Identifying the profile of *Helicobacter pylori* negative gastric cancers: a case only analysis within the Stomach cancer Pooling (StoP) Project

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Running title: *H. pylori* negative gastric cancers in the StoP Project

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Conflict of interest: There are no conflicts of interest to disclose.

Abstract

Background: The prevalence of *Helicobacter pylori* negative gastric cancer (HpNGC) can be as low as 1%, when infection is assessed using more sensitive tests or considering the presence of gastric atrophy. HpNGC may share a high-risk profile contributing to the occurrence of cancer in the absence of infection. We estimated the proportion of HpNGC, using different criteria to define infection status, and compared HpNGC and positive cases regarding gastric cancer risk factors.

Methods: Cases from 12 studies from the Stomach cancer Pooling (StoP) Project providing data on *H. pylori* infection status determined by serological test were included. HpNGC was reclassified as positive (eight studies) when cases presented CagA markers (four studies), gastric atrophy (six studies), or advanced stage at diagnosis (three studies), and were compared with positive cases. A two-stage approach (random-effects models) was used to pool study-specific prevalence and adjusted odds ratios (ORs).

Results: Among non-cardia cases, the pooled prevalence of HpNGC was 22.4% (n=166/853) and decreased to 7.0% (n=55) when considering CagA status; estimates for all criteria were 21.8% (n=276/1325) and 6.6% (n=97), respectively. HpNGC had a family history of gastric cancer more often (OR=2.18, 95% confidence interval [CI]:1.03-4.61) and were current smokers (OR=2.16, 95%CI:0.52-9.02).

Conclusion: This study found a low prevalence of HpNGC, who are more likely to have a family history of gastric cancer in first-degree relatives.

Impact: Our results support that *H. pylori* infection is present in most non-cardia gastric cancers, and suggest that HpNGC may have distinct patterns of exposure to other risk factors.

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

Introduction

Helicobacter pylori (*H. pylori*) infection is the major risk factor for gastric cancer and accounts for the largest proportion of the cases occurring worldwide (1). However, the proportion of patients with gastric cancer testing negative for *H. pylori* infection varies across epidemiological studies (2), and a more accurate definition of the magnitude of the association is needed for reliable estimates of gastric cancer burden due to *H. pylori* infection.

Methodological limitations in the detection of past infection may contribute to an underestimation of the relation between infection and gastric cancer, with at least some of the *H. pylori* negative gastric cancers corresponding to false negative results, due to difficulties in detecting past *H. pylori* infection, especially in retrospective study designs (3). However, the prevalence of *H. pylori* negative gastric cancers can be as low as 1%, when cases likely to have false negative results are reclassified as positive, considering the presence of gastric atrophy or after using more sensitive tests (2).

Although the true proportion of *H. pylori* negative gastric cancer cases is low, these may share a high-risk profile, contributing to the occurrence of cancer in the absence of infection. However, to characterize the risk profile of *H. pylori* negative gastric cancers, large samples from different settings are needed. Furthermore, the analysis of data from populations with varying levels of exposure to gastric cancer determinants may contribute to disclose patterns not identifiable among more homogeneous groups.

Therefore, using a pooled analysis of studies from the Stomach cancer Pooling (StoP) Project (4), this study aimed to estimate the proportion of gastric cancer cases that are *H. pylori* negative, applying different criteria for the definition of *H. pylori* infection status, and to compare *H. pylori* negative and positive gastric cancer cases with regard to the main risk factors for gastric cancer.

Methods

This study is based on the 3.0 version of the StoP Project, which includes 32 case-control or nested within cohort studies for a total of 12511 cases and 29964 controls from 14 countries (4). The original datasets were centralized at the coordinating centre and harmonized according to a pre-specified format before analysis. Ethical approval was obtained by each individual study and the StoP Project was approved by the University of Milan Review Board (Reference 19/15).

In the present analysis, only gastric cancer cases from studies providing data on *H. pylori* infection status determined in blood samples collected before any treatment, overall and among non-cardia cases, were eligible. *H. pylori* infection status was determined by serological tests, namely enzyme-linked immunosorbent assay (ELISA) [12 studies (5-16)] or Western Blot [one study (17)] to determine Immunoglobulin G (IgG) antibodies in serum and multiplex serology [one study (18)], using the same criteria applied in each original study. When anti-*H. pylori* serum IgG titers had been determined using an ELISA-based method, participants with borderline results (n=48) were classified as *H. pylori* positive. Two studies with data on *H. pylori* infection were excluded from the main analyses due to lack of information regarding tumour subsite [China (7) and Mexico (16)] (**Figure 1**), but were kept in a sensitivity analysis.

A total of 12 studies, from Brazil [two studies (12,13)], Iran [three studies (6,9,17)], Japan (14), Latvia (15), Mexico (11), Portugal (8), Russia (5), Spain (18) and Sweden (10), had information on *H. pylori* infection status and tumour subsite, and were therefore considered in the analyses of the summary (pooled) prevalence of negative *H. pylori* infection among all cases and in those classified as non-cardia (described in **Supplementary Table S1**). Heterogeneity between studies was quantified using the I^2 (%) statistic (19).

