"True" *Helicobacter pylori* infection and non-cardia gastric cancer: a pooled analysis within the Stomach cancer Pooling (StoP) Project

Running head: *H. pylori* infection and non-cardia gastric cancer

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Significance statement: This pooled analysis within a global consortium of case-control studies found a significant association between *H. pylori* infection and non-cardia gastric cancer following the reclassification of *H. pylori* negative infection status as positive considering the presence of anti-CagA antibodies, evidence of gastric atrophy or an advanced stage at non-cardia gastric cancer diagnosis. Our classification algorithm may be useful for future studies.

Abstract

Background: Helicobacter pylori (*H. pylori*) is the most important risk factor for non-cardia gastric cancer (NCGC); however, the magnitude of the association varies across epidemiological studies. This study aimed to quantify the association between *H. pylori* infection and NCGC, using different criteria to define infection status.

Methods: A pooled analysis of individual-level *H. pylori* serology data from eight international studies (1325 NCGC and 3121 controls) from the Stomach cancer Pooling (StoP) Consortium was performed. Cases and controls with a negative *H. pylori* infection status were reclassified as positive considering the presence of anti-Cag A antibodies, gastric atrophy or advanced stage at diagnosis, as available and applicable. A two-stage approach was used to pool study-specific adjusted odds ratios (OR), and 95% confidence intervals (95%CI). A meta-analysis of published prospective studies assessing *H. pylori* seropositivity in NCGCs was conducted.

Results: The OR for the association between serology-defined *H. pylori* and NCGC was 1.45 (95%CI:0.87-2.42), which increased to 4.79 (95%CI:2.39-9.60) following the reclassification of negative *H. pylori* infection. The results were consistent across strata of sociodemographic characteristics, clinical features and lifestyle factors, though significant differences were observed according to geographic region – a stronger association in Asian studies. The pooled risk estimates from the literature were 3.01 (95%CI:2.22-4.07) for ELISA or EIA and 9.22 (95%CI:3.12-27.21) for immunoblot or multiplex serology.

Conclusion: The NCGC risk estimate from StoP based on the reclassification of *H. pylori* seronegative individuals is consistent with the risk estimates obtained from the literature. Our classification algorithm may be useful for future studies.

Keywords: Consortium; *Helicobacter pylori*; Pooled analysis; Stomach neoplasms.

Introduction

Helicobacter pylori (*H. pylori*) infection is the most important risk factor for the development of non-cardia gastric cancer (NCGC), and it was estimated to be responsible for nearly 90% of cases worldwide, and approximately 5% of the total burden of all cancers globally.[1] Although there is accumulated evidence suggesting that *H. pylori* infection may be present in most NCGCs, the magnitude of the association varies across epidemiological studies.[2-4]

Methodological limitations in the detection of past *H. pylori* infection may contribute to underestimate the relationship between infection and NCGC. In retrospective studies, individuals with NCGC may test negative following the clearance of infection associated with atrophic gastritis, thus underestimating the prevalence of *H. pylori* infection.[4] As such, case-control studies are often overlooked in the assessment of the association between *H. pylori* infection and gastric cancer.[2] Additionally, *H. pylori* infection status evaluated using immunoblot in prospective studies has yielded higher risk estimates compared to enzyme-linked immunosorbent assay (ELISA).[3] Therefore, the use of more sensitive methods, including considering the presence of gastric atrophy or advanced stage at gastric cancer diagnosis to reclassify potential false-negative results as positive[5,6] may yield a more accurate estimate of the magnitude of the association between *H. pylori* infection and gastric cancer by minimizing the differential misclassification of *H. pylori* infection.

The Stomach Cancer Pooling (StoP) Project, a consortium of case-control and nested case-control studies, which uses an individual participant data approach for the evaluation of the associations between risk factors and gastric cancer,[7] has previously shown the low prevalence (6.6%) of *H. pylori* negative NCGC following the reclassification of serology-defined negative *H. pylori* infection status as positive when they presented either anti-cytotoxin-associated gene A (CagA) antibodies, gastric atrophy or advanced stage at diagnosis.[6] Therefore, the current study aimed to quantify the association between *H. pylori* infection

and NCGC, considering serological test results and additionally following the reclassification of individuals considered more likely to correspond to false-negative results as positive for infection, using an individual participant data meta-analysis of studies participating in the StoP Project.

Methods

The StoP Project

This study is based on the 3.0 version of the StoP Project dataset, which includes a total of 12,511 gastric cancer cases and 29,964 controls from 32 case-control or nested case-control studies.[7] All data were collected and harmonized according to a pre-specified format at the coordinating centre before analysis. The participating studies were conducted in accordance with applicable laws, regulations and guidelines for the protection of human subjects, and the StoP Project was approved by the University of Milan Review Board (Reference 19/15).

The present analysis used data from eight case-control studies with information on *H. pylori* infection status determined in blood samples collected before any treatment for 1390 NCGC cases, and collected at onset of disease, hospital admission or recruitment for 3121 controls. Specifically, data from two studies from Brazil,[8,9] and one study each from Iran,[10] Japan,[11] Latvia,[12] Portugal,[13] Russia[14] and Sweden[15] were included. *H. pylori* infection status was determined using ELISA to measure immunoglobulin G (IgG) antibodies in serum, using the same criteria applied in each original study. Participants with borderline results (n=81, 32 NCGC cases and 49 controls) were classified as *H. pylori* positive.

