.92–6.66) (Table 3). When focused on the two antigens found to be the strongest markers of risk in the HpBCC, compared to individuals sero-negative to both Omp and HP0305, individuals sero-positive to one or to both were at increased odds of a prevalent precancerous lesion (OR, 3.29; 95% CI, 2.43–4.46; and OR, 7.43; 95% CI, 5.59–9.88, respectively). Among *H. pylori*-positive participants only, the strength of these associations were slightly reduced, but all remained statistically significant, with the strongest association still for those sero-positive to both Omp and HP0305 compared to those sero-negative to both (OR, 6.20; 95% CI, 3.97–9.66) (Supplemental Table 2).

Finally, a classification model for prevalent precancerous lesion in Linqu County, including age (continuous), smoking status (current vs. not current), *H. pylori* status (defined as sero-positivity to 4 or more of the 13 *H. pylori* antigens assessed), and Omp and HP0305 antibody status (separately), resulted in an AUC of 0.7510 (95% CI, 0.7245–0.7774). This model was significantly better than one that included age, smoking, *H. pylori* status, and sero-positivity to the established *H. pylori* virulence factor CagA (AUC, 0.7184; 95% CI, 0.6908–0.7461, p for difference in the AUC with the Omp and HP0305 model = 0.0002), as well as to the same model but with *H. pylori* status included only (AUC, 0.7143; 95% CI, 0.6864–0.7422, p for difference in the AUC with the Omp and HP0305 model < 0.0001) (Figure 1 and Table 4). When limiting the population for the model to *H. pylori*-positive individuals only, the stronger predictive ability of Omp and HP0305 as compared to CagA remained (AUC, 0.7139; 95% CI, 0.6773–0.7505, compared to AUC, 0.6623; 95% CI, 0.6248–0.6998, respectively, p value for difference = 0.0004) (see Supplemental Table 3). For a range of probabilities, the positive predictive value (PPV) of the combined status of Hp + Omp+ HP0305+ in our population is fairly high, at >80% for the majority, but these biomarkers did not achieve a similarly high negative predictive value (ranging from 70.5% down to 46.2%).

DISCUSSION

In this cross-sectional study of GC precursor lesions among a high-risk population in China, we validated two *H. pylori* biomarkers we originally identified in a pilot study of urban men in Shanghai, China, and then replicated in a consortium of prospective cohort studies of men and women in China, Japan, and Korea. The consistency and strength of the associations with antibody sero-positivity to the *H. pylori* proteins Omp and HP0305 suggest that these biomarkers could substantially add to a screening program that seeks to identify individuals at highest risk for GC for closer surveillance and to be targeted for *H. pylori* eradication, an established method for reducing GC risk (15, 16). Adding motivation for this plan is the data from China showing that *H. pylori* eradication, even among individuals who have already developed precancerous lesions (particularly IM or DYS, the outcomes in the present study) is as, if not more, effective in reducing GC incidence than among those with normal or CAG histopathology (17).

Examining antibodies to multiple *H. pylori*-specific proteins to uncover potential biomarkers of GC risk has been performed in other populations, the original of which was a German case-control study that found significant associations between antibodies to eight individual *H. pylori* proteins, including HP0305, with an OR of 2.34 (95% CI, 1.46–3.74), but not Omp (OR, 1.41; 95% CI, 0.89–2.26) (18). Since our original publication of our pilot study in 2012 (8), second to be performed only to the study above, there have been numerous additional studies, with findings generally strongest for the known virulence factors VacA and CagA. Specifically, recently in the MCC-Spain case-control study, only sero-positivity to CagA and VacA were found to be individual predictors of non-cardia GC risk, with no associations found for Omp or HP0305 (19). In a case-control study in northeastern Iran, a population with a high risk of GC and a high prevalence of *H. pylori*, again only antibodies to CagA and VacA were found to be associated with risk, with no associations found for Omp or HP0305 (20). In a Swedish case-control study, all antigens were significantly associated with GC risk, and after performing principal component analysis, the authors derived two factors associated with increased risk of non-cardia GC: the first, and most strongly associated with risk, included CagA, VacA, and Omp; the second included NapA and Catalase (21). The only other study to explore results from the German Cancer Research Center *H. pylori* multiplex serology assay in East Asian populations – the geographic region in which the greatest number of GCs occur each year – was in the Linxian Nutrition Intervention Trial cohort (9). In this study of one population in China, and the only other prospective investigation, only two *H. pylori* antigens passed the Bonferroni correction for multiple testing – Omp and HP0305 (OR, 2.30; 95% CI, 2.36–3.88; and OR, 2.16; 95% CI, 1.40–3.33) (22). Also, previously, a subset of Linqu County samples were assayed by recomLine analysis (Mikrogen, Munich, Germany) (23) to determine sero-positivity to six *H. pylori* antigens (CagA, VacA, GroEL, UreA, HcpC, and gGT), and found that CagA was an independent predictor of advanced gastric lesions (24). Longitudinally, both CagA and GroEL were also seen to predict progression of gastric lesions, although neither Omp nor HP0305 were included in these analyses.