To reduce the probability of false negative results due to misclassification of infected subjects as non-infected among non-cardia gastric cancer cases, a negative serological result for *H. pylori* infection status was reclassified as positive when a positive result had been obtained for cytotoxin associated-gene A (CagA) status independently of the detection of surface antibodies

against *H. pylori*. Additional analyses were conducted, in which a negative *H. pylori* infection status was reclassified as positive when gastric atrophy was present as evaluated through histological examination or measured by serum pepsinogen (PG) levels [PGI/II≤3 (2,20)], or tumour stage was advanced at diagnosis, *i.e.*, stage IV, according to the *TNM Classification of Malignant Tumours* (21). If more than one criterion could be applied to define *H. pylori* infection status in each study but the necessary information was not available for all non-cardia gastric cancer cases, only cases for which at least one criterion could be applied were included to ensure comparability. As such, analyses of the pooled prevalence of negative *H. pylori* infection among non-cardia cancers included 1325 cases from the eight studies whose *H. pylori* infection status could be reclassified using at least one of the criteria described above, including 853 cases from four studies (8,10,12,13), 974 cases from six studies (8,9,12-15) and 654 cases from three studies (5,8,15) with infection status reclassified based on CagA status, gastric atrophy and advanced tumour stage, respectively (**Figure 1**). Overall, excluded gastric cancer cases (n=9627) were similar to included gastric cancer cases (n=2884, described in **Supplementary Table S1**) regarding sex (males: 64.9% *vs.* 62.8%; p-value=0.043), age (≤65 years: 55.3% *vs.* 53.3%; p-value=0.055) and family history of gastric cancer (yes: 15.3% *vs.* 16.2%; p-value=0.359). The largest differences were observed considering geographic region (more participants from Europe [34.3% *vs.* 19.3%], and fewer participants from the Americas [47.9% *vs.* 55.6%] and Asia [17.7% *vs.* 25.0%], p<0.001), as well as differences in social class (fewer intermediate [28.7% *vs.* 33.5%], and more low [57.5% *vs.* 53.0%] and high [13.8% *vs.* 11.5%], p-value<0.001) and lifestyle factors: more ever smokers or drinkers (58.5% *vs.* 49.4%, p-value<0.001, and 73.4% *vs.* 60.7%, p-value<0.001, respectively), a lower fruit and vegetable intake (34.5% *vs.* 23.9%, p-value<0.001), and a greater intermediate/higher salt intake (65.1% *vs.* 46.5%, p-value<0.001).

A two-stage modeling approach (22) was used to estimate the association between sociodemographic characteristics (sex, age and social class), clinical features (histological type, family history of gastric cancer in first-degree relatives and body mass index), and lifestyle factors (smoking

status, alcohol drinking, fruit and vegetable intake, and salt intake) with *H. pylori* infection status (*H. pylori* positive gastric cancers were the reference group). First, logistic regression models were used to compute odds ratios (ORs) and the corresponding 95% confidence intervals (95%CI) for the comparison between *H. pylori* negative and positive gastric cancers, adjusting for sex, age (continuous), social class (low, intermediate, high, as defined in each original study considering education, income or occupation) and study centre (for multicentre studies), when appropriate and available (**Supplementary Table S2**). Second, summary (pooled) effect estimates were computed using the DerSimonian-Laird method, assuming a random-effects model (23). Heterogeneity between studies was quantified using the I^2 (%) statistic (19).

Statistical analyses were performed using STATA version 15.1.

Results

The overall pooled prevalence of *H. pylori* negative gastric cancer cases was 19.7% (n=624), being highest in the Americas (28.8%, I^2=57.9%), and lower in Asia (10.8%, I^2=95.2%) and Europe (22.2%, I^2=98.1%). The analyses restricted to non-cardia gastric cancer cases yielded a pooled proportion of *H. pylori* negatives of 17.5% (n=335; **Figure 2**).

When *H. pylori* infection status was reclassified from negative to positive considering positive CagA status, the pooled prevalence of negative *H. pylori* among non-cardia gastric cancer cases was 7.0% (n=55; *vs.* 22.4% before reclassification, four studies, **Figure 3**), being highest in the Americas (11.0%, I^2=86.6%), and lowest in Europe (7.5%, I^2=92.4%). No study from Asia had information on CagA status. Additionally, as depicted in **Supplementary Fig. S1,** when the presence of gastric atrophy or advanced tumor stage at diagnosis were considered, the pooled prevalences of negative *H. pylori* were 8.5% (n=87; *vs.* 19.0% before reclassification, six studies) and 20.4% (n=113; *vs.* 24.5% before reclassification, three studies), respectively. When all available criteria were used to define *H. pylori* infection, the pooled prevalence decreased to 6.6% (n=97; *vs.* 21.8% before reclassification, eight studies), being highest in Europe (10.0%, I^2=97.1%), and lower in Asia (3.4%, I^2=87.5%) and the Americas (4.2%, I^2=87.9%).

Table 1 presents the pooled ORs and 95%CI comparing *H. pylori* negative and *H. pylori* positive gastric cancer cases considering the presence of CagA, and **Supplementary Table S3** shows the number of gastric cancer cases infected and not infected with *H. pylori* considered. Patients with family history of gastric cancer in first-degree relatives were more frequently observed among *H. pylori* negative cases (OR=2.18, 95%CI:1.03-4.61). Additionally, although no statistically significant associations were observed, current smoking (OR=2.16, 95%CI:0.52-9.02) was associated with *H. pylori* negative cases. Likewise, older age (OR=1.32, 95%CI:0.48-3.68), overweight/obesity (OR=1.14, 95%:0.26-5.05), and intermediate/high salt intake (OR=1.31, 95%CI:0.58-2.96) were more frequent among those with *H. pylori* negative infection status. On the contrary, females (OR=0.78,

95%CI:0.42-1.47) and ever drinkers (OR=0.68, 95%CI:0.20-2.32) were less frequent among those with *H. pylori* negative infection.