A negative serological result for *H. pylori* infection status was reclassified as positive when: a) a positive result had been obtained for CagA serology status independently of the detection of surface antibodies against *H. pylori* among cases and controls; b) gastric atrophy was present as evaluated through histological examination among NCGC cases only, or measured by serum pepsinogen (PG) levels [PGI/II≤3][5,16-18] among cases and controls; or c) tumour stage was advanced at diagnosis of NCGC, *i.e.*, stage IV, according to the *TNM Classification of Malignant Tumours*.[19] Considering the higher probability of false-negative results due to misclassification of infected subjects as non-infected among NCGC

cases,[20,21] only cases for which at least one criterion could be applied to define *H. pylori* infection status were included. As such, analyses included 1325 NCGC cases whose *H. pylori* infection status could be reclassified using at least one of the criteria described above: 853 cases from four studies,[8,9,13,15] 974 cases from six studies[8-13] and 654 cases from three studies[12-14] with data on CagA serostatus, gastric atrophy and advanced tumour stage, respectively. All controls (n=3121) were included in this analysis even if information was not available for the reclassification of *H. pylori* negative infection status; 1635 controls had information for at least one of the criteria: 1107 controls from four studies[8,9,13,15] with information on CagA serostatus and 940 controls from four studies[8,9,11,12] with information on the presence of gastric atrophy.

A two-stage modeling approach[22] was used to estimate the association between *H. pylori* infection and NCGC, considering serological test results and after reclassification of *H. pylori* infection. First, logistic regression models were used to compute study-specific odds ratios (ORs) and the corresponding 95% confidence intervals (95%CI) for the association between *H. pylori* infection and NCGC. Models were adjusted for sex, age (five-year groups: <40; 40-44; …; 70-74; ≥75), socioeconomic status (low, intermediate, or high, as defined in each original study based on education, income or occupation) and study center (for multicenter studies), when appropriate and available as described in detail in **Supplementary Table 1**. Second, summary (pooled) effect estimates were computed using random-effects models [23]. Heterogeneity between studies was quantified using the I^2 (%) statistic.[24]

Stratified analyses were also carried out to further explore the effect of *H. pylori* infection across strata of sex, age (≤65, >65), geographic region, socioeconomic status, family history of gastric cancer, smoking status (never, ever), alcohol drinking (never, ever), fruits and vegetables intake (low, intermediate/high), salt intake (low, intermediate/high), type of controls (hospital-based, population-based), and cancer histological type (intestinal, diffuse, unspecified). Multinomial logistic regression models were used to

estimate the ORs for cancer of each histological type of cancer separately (i.e., intestinal, diffuse, unspecified). The heterogeneity between groups was assessed through the Q test for heterogeneity.[25] Visual inspection of the funnel plots and Egger's regression asymmetry test were used for the evaluation of publication bias.[26] Leave-one-out analyses were carried out to assess the influence of any given study.

Sensitivity analyses were conducted considering only the 1635 controls whose *H. pylori* infection status could be reclassified using at least one of the criteria as well as the NCGC cases from the respective studies: 853 cases and 1107 controls from four studies,[8,9,13,15] 554 cases and 940 controls from four studies,[8,9,11,12] and 528 cases from two studies[12,13] with data on CagA serostatus, gastric atrophy and advanced tumour stage, respectively. An additional sensitivity analysis was carried out in which cases and controls negative for *H. pylori* by serological test results and considered positive if meeting the three reclassification criteria were removed from the reference (negative) group.

Literature review

PubMed was searched from inception until 31 May 2021 for publications in English using the following search expression: ("gastric cancer" OR "stomach cancer") AND "*Helicobacter pylori*" AND ("prospective studies" OR "cohort studies" OR "systematic review" OR "meta-analysis"). The reference lists of relevant review articles were also screened.[3,4,27,28]

Studies were included when they evaluated the association between *H. pylori* infection and NCGC considering *H. pylori* serology collected prior to the diagnosis of NCGC in the cases.

Data on study design characteristics [author names, country and study name, follow-up time in years, number of NCGC cases included, percentage of cases positive for *H. pylori*, and information on the

assessment of *H. pylori* infection (ELISA or enzyme immunoassay [EIA] or immunoblot/multiplex serology)] and relative risk (RR) or OR estimates for the association between *H. pylori* serology and NCGC were extracted. Whenever available, adjusted estimates were considered.

Results from studies with information on the method of assessment of *H. pylori* infection status were summarized by meta-analysis. If a particular study provided estimates for more than one of the same method [ELISA or EIA (IgA, IgG, CagA, etc.), or immunoblot (IgA, IgG, CagA, multiplex serology)], estimates for IgG and/or CagA, or multiplex serology were included in the meta-analysis. Combined estimates and respective 95%CIs were calculated using random effects models considering the method of *H. pylori* infection assessment (A) ELISA or EIA, or B) immunoblot) and follow-up time in years (<10, ≥10, not specified). The I^2 statistic was computed to quantify heterogeneity.[24] Visual inspection of the funnel plots and Egger's regression asymmetry test were used for assessment of publication bias.[26] Leave-one-out analyses were used to evaluate the influence of any given study.