In all of our analyses of *H. pylori* antigen-specific association with GC risk and within each individual East Asian study population – in the Shanghai Men's Health Study (8); the individual cohorts that comprise the HpBCC (Japan Public Health Center-based Prospective Study I and II; Korean Cancer Prevention Study II; Korean Multicenter Cancer Cohort I; Linxian Nutrition Intervention Trial; Shanghai Men's Health Study newly-identified cases; and the Shanghai Women's Health Study) (9); and now the Linqu County trial, reported on in the present manuscript – antibodies to Omp and HP0305 have significantly been associated with GC risk. This consistency highly suggests that these are replicable markers of risk for East Asian populations. These results also support more research into the mechanisms of Omp (an outer membrane protein, known as HP1564) and HP0305 (a hypothetical protein, also shown to be expressed in outer membrane vesicles). Previously, studies have been conducted to characterize these proteins, suggesting their roles in bacterial colonization and as pro-inflammatory agents (25–29), but examination of the role of these proteins in carcinogenesis on the molecular level have not yet been performed.

Other groups have also looked to develop risk prediction models for GC that include biomarkers, such as two from Japan: in Fukuoka, for which the model included a dual

measure incorporating *H. pylori* IgG antibodies and pepsinogen levels, and hemoglobin A1c levels, in addition to age, sex, and smoking status (30); and from the National Cancer Center in Tokyo, whose model included again *H. pylori* titers and pepsinogen levels, along with age, sex, smoking status, family history of GC, and consumption of highly salted food (31). Both of these models produced c-statistics above 0.70 but below 0.80, as in our current study. In a rural county of Northern China with high GC mortality, a risk prediction model for gastric precancerous lesions of five circulating biomarkers (pepsinogen I, pepsinogen II, pepsinogen I/II ratio, *H. pylori* IgG status, and gastrin-17 levels) produced a c-statistic of 0.803 (32). In the present study, pepsinogen levels did not significantly improve the predictive capability of our classification model for prevalent precancerous lesion: among individuals with a valid pepsinogen assay, the model including age, smoking, *H. pylori* status, and Omp and HP0305 sero-positivity, the AUC was 0.7454; adding pepsinogen moved it only slightly to 0.7477. Among *H. pylori*-positive individuals, there was also no appreciable difference (AUCs of 0.7043 and 0.7070, respectively), and pepsinogen was no longer significantly associated with prevalence (OR, 1.92; 95% CI, 0.87–4.20). Furthermore, pepsinogen was highly correlated with Omp and HP0305 in this study, with pepsinogen I:II ratios decreasing (indicating greater gastric atrophy) with increasing Omp/HP0305 category, so that for individuals sero-negative to both Omp and HP0305 the median pepsinogen ratio was 15, compared to 12 for individuals sero-positive to only one, and 7 for individuals sero-positive to both (all p-values comparing pepsinogen ratios by the Wilcoxon rank sum test <0.01).