When considering all criteria (CagA status, gastric atrophy and advanced stage at diagnosis; **Supplementary Table S4**) to reclassify infection status, although no statistically significant associations were observed, female gender (OR=1.29, 95%CI:0.80-2.10), high social class (OR=1.55, 95%CI:0.53-4.54), gastric cancers of diffuse (OR=1.92, 95%CI:0.99-3.72) and mixed or unclassifiable (OR=1.32, 95%CI:0.39-4.42) histological type, family history of gastric cancer in first-degree relatives (OR=1.28, 95%CI:0.69-2.35), and intermediate/high salt intake (OR=1.42, 95%CI:0.64-3.11) were associated with *H. pylori* negative cases. On the contrary, overweight/obesity (OR=0.88, 95%:0.54-1.40), ever drinking (OR=0.78, 95%CI:0.43-1.43), and a low fruit and vegetable intake (OR=0.48, 95%CI:0.19-1.23) were less frequent among those with *H. pylori* negative infection.

Discussion

In this study, the prevalence of *H. pylori* negative cases decreased from 22.4% among non-cardia gastric cancers to 7.0% after reclassifying as *H. pylori* positive the cases with CagA markers of infection.

The pooled prevalence of *H. pylori* negative gastric cancers in this study is similar to that reported previously in one of the few studies conducted in Europe, after combining the results from different tests to assess *H. pylori* infection status [prevalence of *H. pylori* negative infection of 13.8% (24)]. However, most of the evidence on this topic comes from studies from Asia, mainly performed in Japan (25-28) or South Korea (29,30). In these settings, the prevalence of *H. pylori* negative gastric cancers is much lower, ranging between less than 1% and 5% (2). This is in line with our results showing the pooled prevalence of *H. pylori* negatives considering several criteria to be highest in studies conducted in Europe and lowest in Asia. However, only two countries from Asia were included in this specific analysis, i.e., from Iran (9) and Japan (14), and results showed considerable heterogeneity (I^2=87.5%). Furthermore, these two studies did not have information regarding CagA status and were not included in the main analysis. This limits robust conclusions regarding the geographical distribution of *H. pylori* negative gastric cancers. Nevertheless, our study adds to the existing literature by quantifying the prevalence of *H. pylori* negative gastric cancers in South America, which was lower than the observed in Europe and higher than in Asia; however, one of the studies conducted in Brazil includes individuals of Japanese origin only (13).

A previous review on the characteristics of *H. pylori* negative gastric cancers defined a set of minimum criteria for their definition, *i.e.*, negative findings in two or more methods including endoscopic or pathological findings or serum PG test, a negative urea breath test or serum IgG test, and no history of *H. pylori* eradication (2). The authors also recommended stricter criteria that require assessment by endoscopic, pathological (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. In the current analysis, *H. pylori* infection status

was initially determined by serological tests, which are useful to detect cases with past, but not current, infection. Nevertheless, a significant proportion of previously infected individuals may remain undetected as cases are more likely to have been infected in the distant past and tend to clear the infection as cancer progresses (31,32). As such, the present study first considered CagA markers to reclassify *H. pylori* infection status. In previous studies, CagA status independently of *H. pylori* infection status was used as a more sensible marker of past infection (31,32). The main analysis was complemented by considering the presence of gastric atrophy or tumour stage at diagnosis. As per the model for the development of intestinal type tumours proposed by Correa (33), successive histological changes, from superficial gastritis, atrophic gastritis, intestinal metaplasia, to dysplasia, and finally, adenocarcinoma occur. As such, other biomarkers or histological analyses can be used to evaluate the presence of gastric precancerous lesions, namely gastric atrophy (34). These are strongly associated with *H. pylori* infection but constitute an unfavourable environment for its persistence, contributing to *H. pylori* clearance as carcinogenesis progresses (35). In this case, histological examination of gastric atrophy or the measurement of serum PG I and II levels may also be used to reclassify *H. pylori* infection status, as there is a higher probability of a false negative result in the presence of gastric atrophy (28,36). Serum PGI/II>3.0 indicates no atrophic change, while serum PGI/II≤3.0 indicates the presence of atrophic gastritis (2,20). Therefore, serum PG levels may be used as a non-invasive method for predicting atrophic gastritis (2). Additionally, several validation studies have been published, which showed that endoscopic, histological and serological atrophic gastritis have relatively good correlations (20,37,38). Previous studies showed that *H. pylori* infection tends to clear as cancer progresses with previously infected individuals remaining undetected at the time of diagnosis (31,32). Consequently, the titer of *H. pylori* antibodies shows a decreasing trend as the stage of gastric mucosa becomes more advanced (39). Previous studies have shown that advanced gastric cancer cases have lower *H. pylori* IgA or IgG antibody titers compared to early-stage gastric cancers (40,41). Therefore, we included advanced stage at diagnosis as one of the criteria to define *H. pylori* infection. Finally, we also considered the timing of blood collection by

excluding patients evaluated following any gastric cancer treatment. In fact, there is a relatively high probability for spontaneous regression and dynamic changes in *H. pylori* infection even after partial gastrectomy (42). Another criterion that has been proposed is information on history of *H. pylori* eradication, but the lack of data precluded its use in the present analysis.