The quality of studies included in the current manuscript was assessed using the Newcastle-Ottawa Scale (NOS) for quality assessment of case-control and cohort studies.[29] The scale evaluates the quality of studies based on three different categories: selection, comparability and exposure (case-control studies) or outcome (nested case-control studies). A study can be awarded a maximum of nine stars, which indicates the highest quality. When more than one report referred to the same study, any of the reports could be used to obtain information on the study characteristics for the quality assessment.

All statistical analyses were performed using STATA version 15.1 (STATA Corporation, College Station, Texas, USA). A p-value less than 0.05 was considered significant.

Results

The StoP Project

The main characteristics of the NCGC cases and controls are described in **Supplementary Table 2**, and the number of *H. pylori* negative and positive NCGC cases and controls, considering serological test results and additionally reclassifying as positive individuals more likely to correspond to false-negative results is shown in **Supplementary Table 3**. The eight studies from the StoP project were awarded seven or more stars when applying the NOS (**Supplementary Table 4**).

The study-specific and pooled adjusted ORs for NCGC considering serology-defined *H. pylori* infection and after reclassifying potentially false-negative results are presented in **Figure 1**. Although not statistically significant, a positive serology-defined *H. pylori* infection status was associated with higher odds of NCGC (OR=1.45; 95%CI:0.87-2.42, I^2=87.8%). Following the reclassification of *H. pylori* status, the pooled analysis yielded significantly higher odds of NCGC (OR=4.79; 95%CI:2.39-9.60, I^2=84.3%). **Supplementary Table 5** provides the pooled estimates considering each criterion used. Reclassifying negative *H. pylori* individuals considering CagA status had the greatest effect on the pooled OR (3.18, 95%CI:0.88-11.44 after vs. OR=0.96, 95%CI:0.51-1.83 before, four studies). **Figure 2** presents the sensitivity analyses considering only cases and controls whose *H. pylori* infection status could be reclassified using at least one of the criteria considered. The pooled adjusted ORs remained essentially unchanged (serology-defined – OR=1.45; 95%CI:0.84-2.49, I^2=86.3%; reclassification of *H. pylori* status – OR=5.44; 95%CI:2.50-11.79, I^2=82.8%). Furthermore, the sensitivity analysis removing reclassified negative to positive *H. pylori* cases and controls from the reference group yielded an adjusted OR of 4.16 (95%CI:2.06-8.37, I^2=84.3%).

The effect of *H. pylori* infection status was consistent across most strata of sociodemographic characteristics, clinical features and lifestyle factors (**Table 1**). Significant differences according to

geographic region were observed when considering *H. pylori* infection status before and after reclassification (p for interaction: ≤0.001 and 0.038, respectively), with a stronger and significant association found among studies conducted in Asia (OR=4.96; 95%CI:3.01-8.19, I^2=0.0%, and OR=11.75; 95%CI:5.86-23.55, I^2=0.0%, respectively). Additional analyses considering histological type yielded statistically significant OR estimates following *H. pylori* infection status reclassification (intestinal OR=4.42; 95%CI:2.12-9.22, I^2=61.9%; diffuse OR=3.45; 95%CI:1.60-7.46, I^2=64.1%; unspecified OR=2.25; 95%CI:1.04-4.87, I^2=34.8%).

Visual inspection of the funnel plot (**Supplementary Figure 1**) suggests no relevant asymmetry, and Egger's regression asymmetry test (p=0.319 for serological test results and p=0.129 for the reclassification of *H. pylori* status) showed no statistically significant bias. The leave-one-out analyses showed that no study considerably influenced the pooled estimates obtained (**Supplementary Figure 2**).

Literature review

A total of 27 studies with information from 24 cohorts were included in the current literature review. None of the studies included in the StoP Project overlapped with the cohort studies obtained from the literature review. Additional information regarding each study in provided in **Supplementary Table 6**. The studies included in the literature review were awarded between four stars (one study) and nine stars (six studies; **Supplementary Table 7**).

The association between *H. pylori* infection and NCGC ranged from 1.07 (95%CI:0.77-1.49)[30] to 17.10 (95%CI:4.00-72.90)[31] when considering *H. pylori* assessment by ELISA or EIA, which yielded a pooled estimate of 3.01 (95%CI:2.22-4.07, I^2=74.4%; **Figure 3**). Higher pooled estimates were obtained when considering a follow-up time greater than or equal to 10 years (OR=3.82; 95%CI:2.46-5.95, I^2=74.2%)

compared to a shorter follow-up time (OR=2.49; 95%CI:1.60-3.87, I^2=76.3%). The magnitude of the association ranged from 2.80 (95%CI:2.25-3.48)[32] to 21.40 (95%CI:7.10-64.60)[33] when immunoblot or multiplex serology were used for the detection of *H. pylori*, yielding a pooled OR estimate of 9.22 (95%CI:3.12-27.21, I^2=81.5%), with lower pooled estimates being obtained when a shorter follow-up time was considered (OR=2.80; 95%CI:2.25-3.48, *Helicobacter pylori* Biomarker Cohort Consortium)[32] compared to a follow-up time greater than or equal to 10 years (OR=14.65; 95%CI:7.44-28.85, I^2=0.0%). A pooled analysis including all serology results yielded an overall estimate of 3.17 (95%CI:2.39-4.20, I^2=75.6%). Higher pooled estimates were obtained when considering a follow-up time greater than or equal to 10 years (OR=4.87; 95%CI:3.07-7.73, I^2=77.0%) compared to a shorter follow-up time (OR=2.17; 95%CI:1.50-3.13, I^2=74.0%).