While it is a limitation of the present study that we do not have pepsinogen successfully measured on all participants, our results on the subset of individuals with valid pepsinogen measurements suggests adding pepsinogen does not improve the classification ability of the model. In Japan and Korea where pepsinogen is assayed regularly, different technologies are used (including latex agglutination and immunoradiometric assay) (33), and as part of their large screening programs can take place soon after blood draw, thus avoiding the degradation of samples. Furthermore, a recent study to explore differences in results comparing three different pepsinogen tests did find some significant differences comparing the Biohit ELISA assay (similar to our Eagle BioSciences ELISA) with the Japanese Eiken latex agglutination system, although in general concluded that the assays have “good relative agreement” (34). Finally, it is possible that pepsinogen does not add value to our classification model in that unlike the models presented above that sought to predict future GC, ours was developed to determine prevalent precancerous lesion, which may be a state when atrophy is no longer the strongest signal.

Our finding of a validated biomarker that includes antibodies to just two specific *H. pylori* antigens, that can be determined in one assay, versus the previous models above all requiring at least 3 assays (2 for pepsinogen I and II and 1 for *H. pylori*, plus HbA1c), as well as other lifestyle characteristics not always available in electronic medical records, suggests the Omp and HP0305 markers could potentially be part of a feasible test for determining risk among a large population base. Furthermore, none of these previous risk prediction models considered the heterogeneity of *H. pylori*, which is particularly important in East Asia, where the majority of the population is *H. pylori*-positive. In fact, when we compared our precancerous lesion classification model including the Omp and HP0305 biomarker to the

same model with *H. pylori* dichotomous status alone, the Omp/HP0305 model performs significantly better (AUC, 0.7510; 95% CI, 0.7245–0.7774 compared to AUC, 0.7143; 95% CI, 0.6864–0.7422, p for difference<0.0001; Supplemental Figure 1). Another limitation is that we did not additionally have a conventional measure of *H. pylori* such as an ELISA or immunoblot assay for comparison to our serological measures. However, in our previous work in the Shanghai Women's Health Study we found a similar if slightly higher prevalence of *H. pylori* among controls by multiplex serology (94.6%) than using conventional methods (92.2%), which is what might be expected as it is possible that multiplex serology is more sensitive than standard ELISA (9, 35). Additionally, in the study in the Linxian Nutrition Intervention Trial alone, adjustment for conventional ELISA *H. pylori* sero-positivity did not change the significant results found using multiplex serology with Omp and HP0305 (22). Finally, we must also note that a limitation of the present study is that we did not produce a prediction model for risk of incidence GC, but rather one for prevalent gastric precancerous lesion. However, the findings all validate what we found previously in prospective cohort studies, and it is well established that individuals with precancerous lesions are at highest risk for GC. The present study also was performed among a population at high risk in East Asia, as with the previous studies in our group. The ability to generalize our results to non-East Asian populations is not known.

In conclusion, in populations with a high prevalence of the known carcinogen *H. pylori* and a high incidence of GC, it has been established that biomarkers are needed to identify individuals at highest risk of developing cancer for targeted eradication. Our identification, replication, and now validation of two novel *H. pylori* biomarkers for GC risk in East Asia, Omp and HP0305, have specific importance for contributing to targeted *H. pylori* eradication schemes, as the treatment for this cancer-causing bacteria is a relatively straight-forward course of 10 to 14 days of two to three antibiotics and a proton pump inhibitor, which has already been shown in clinical trials to reduce gastric cancer risk by 50%. Furthermore, the evidence presented here suggests that these two markers can contribute to a high-risk classification model in East Asia, as they predict prevalence of precancerous gastric lesions beyond the established, and highly prevalent, known virulence marker of CagA. Moreover, they can be easily measured to then result in AUCs at a similar level to risk prediction models that include larger panels of biomarkers that are also more cost- and time-intensive to assay.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AUC	area under the curve

CI	confidence interval
OR	odds ratio
HpBCC	*Helicobacter pylori* Biomarker Cohort Consortium