The increase in the prevalence of *H. pylori* positive cases observed following the reclassification performed in this study, based on the presence of CagA markers of infection, as well as the combination of all criteria, points to the underestimation of *H. pylori* associated gastric cancer risk. The pooled prevalence of *H. pylori* negatives was higher when the advanced tumour stage criterion was added compared to that observed when restricted to non-cardia cases (20.4% vs. 17.5%, respectively). However, only three studies had information on tumour stage at diagnosis and considering only these studies, the prevalence of *H. pylori* negatives among non-cardia cases was 24.5%. Adding more criteria, such as history of *H. pylori* eradication, as well as uniformly applying endoscopic, pathologic, and additional *H. pylori* tests to all included studies (2), would have resulted in an even higher proportion of *H. pylori* infected gastric cancers.

Regarding the sociodemographic characteristics of *H. pylori* negative gastric cancers, previous reports have generally found that these develop similarly in both genders (24,26,28-30,36). Although a slight female predominance was observed when considering gastric atrophy, advanced stage at diagnosis and all criteria to reclassify *H. pylori* infection, this association was not significant, and the opposite was observed when CagA was used. Some studies have described *H. pylori* negative gastric cancers to be more frequent among younger individuals (26,28,36), while others report no age differences (24,29,30). In the present study, no statistically significant differences were observed though we obtained somewhat inconsistent results. We found older age to be associated with *H. pylori* negative cases after the reclassification of infection status considering the presence of CagA and gastric atrophy, while the opposite was observed when considering advanced stage at diagnosis. Finally, there was no association between age at diagnosis and *H. pylori* infection status following reclassification considering all criteria.

Several studies have reported that *H. pylori* negative cancers were more frequently of the diffuse type (25-28,30,36,43), which may suggest a different carcinogenic pathway among *H. pylori* negative gastric cancers (26,44). However, previous prospective studies have shown no significant differences between intestinal and diffuse histological type gastric cancer in the association of *H. pylori* and distal gastric cancer (45,46). In the current study, though not statistically significant, a higher proportion of diffuse and mixed or unclassifiable cancers were found among *H. pylori* negative cases, particularly following reclassification considering advanced stage at diagnosis and all criteria.

Another study, which examined smoking and alcohol intake and *H. pylori* negative gastric cancer cases, found no differences (29). Although not statistically significant, when considering CagA status to define *H. pylori* infection status, we found that current smokers were more frequent among *H. pylori* negative gastric cancers. Tobacco smoking has been linked to gastric cancer, with recent estimates indicating that over 10% of gastric cancers worldwide are attributable to tobacco use (47). We also evaluated alcohol drinking, fruit and vegetable intake, and salt intake, with no significant results observed when defining *H. pylori* infection status according to different criteria. However, we observed an association between higher social class and *H. pylori* negative gastric cancers (advanced stage and all criteria), which suggests that the behavioral risk factors that mediate the relationship between socioeconomic status and gastric cancer may be different between *H. pylori* negative and positive gastric cancers. Indeed, previous studies found a strong association between low socioeconomic status and gastric cancer risk (48-50), which may be related to selected dietary habits, smoking and alcohol intake.

Previous studies have shown that the aetiology of *H. pylori* negative gastric cancers may also be determined by genetic predisposition (51,52); further, familial clustering is responsible for *H. pylori* transmission between family members (53). Although we did not have information regarding genetic predisposition, we found a statistically significant association between family history of

gastric cancer in first-degree relatives and *H. pylori* negative infection following reclassification with CagA status. However, this association was no longer significant once all criteria were considered.

In the current study, nearly 70% of gastric cancer cases from eighteen studies could not be considered because they did not have information on *H. pylori* infection status, and there were only four studies included in the main analysis considering CagA status as the reclassification criterion. Significant differences were observed between included and excluded participants regarding geographic region with fewer participants from Asian countries being included. This may have led to an overestimation of the prevalence of *H. pylori* negative gastric cancer cases given that studies from Asia presented the lowest prevalence of *H. pylori* negative gastric cancer. Nevertheless, all studies that included blood samples to detect *H. pylori* infection were considered in the present study, which allowed us to use raw, individual-participant data rather than published studies only, thus minimizing publication bias (54,55). Additionally, the methodology used in the present study has been shown to have several advantages (56-58), including the ability to use all the available information in each study as necessary, and allowing pooled analyses based on complete and more homogenous data.

The retrospective nature of the included studies has methodological limitations in detecting the past *H. pylori* infection status of patients with different tumour stages. Previous studies have shown that *H. pylori* infection tends to clear in previously infected individuals as the cascade of histological changes in the gastric mucosa progresses (59,60). To overcome this, we reclassified negative *H. pylori* infection status to better quantify the prevalence of infection among non-cardia gastric cancer cases. Additionally, we did not have information regarding a history of *H. pylori* eradication as well as the inability to apply endoscopic, pathological and additional *H. pylori* tests uniformly to all included studies. Nevertheless, we reclassified *H. pylori* negative cases as positive considering characteristics of the cases that could contribute for false negative results. However, this may have led to increase misclassification among *H. pylori* positive cases and to underestimate the prevalence of *H. pylori* negative patients. Although the current study compared *H. pylori* infection

negative and positive cases regarding several known gastric cancer risk factors, Epstein-Barr virus (EBV) has also been proposed to be related to the development of *H. pylori* negative gastric cancer (34). However, only one study included in the present analysis included information on both *H. pylori* and EBV infection status (15). Nevertheless, we found nearly two-fold higher odds of EBV infection among *H. pylori* negative gastric cancers, regardless of the criteria used to define *H. pylori* infection status.