The visual inspection of the funnel plot and Egger's test (p=0.006 for ELISA or EIA and p=0.021 for immunoblot or multiplex serology) are suggestive of publication bias (**Supplementary Figure 3**), suggesting an underrepresentation of studies with weaker associations in both cases. The leave-one-out analyses showed that no study considerably influenced the pooled estimates obtained (**Supplementary Figure 4**).

Discussion

In this study within the StoP Consortium, a significant association between *H. pylori* infection and NCGC was observed following the reclassification of *H. pylori* negative infection status as positive considering the presence of anti-CagA antibodies, evidence of gastric atrophy or an advanced stage at NCGC diagnosis. The results were generally consistent across strata of sociodemographic characteristics, clinical features and lifestyle factors, except for differences according to geographic region, as a stronger association was found for studies from Asia.

The pooled estimates obtained in the current study following the reclassification of negative *H. pylori* individuals are in line with results obtained from case-control studies nested within prospective cohorts, which yielded a pooled estimate of 3.01. However, when a more sensitive method for the detection of anti-*H. pylori* antibodies, such as immunoblot or multiplex serology, is used, the magnitude of the association increased, yielding a pooled estimate of 9.22. Likewise, a recent report quantifying the burden of gastric cancer attributable to *H. pylori*, and which performed a review of the literature for studies comparing the risk of NCGC, using both ELISA and immunoblot for detection of *H. pylori* infection,[3] found consistently higher estimates among prospective studies using immunoblot compared with ELISA,[33-36] whereas the only case-control study showed no difference between results by ELISA and immunoblot.[3,28] Furthermore, the use of a 116kDa (CagA) band also led to an increase in the prevalence of *H. pylori* among gastric cancer cases.[3] In particular, the case-control study found a strong association (OR=11.3; 95% CI:5.64-22.7) in contrast to the low prevalence and null estimates for *H. pylori* overall.[3,28] In the present study, only case-control studies in which *H. pylori* infection status was initially determined by serological tests, which are useful to detect past infection, were included. However, a relevant proportion of previously infected individuals may remain undetected in serological tests, particularly gastric cancer cases as they are more likely to have been infected in the past and infection tends to clear as cancer

progresses.[20,21] In fact, a previous review outlined a minimum set of criteria to define *H. pylori*-negative gastric cancer cases, namely negative findings in two or more methods including endoscopic or pathologic findings or serum PG test, a negative urea breath test or serum IgG test, and no history of *H. pylori* eradication.[5] Stricter criteria were also provided, including assessment by endoscopic, pathologic (updated Sydney System), as well as two or more *H. pylori* tests (e.g., rapid urease test, urease breath test, serum IgG, or stool antigen), a serum PG test, and determination of *H. pylori* eradication history.[5] On the contrary, *H. pylori* infection status determined by serological tests is not expected to remain undetected to the same extent among controls and will lead to differential misclassification contributing to biased downward estimates of the association between *H. pylori* infection and gastric cancer.

Therefore, to better quantify the association between *H. pylori* infection and NCGC, the present study considered anti-CagA antibodies, the presence of gastric atrophy or tumour stage at gastric cancer diagnosis to reclassify *H. pylori* negative infection status. As described above, CagA serostatus independently of *H. pylori* infection status has been used as a more sensitive marker of past infection in previous studies.[20,21,33,37] Furthermore, the carcinogenic cascade originally proposed by Correa reflects successive histological changes from superficial gastritis, atrophic gastritis, intestinal metaplasia, to dysplasia, and finally, adenocarcinoma, with *H. pylori* infection being the main factor for gastric cancer development.[38] Nevertheless, the presence of gastric precancerous lesions represents an unfavourable environment for its persistence over time, contributing to the clearance of infection as carcinogenesis progresses.[39] As such, the use of other biomarkers, including the measurement of circulating PG I and II levels, or histological examination of gastric atrophy have also been used to reclassify *H. pylori* infection status, since there is a high probability of a false negative result in the presence of gastric atrophy.[40-42] In particular, PG levels may be used as a non-invasive method for predicting atrophic gastritis,[5] with a PGI/II≤3.0 generally indicating the presence of gastric atrophy.[5,16-18] Moreover, previous studies have shown that gastric atrophy evaluated through endoscopic or histological examination or measured by PG

levels have relatively good correlations.[18,43,44] Advanced stage at diagnosis was also used to reclassify negative *H. pylori* infection status among the NCGC included in the present study. This criterion was considered since *H. pylori* antibody titers show a decreasing trend as the stage of gastric mucosa becomes more advanced,[45] which leads previously infected individuals to present a negative *H. pylori* infection status at the time of diagnosis.[20,21,46] Lower *H. pylori* IgA or IgG antibody titers have been observed among advanced compared to early-stage gastric cancers.[47,48] In fact, our meta-analyses conducted following a literature review showed that the association between *H. pylori* seroinfection and NCGC is stronger when considering cohort studies with a longer follow-up compared to those with a shorter follow-up, highlighting the higher potential misclassification of *H. pylori* seroinfection status over time. Further, the timing of blood collection was also considered by excluding NCGC patients evaluated following any gastric cancer treatment. Indeed, there is a relatively high probability for spontaneous regression and dynamic changes in *H. pylori* infection even after partial gastrectomy.[49]