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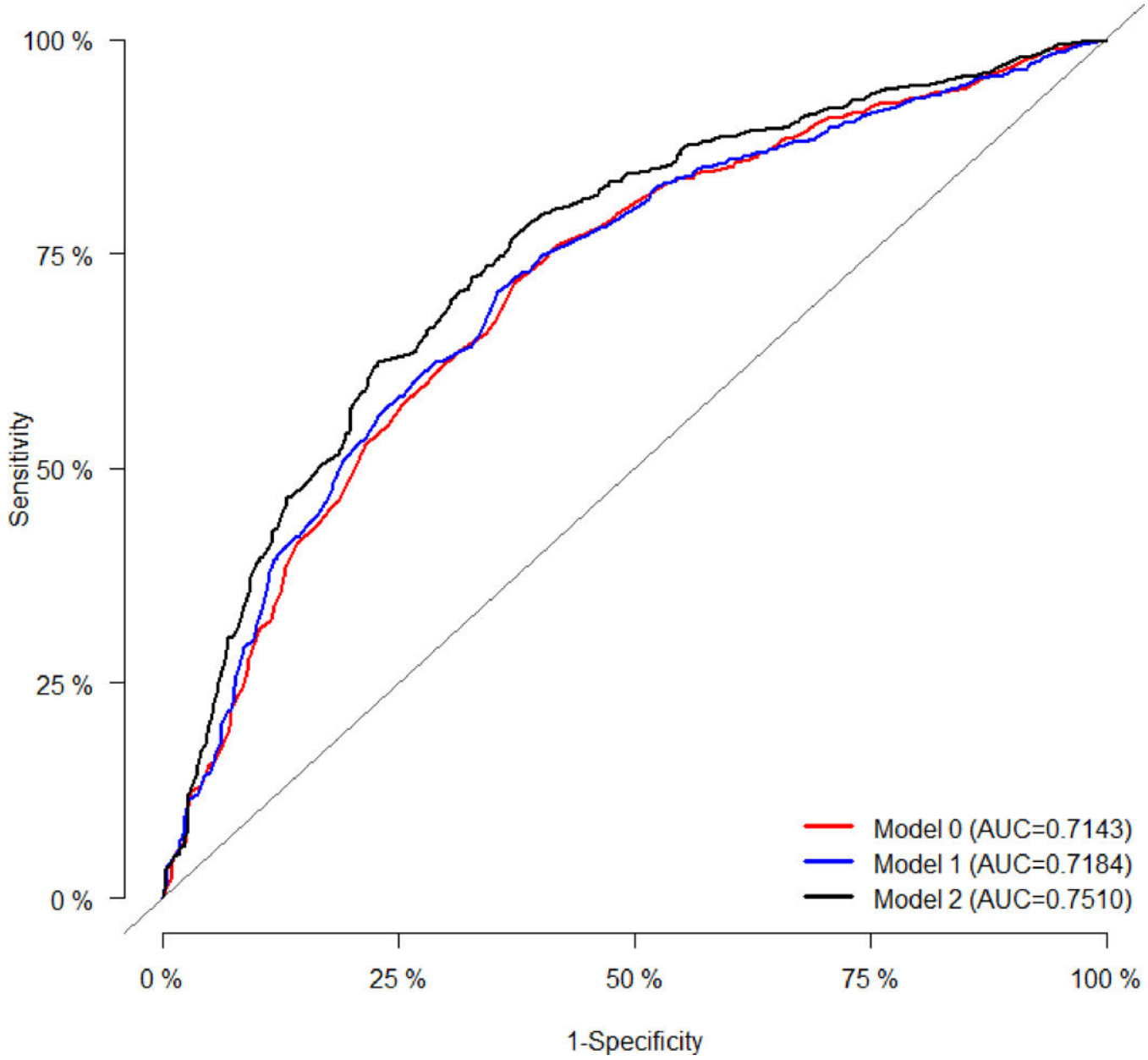

Figure 1:

Receiver-operator characteristic curves for discriminating controls (superficial or mild atrophic gastritis) from individuals with gastric pre-cancerous lesions (intestinal metaplasia, indefinite dysplasia, and dysplasia) by model. Model 0: age, smoking, and *H. pylori* sero-positivity; Model 1: age, smoking, *H. pylori* sero-positivity, and CagA sero-positivity; and Model 2: age, smoking, *H. pylori* sero-positivity, Omp sero-positivity, and HP0305 sero-positivity.