Additionally, we were unable to ensure a standardized pathologic classification within studies participating in the StoP Project. We restricted our subgroup analyses to non-cardia gastric cancers as they are more often associated with *H. pylori* infection (24,42); consequently, we excluded two studies (7,16) without information on tumour subsite. A sensitivity analysis including all 14 studies yielded a similar pooled prevalence of negative *H. pylori* infection (20.6% *vs.* 19.7% for 12 studies). Furthermore, the harmonization of adjustment strategies and control of confounding in studies of the StoP Project contribute to the validity of our findings.

H. pylori negative gastric cancers may reflect misclassification of infection status, which was minimized in our analyses, but may also correspond to a subgroup of cases occurring because of exposures other than *H. pylori* infection. In particular, our results suggest that *H. pylori* negative gastric cancers may be more likely of diffuse histological type, and gastric cancers of different histological subtypes were proposed by Correa (33) to have distinct aetiologies. In fact, the carcinogenic cascade proposed (33) for intestinal gastric adenocarcinomas reflects successive histological changes with *H. pylori* positive infection being the main factor for gastric cancer development, and there appears to be a relatively greater impact of environmental factors in the etiology of intestinal type carcinomas, while the diffuse type has been considered to be more dependent on the individuals' genetic profile (61). Furthermore, our analyses considering gastric cancer risk factors showed that current smokers were more frequent among *H. pylori* negative gastric cancers. Tobacco smoking has been associated with the development of precursor lesions, such as chronic atrophic gastritis (62), intestinal metaplasia (63) and dysplasia (62), and it is an

established risk factor for invasive cancer (64), including both intestinal and diffuse gastric adenocarcinomas (65,66). Overall, our study has contributed to better estimate the prevalence of *H. pylori* negative gastric cancer and explored differences between cases of gastric cancer with or without evidence of infection regarding the exposure to several risk factors. Future studies must further reduce the misclassification of *H. pylori* negative gastric cancers using other *H. pylori* antibodies or other markers of infection in stomach tissue, and evaluate additional risk factors including EBV using larger sample sizes for more robust conclusions.

In conclusion, the current study found a low prevalence of *H. pylori* negative gastric cancers following the reclassification of infection status. Although our results further support that *H. pylori* infection is present in most non-cardia gastric cancers, they also suggest that *H. pylori* negative gastric cancers may have distinct patterns of exposure to the risk factors for gastric cancer.

Acknowledgments: S Morais, B Peleteiro, N Araújo and N Lunet received national funding from the Foundation for Science and Technology – FCT (Portuguese Ministry of Science, Technology and Higher Education), under the Unidade de Investigação em Epidemiologia – Instituto de Saúde Pública da Universidade do Porto (EPIUnit; UIDB/04750/2020). S Morais received funding under the scope of the project "NEON-PC - Neuro-oncological complications of prostate cancer: longitudinal study of cognitive decline" (POCI-01-0145-FEDER-032358; ref. PTDC/SAU-EPI/32358/2017) and the EPIunit – Junior Research – Prog Financing (UIDP/04750/2020). N Araújo received an individual grant (SFRH/BD/119390/2016) funded by FCT and the 'Programa Operacional Capital Humano' (POCH/FSE). C La Vecchia received funding from the Italian Association for Cancer Research (AIRC, investigator grant no. 21378). All authors received support from the European Cancer Prevention (ECP) Organization for project meetings. All authors thank all MCC-Spain study collaborators (CIBERESP, ISCIII, ISGlobal, ICO, University of Huelva, University of Oviedo, University of Cantabria, University of León, ibs. Granada, Instituto Salud Pública de Navarra, FISABIO, Murcia Regional Health Authority and cols).

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Table 1. Pooled odds ratios and 95% confidence intervals (random-effects model) comparing *Helicobacter pylori* negative and *H. pylori* positive gastric cancer cases with regard to sociodemographic characteristics, clinical features and lifestyles factors among all gastric cancer cases and non-cardia gastric cancer cases only, considering serological test results and additionally reclassifying as positive the *H. pylori* infection status of cases likely to correspond to false negative results of the serological test.