A previous systematic review and meta-analysis of *H. pylori* infection and NCGC across populations with different gastric cancer risk, found that the summary relative risk (RR) was similar in both low- and high-risk populations [RR=2.56; 95%CI:1.99-3.29 and RR=2.81; 95%CI:1.92-4.74, respectively].[28] High- and low-risk populations were defined according to the risk of gastric cancer, with China, Japan and Korea being included in the former, and Australia, Finland, Germany, Norway, Sweden, the USA and a European multicenter study included in the latter. In the present StoP study, we found that the pooled OR considering the more sensitive criteria was highest in studies conducted in Asia, and lowest in the Americas and Europe. However, only two countries from Asia were included in this analysis, i.e., from Iran [10] and Japan [11], and this limits robust conclusions regarding differences in the geographical distribution of the association between *H. pylori* infection and NCGC. Nevertheless, our study adds to the existing literature by quantifying the association between *H. pylori* and NCGC in South America, which was lower

than the one observed in Asia; however, one of the studies conducted in Brazil was restricted to individuals of Japanese origin.[8]

Regarding sociodemographic characteristics, a previous systematic review found that the association between *H. pylori* infection and NCGC did not differ by sex.[4] This is in line with the results obtained in the present study as no significant differences were observed between males and females following the reclassification of *H. pylori* infection. Previous systematic reviews found that the magnitude of the association between *H. pylori* infection and NCGC varies with age as the effect is reduced in older age groups,[4,27] which was also observed in the current study, though not significant. These differences may be due to the increased prevalence of *H. pylori* infection with age,[50] and a greater potential misclassification of infection status due to age-related gastric atrophy,[51,52] particularly among controls.

Although several studies have suggested a different carcinogenic pathway considering histological type,[38,53] previous systematic reviews found that the association between *H. pylori* infection and NCGC did not differ between intestinal and diffuse type cancers.[4,27] Likewise, no significant difference in the association between *H. pylori* infection and NCGC was observed for cancers with different histological types. Nevertheless, the association was stronger among intestinal type cancers after the reclassification of infection status.

We also evaluated smoking status, alcohol drinking, fruit and vegetable intake, and salt intake, with no significant differences being observed across these strata. Nevertheless, a stronger association between *H. pylori* infection and NCGC was observed among ever drinkers, and individuals with a low fruit and vegetable intake; despite interaction terms not being significant. Within the StoP Project, a previous study that aimed to explore the interaction between *H. pylori* infection and several gastric cancer risk factors found a more than multiplicative interaction between infection and alcohol drinking (OR=1.38, 95%CI:1.07-1.77, p-interaction=0.02).[54] The higher risk of NCGC among ever drinkers may be due to

damage to the gastric mucosa caused by the bacterium, which facilitates the genotoxic effect of acetaldehyde that is the primary metabolite of ethanol.[55] Moreover, a higher risk of gastric cancer has been observed among individuals with lower intakes of fruits and vegetables.[56,57] In particular, several fruits, such as citrus,[58,59] apples[60] or berries[61] have been shown to contain flavanones that have anti-oxidant activity, and fruits and vegetables are also rich in fiber, which can act as a scavenger of nitrates, preventing the formation of carcinogenic N-nitroso compounds.[62]

The current study is based on a uniquely large individual participant data meta-analysis of eight studies participating in the StoP Project Consortium, including data from Asia, Europe, and Central and Latin America. Although substantial heterogeneity was observed, which may be largely due to the different methods and cut-offs used to define *H. pylori* infection status, the harmonization of adjustment strategies and control of confounding in studies of the StoP Project contribute to the validity of our findings. We also conducted several sensitivity analyses to assess the robustness of the results, and addressed differential misclassification of *H. pylori* infection status by reclassifying potential false negatives, as well as removing them from the reference group. Although not all controls included in the current study had information regarding CagA serostatus or gastric atrophy, we conducted a sensitivity analysis using only NCGC and controls who could be reclassified using at least one criterion, and the results remained essentially the same. Additionally, it was not possible to apply endoscopic, pathological and additional *H. pylori* tests uniformly to all included studies, and we did not have information regarding past *H. pylori* eradication to include in the current study.