Table 1:

Demographic characteristics of the validation population, Linqu County, Shandong Province, China, 2002–2004 (N=1,402)

	Controls		Precancerous gastric lesions					
	Superficial gastritis/ mild CAG (N=512)		Intestinal metaplasia (N=412)		Indefinite dysplasia (N=333)		Dysplasia (N=145)	
	N	%	N	%	N	%	N	%
Sex								
Female	304	59%	232	56%	152	46%	58	40%
Male	208	41%	180	44%	181	54%	87	60%
Age								
≤40	62	12%	32	8%	22	7%	6	4%
40–49	269	53%	210	51%	150	45%	70	48%
50–59	150	29%	139	34%	126	38%	55	38%
≥60	31	6%	31	8%	35	11%	14	10%
Current smoker								
No	341	67%	269	65%	167	50%	66	46%
Yes	171	33%	143	35%	166	50%	79	54%
Family history								
No	493	96%	387	94%	313	94%	137	94%
Yes	19	4%	25	6%	20	6%	8	6%

Table 2:

Prevalence odds ratios for precancerous gastric lesions by previously identified *H. pylori* antigens (N=1,402)

	Controls		Precancerous gastric lesions				
	N	%	N	%	OR	95% CI	p-value
H. pylori+[a]	274	54%	738	83%	4.51	3.50–5.81	<0.0001
Omp +	231	45%	720	81%	5.37	4.20–6.89	<0.0001
CagA +	307	60%	724	81%	3.23	2.51–4.15	<0.0001
VacA +	304	59%	740	83%	3.75	2.90–4.85	<0.0001
HcpC +	178	35%	624	70%	4.50	3.56–5.70	<0.0001
HP0305 +	149	29%	547	61%	3.85	3.04–4.88	<0.0001
GroEl +	223	44%	640	72%	3.37	2.68–4.25	<0.0001
NapA +	153	30%	409	46%	2.02	1.60–2.55	<0.0001
HyuA +	165	32%	390	44%	1.61	1.28–2.03	<0.0001
Cad +	88	17%	285	32%	2.25	1.72–2.96	<0.0001
HpaA +	110	21%	273	31%	1.69	1.31–2.19	<0.0001
HP 0231 +	35	13%	547	61%	1.53	1.11–2.09	0.0084
Catalase +	191	37%	367	41%	1.18	0.94–1.48	0.1466
UreA +	146	29%	288	32%	1.15	0.91–1.47	0.2358

NOTE: Odds ratios adjusted for age and smoking status, reference groups comprises those antigen-negative

[a] defined as sero-positive to ≥4 *H. pylori* antigens of 13-plex

Table 3:

odds of precancerous gastric lesions, all patients (n=1,402)

	Controls		Precancerous gastric lesions				
	N	%	N	%	OR	95% CI	p-value
Hp panel							
0–3 sero+	360	70	307	35	(ref)		
4–5 sero+	118	23	457	51	4.69	3.63–6.07	<0.001
6 sero+	34	7	126	14	4.41	2.92–6.66	<0.001
P for trend[a]							<0.001
Omp & HP0305							
Omp- and HP0305-	260	51	147	17	(ref)	-	-
Omp+ or HP0305+	124	24	219	25	3.29	2.43–4.46	<0.001
Omp+ and HP0305+	128	25	524	59	7.43	5.59–9.88	<0.001

NOTE: Adjusted for age and smoking status

[a]Cochrane-Armitage trend test

Table 4:

Prevalence odds ratios for classification model of precancerous gastric lesions

	Model 0		**Model 1**		**Model 2**	
	AUC = 0.7143		**AUC = 0.7184**		**AUC = 0.7510**	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (continuous)	1.04	1.03–1.06	1.05	1.03–1.06	1.05	1.03–1.07
Current smoker	1.66	1.30–2.11	1.68	1.32–2.15	1.45	1.13–1.86
H. pylori+[a]	4.51	3.50–5.81	3.62	2.62–4.98	1.68	1.20–2.36
CagA +	—	—	1.44	1.03–2.00	—	—
Omp +	—	—	—	—	2.98	2.17–4.07
HP0305 +	—	—	—	—	1.73	1.28–2.34

NOTE: Odds ratios adjusted for all variables in the model

[a]defined as sero-positive to ≥4 *H. pylori* antigens,

p-value for difference in the AUCs of Model 0 vs. Model 1 = 0.1524

p-value for difference in the AUCs of Model 0 vs. Model 2 = <0.001

p-value for difference in the AUCs of Model 1 vs. Model 2 = 0.0002