	***H. pylori* negative *vs H. pylori* positive gastric cancer cases[a]**					
	All gastric cancer cases		**Non-cardia gastric cancer cases only**			
	Serological test results[b]		**Serological test results[b]**		**After reclassification of *H. pylori* status[c]**	
	aOR[d] (95%CI)	**I^2 (%)**	**aOR[d] (95%CI)**	**I^2 (%)**	**aOR[d] (95%CI)**	**I^2 (%)**
Sex[e]						
Males	1		1		1	
Females	1.20 (0.87-1.64)	0.0	1.17 (0.81-1.69)	0.0	0.78 (0.42-1.47)	0.0
Age (years)						
≤65	1		1		1	
>65	0.95 (0.55-1.63)	0.0	0.99 (0.52-1.89)	0.0	1.32 (0.48-3.68)	0.0
Social class[f]						
Low	1		1		1	
Intermediate	0.85 (0.46-1.56)	38.7	1.13 (0.42-3.02)	71.6	1.34 (0.64-2.82)	0.0
High	0.81 (0.44-1.47)	0.0	0.91 (0.31-2.65)	57.4	0.88 (0.09-8.61)	60.1
Histological type						
Intestinal	1		1		1	
Diffuse	1.22 (0.84-1.75)	0.0	1.17 (0.77-1.78)	0.0	1.05 (0.43-2.58)	26.4
Mixed/unclassifiable	0.78 (0.40-1.55)	0.0	1.00 (0.48-2.07)	0.0	1.37 (0.39-4.81)	--[g]
Family history in first-degree relatives						
No	1		1		1	
Yes	1.19 (0.62-2.25)	48.4	1.29 (0.63-2.62)	42.2	2.18 (1.03-4.61)	0.0
Body mass index						
Underweight/Normal	1		1		1	
Overweight/Obese	1.02 (0.54-1.91)	55.9	0.97 (0.39-2.44)	69.3	1.14 (0.26-5.05)	37.9
Smoking status						
Never	1		1		1	
Ever	0.80 (0.43-1.47)	63.1	0.93 (0.37-2.35)	77.8	0.92 (0.46-1.82)	0.0
Former	0.78 (0.44-1.38)	48.6	0.93 (0.34-2.53)	76.4	0.84 (0.39-1.81)	0.0
Current	0.85 (0.40-1.78)	54.1	0.97 (0.37-2.55)	63.0	2.16 (0.52-9.02)	34.9
Alcohol drinking						
Never	1		1		1	
Ever	0.89 (0.57-1.38)	0.0	0.92 (0.53-1.59)	6.3	0.68 (0.20-2.32)	24.6
Former	0.86 (0.50-1.47)	0.0	0.82 (0.42-1.60)	0.0	0.70 (0.17-2.79)	0.0
Current	0.83 (0.41-1.67)	19.6	0.74 (0.23-2.39)	53.0	2.29 (0.57-9.22)	--[g]
Fruit and vegetable intake						
Intermediate/High	1		1		1	
Low	1.27 (0.55-2.93)	68.1	1.60 (0.43-5.96)	77.0	2.73 (1.04-7.32)	--[g]
Salt intake						
Low	1		1		1	
Intermediate/High	1.02 (0.58-1.81)	0.0	0.94 (0.50-1.78)	0.0	1.31 (0.58-2.96)	0.0

aOR – Adjusted odds ratio; CI – Confidence interval.

[a] Including four studies with information on CagA status: BRAZIL 1 and 2 [Nishimoto et al., 2002 (12), Hamada et al., 2002 (13)], PORTUGAL [Lunet et al., 2007 (8)], and SWEDEN [Ye et al., 1999 (10)].

[b] Among all gastric cancer cases and non-cardia gastric cancer cases only, *H. pylori* infection status was defined considering serological tests using the same criteria applied in each original study.

[c] Among non-cardia gastric cancer cases only, 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.

[d] Adjusted for sex, age (continuous), social class and study centre (for multicentre studies), except if otherwise specified.

[e] Adjusted for age (continuous), social class and study centre (for multicentre studies).

[f] Adjusted for sex, age (continuous) and study centre (for multicentre studies).

[g] OR estimates could only be estimated for one study, PORTUGAL [Lunet et al., 2007 (8)], due to the small number of *H. pylori* negative cases in each strata.

Figure legends:

Figure 1. Flow chart of sample definition considering different criteria to define *Helicobacter pylori* infection status as negative.

Figure 2. Prevalence and pooled prevalence of *Helicobacter pylori* negative gastric cancer cases among all gastric cancer cases and non-cardia gastric cancer cases only, considering serological test results[a] to define *H. pylori* infection status as negative.

CagA – Cytotoxin associated-gene A; CI – Confidence interval; DL – Dersimonian–Laird random-effects model; *H. pylori* + – infected with *Helicobacter pylori*; *H. pylori* - – not infected with *Helicobacter pylori*.

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

Figure 3. Prevalence and pooled prevalence of *Helicobacter pylori* negative gastric cancer cases among all gastric cancer cases and non-cardia gastric cancer cases only, considering serological test results[a] and following the reclassification of a negative serological result for *H. pylori* infection status as positive if a positive result was obtained for CagA status.

CagA – Cytotoxin associated-gene A; CI – Confidence interval; DL – Dersimonian-Laird random-effects model; *H. pylori* + – infected with *Helicobacter pylori*; *H. pylori* - – not infected with *Helicobacter pylori*.

[a] Among all gastric cancer cases and non-cardia gastric cancer cases only, *H. pylori* infection status was defined considering serological tests using the same criteria applied in each original study.

Author Manuscript Published OnlineFirst on November 2, 2021; DOI: 10.1158/1055-9965.EPI-21-0402
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Figure 1