The retrospective design of the studies included may affect the validity of the information regarding lifestyle factors, including smoking status, alcohol drinking, fruit and vegetable intake, and salt intake. Additionally, as past dietary habits were reported by patients, recall bias may have occurred since changes in lifestyle may occur as cancer develops and becomes symptomatic.[63] However, we included only incident

gastric cancer cases. Furthermore, NCGC cases may clear *H. pylori* infection as carcinogenesis progresses,[39] resulting in a seronegative status. As such, we considered several criteria to reclassify infection status including anti-CagA antibodies, the presence of gastric atrophy or tumour stage at gastric cancer diagnosis.

Four studies in the analysis included hospital-based controls,[9-12] which may result in selection bias. It is possible that hospital-based controls include individuals with conditions that could be related to *H. pylori* infection status or lifestyle factors, while population-based controls are more likely to be representative of the study base. Nevertheless, the results of our stratified analysis by type of controls showed that the overall conclusions are not driven by the studies with hospital- *vs*. population-based controls.

In conclusion, the current large-scale StoP study further confirms the nearly five times higher odds of NCGC among *H. pylori* infected individuals when considering additional criteria to define *H. pylori* infection status, being in line with results obtained from prospective cohort studies.

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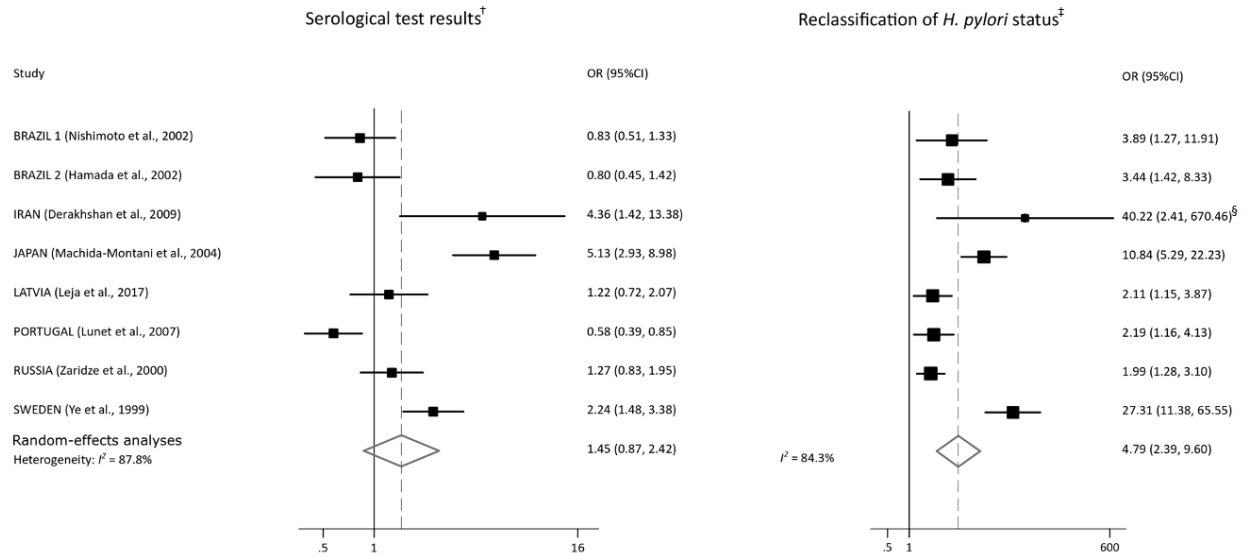

Figure 1. Forest plots describing the association between *H. pylori* infection status, considering serological test results† and additionally reclassifying as positive the *H. pylori* infection status of those likely to correspond to false-negative results of the serological test‡, and non-cardia gastric cancer using estimates from the Stomach Cancer Pooling (StoP) Project database.

95%CI – 95% confidence interval; OR – odds ratio.

† *H. pylori* infection status was defined considering serological tests using the same criteria applied in each original study.

‡ Additional criteria were used to reclassify *H.* pylori infection status: a negative serological test result for *H. pylori* infection was reclassified as positive if a positive result was obtained for cytotoxin-associated gene A (CagA) status (cases and controls when available) or gastric atrophy was present (cases and controls when available) or tumour stage at diagnosis was advanced (cases only when available).

§ The crude OR and 95%CI for the study from IRAN (Derakhshan et al., 2009)[10] was calculated by adding 0.5 to each cell as all cases were *H. pylori* positive following the reclassification of infection status.

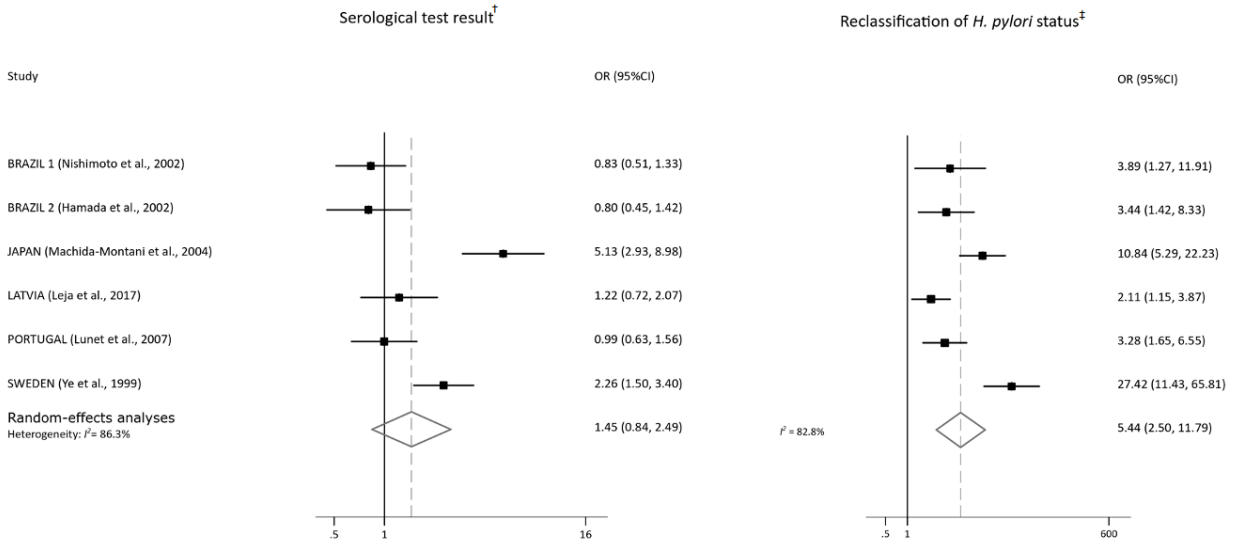

Figure 2. Forest plots describing the association between *H. pylori* infection status, considering serological test results† and additionally reclassifying as positive the *H. pylori* infection status of those likely to correspond to false-negative results of the serological test‡, and non-cardia gastric cancer using estimates from the Stomach Cancer Pooling (StoP) Project database **considering cases (n=1325) and controls (n=1635) who could be reclassified based on at least one criterion**§.

95%CI – 95% confidence interval; OR – odds ratio.

† *H. pylori* infection status was defined considering serological tests using the same criteria applied in each original study.

‡ Additional criteria were used to reclassify *H.* pylori infection status: a negative serological test result for *H. pylori* infection was reclassified as positive if a positive result was obtained for cytotoxin-associated gene A (CagA) status (cases and controls when available) or gastric atrophy was present (cases and controls when available) or tumour stage at diagnosis was advanced (cases only when available).

§ Excluding studies: IRAN (Derakhshan et al., 2009)[10] and RUSSIA (Zaridze et al., 2000)[14] as no controls could be reclassified at least once.

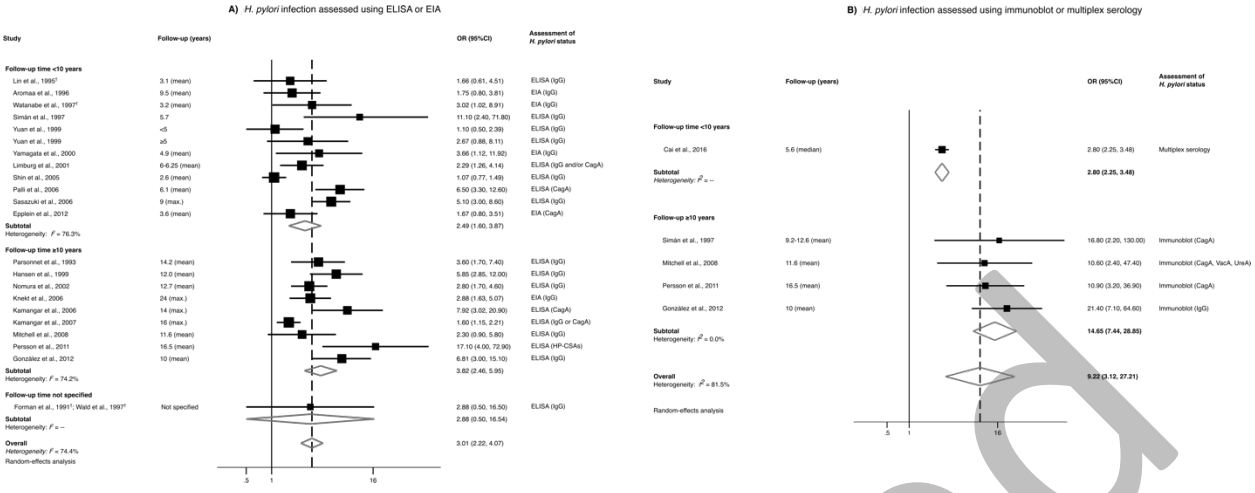

Figure 3. Meta-analysis of the literature review of prospective studies of non-cardia gastric cancer quantifying the association with *H. pylori* infection considering the method of *H. pylori* infection assessment [A) ELISA or EIA, or B) immunoblot or multiplex serology] and follow-up time in years (<10, ≥10, not specified).

95%CI – 95% confidence interval; CagA – cytotoxin-associated gene A; EIA – enzyme immunoassay; ELISA – enzyme-linked immunosorbent assay; HP-CSAs – *H. pylori* cell-surface antigens; IgG – immunoglobulin G; Max. – maximum; OR – odds ratio; UreA – urease A; VacA – vacuolating cytotoxin A.