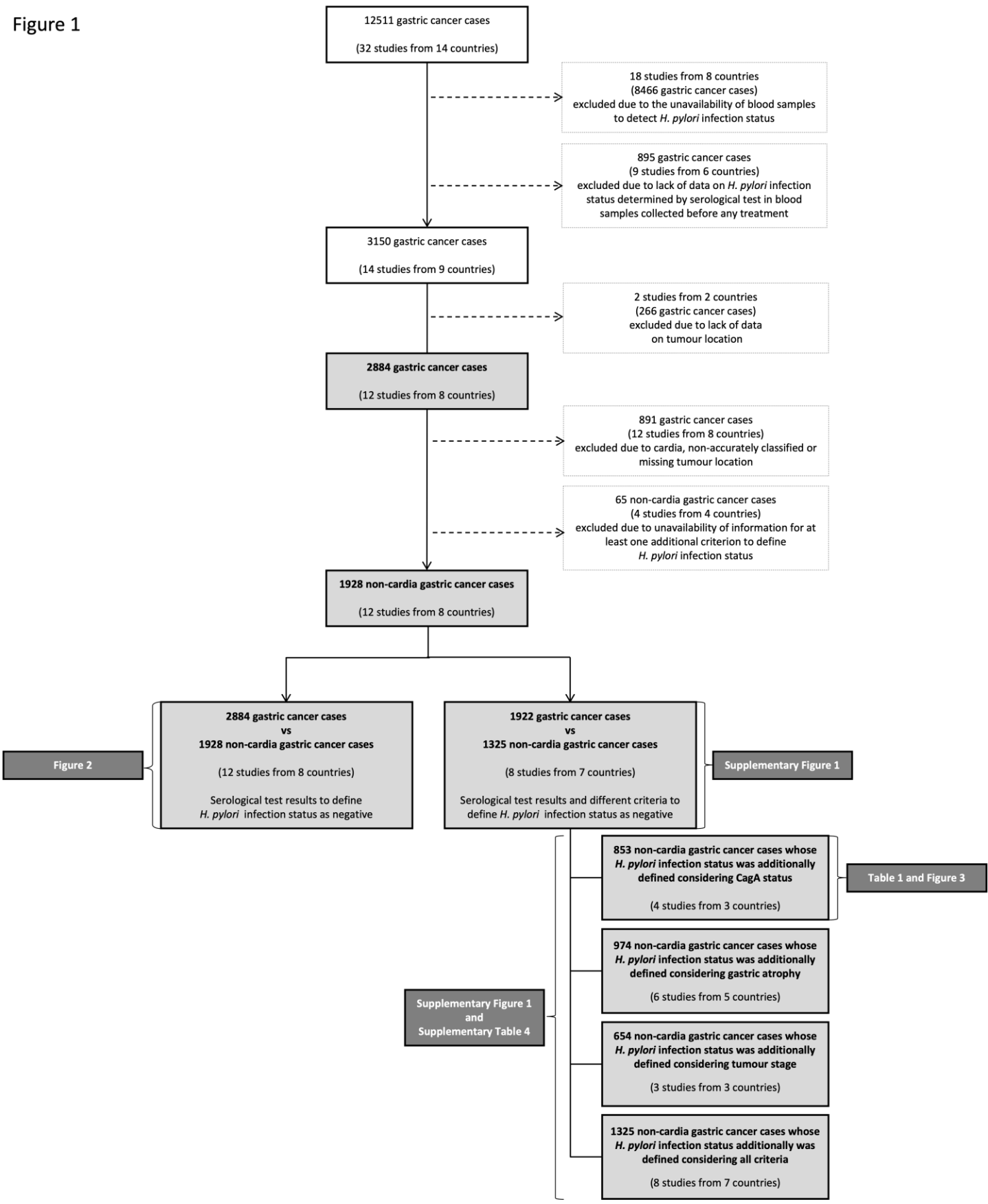


Figure 2

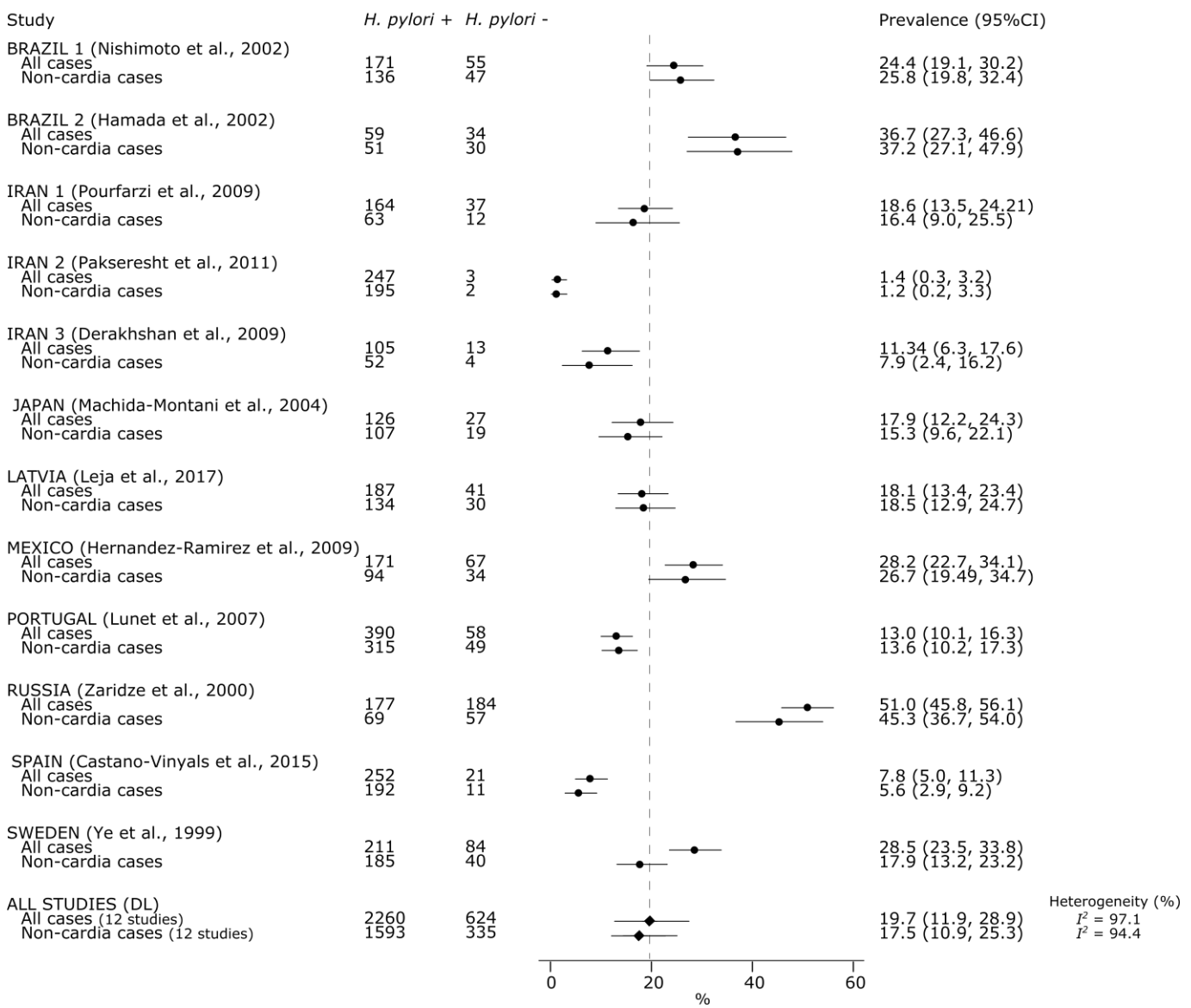
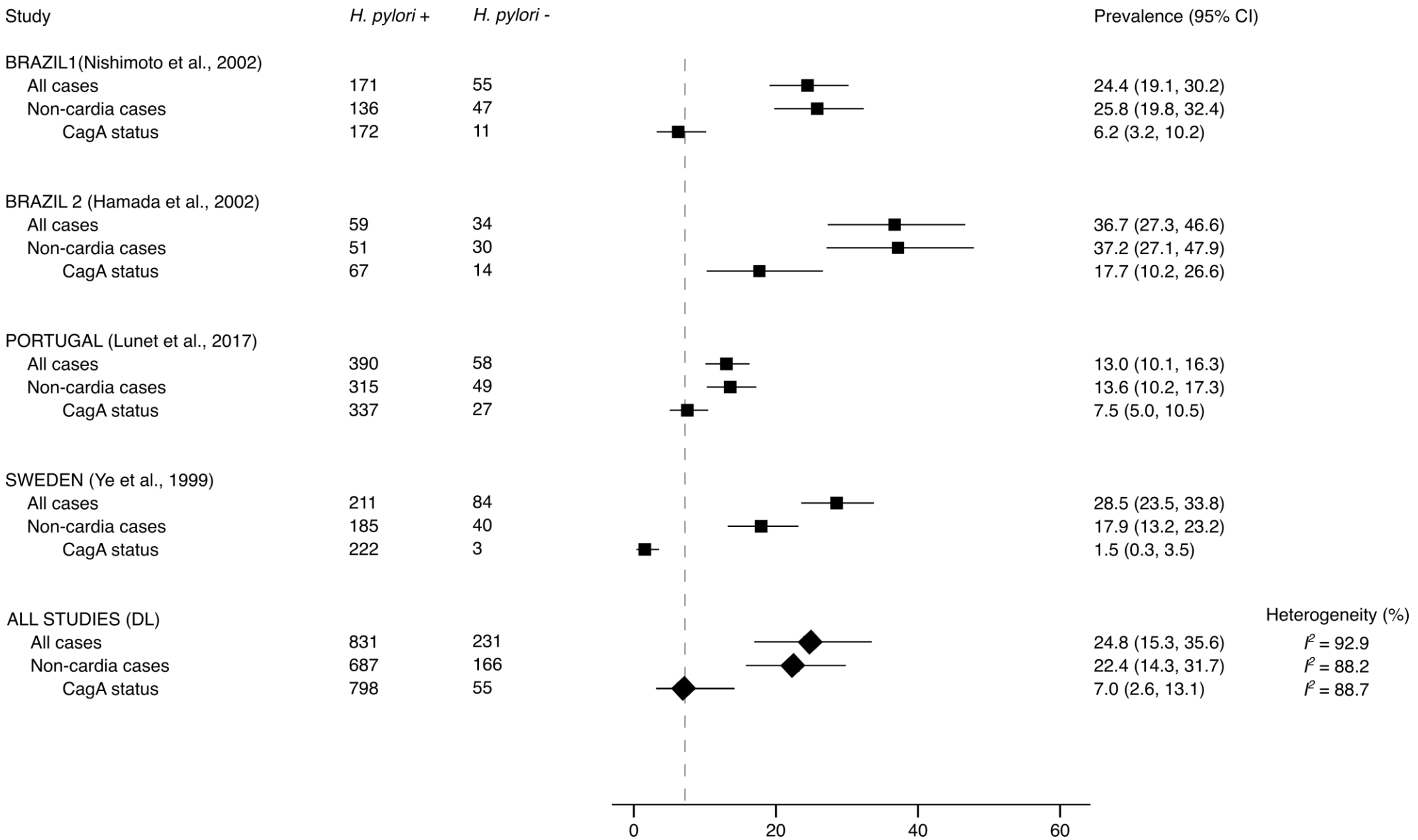


Figure 3



Cancer Epidemiology, Biomarkers & Prevention

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Identifying the profile of Helicobacter pylori negative gastric cancers: a case only analysis within the Stomach cancer Pooling (StoP) Project

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Cancer Epidemiol Biomarkers Prev Published OnlineFirst November 2, 2021.