† Data obtained from the *Helicobacter* and Cancer Collaborative Group.[4]

Table 1. Pooled odds ratios and 95% confidence intervals (Dersimonian-Laird random-effects model) for non-cardia gastric cancer considering *H. pylori* infection status defined according to serological test results[†] and additionally reclassifying as positive the *H. pylori* infection status of those likely to correspond to false-negative results of the serological test[‡], stratified by sociodemographic characteristics, clinical features and lifestyles factors.

	Serological test results[†]		**Reclassification of *H. pylori* status[‡]**	
	aOR[§] (95%CI)	***I²* (%)**	**aOR[§,¶] (95%CI)**	***I²* (%)**
Overall	1.45 (0.87-2.42)	87.8	4.79 (2.39-9.60)	84.3
Sex				
Males	1.38 (0.78-2.44)	81.5	4.55 (2.12-9.78)	73.8
Females	1.37 (0.78-2.40)	73.5	4.68 (2.26-9.67)	68.0
p for interaction	*0.986*		*0.958*	
Age (years)				
≤65	1.77 (0.97-3.23)	82.6	5.50 (2.49-12.11)	77.7
>65	1.10 (0.68-1.78)	67.1	3.32 (1.68-6.56)	61.3
p for interaction	*0.229*		*0.343*	
Geographic region				
Americas	0.82 (0.56-1.18)	0.0	3.60 (1.80-7.22)	0.0
Asia	4.96 (3.01-8.19)	0.0	11.75 (5.86-23.55)	0.0
Europe	1.19 (0.66-2.15)	86.6	3.77 (1.44-9.89)	89.9
p for interaction	***<0.001***		***0.038***	
Socioeconomic status[††]				
Low	0.95 (0.46-1.96)	79.1	3.64 (1.12-11.87)	76.3
Intermediate	1.54 (0.76-3.12)	75.9	5.39 (2.24-12.98)	64.4
High	1.58 (0.56-4.43)	59.9	4.21 (1.63-10.87)	16.8
p for interaction	*0.585*		*0.859*	
Family history of cancer[‡‡]				
No	1.35 (0.83-2.20)	82.0	4.49 (2.24-8.99)	80.5
Yes	1.27 (0.46-3.52)	62.3	3.66 (1.17-11.49)	57.0
p for interaction	*0.915*		*0.764*	
Smoking status[‡‡]				
Never	1.27 (0.71-2.28)	81.1	4.12 (2.22-7.67)	63.5
Ever	1.19 (0.62-2.28)	80.9	3.94 (1.70-9.18)	75.7
p for interaction	*0.884*		*0.933*	
Alcohol drinking[‡‡]				
Never	1.42 (0.81-2.50)	66.3	3.61 (2.21-5.90)	13.7
Ever	1.35 (0.61-2.97)	87.2	6.32 (2.02-19.79)	84.0
p for interaction	*0.919*		*0.377*	
Fruit and vegetable intake[‡‡]				
Low	2.23 (0.59-8.46)	66.6	7.57 (2.78-20.59)	0.0
Intermediate/High	1.34 (0.76-2.37)	86.3	4.73 (2.01-11.14)	85.8
p for interaction	*0.491*		*0.426*	

Salt intake‡‡				
Low	1.25 (0.61-2.57)	83.0	3.99 (1.91-8.35)	54.8
Intermediate/High	1.12 (0.45-2.78)	71.9	2.70 (1.13-6.44)	53.1
p for interaction	*0.853*		*0.502*	
Controls				
Hospital-based§§	2.08 (0.82-5.25)	89.2	5.60 (1.91-16.43)	78.8
Population-based¶¶	1.13 (0.30-4.29)	95.5	7.59 (0.64-89.98)	95.2
p for interaction	*0.464*		*0.825*	

95%CI – 95% confidence interval; aOR – adjusted odds ratio.

† *H. pylori* infection status was defined considering serological tests using the same criteria applied in each original study.

‡ Additional criteria were used to reclassify *H.* pylori infection status: a negative serological test result for *H. pylori* infection was reclassified as positive if a positive result was obtained for cytotoxin-associated gene A (CagA) status (cases and controls when available) or gastric atrophy was present (cases and controls when available) or tumour stage at diagnosis was advanced (cases only when available).

§ Pooled ORs were computed using random-effects models. Study-specific ORs were adjusted, when available and applicable, for sex, age (five-year age groups: <40; 40-44; …; 70-74; ≥75), socioeconomic status (low, intermediate, or high, as defined in each original study based on education, income or occupation) and study center (for multicenter studies).

¶ The crude OR and 95%CI for the study from IRAN (Derakhshan et al., 2009)[10] was calculated by adding 0.5 to each cell as all cases were *H. pylori* positive following the reclassification of infection status.

†† As defined in each original study based on education, income or occupation. No information available for study: LATVIA (Leja et al., 2017).[12]

‡‡ No information available for study: IRAN (Derakhshan et al., 2009).[10]

§§ Including studies: BRAZIL 1 (Nishimoto et al., 2002);[9] IRAN (Derakhshan et al., 2009);[10] JAPAN (Machida-Montani et al., 2004);[11] LATVIA (Leja et al., 2017).[12]

¶¶ Including studies: PORTUGAL (Lunet et al., 2007);[13] SWEDEN (Ye et al., 1999).[15]