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Relationship between *Helicobacter pylori* infection and gastric cardia cancer

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1. Gastric cancer incidence, mortality and trends

Gastric cancer is now the fourth most common cancer worldwide, with around 989 000 new cases estimated for 2008 (1).

On the American continent, sharp regional differences are observed in the gastric cancer age-standardized incidence rates. North America has the lowest incidence, in Central America the rates are approximately twice higher and in South America about three-fold higher (figure 1) (1). In Europe, Northern and Western countries have the lowest incidence followed by the Southern and Central/Eastern regions (1). Worldwide, the highest age-standardized incidence rates are observed in Eastern Asia, and the lowest in Western Africa (figure 1) (1).

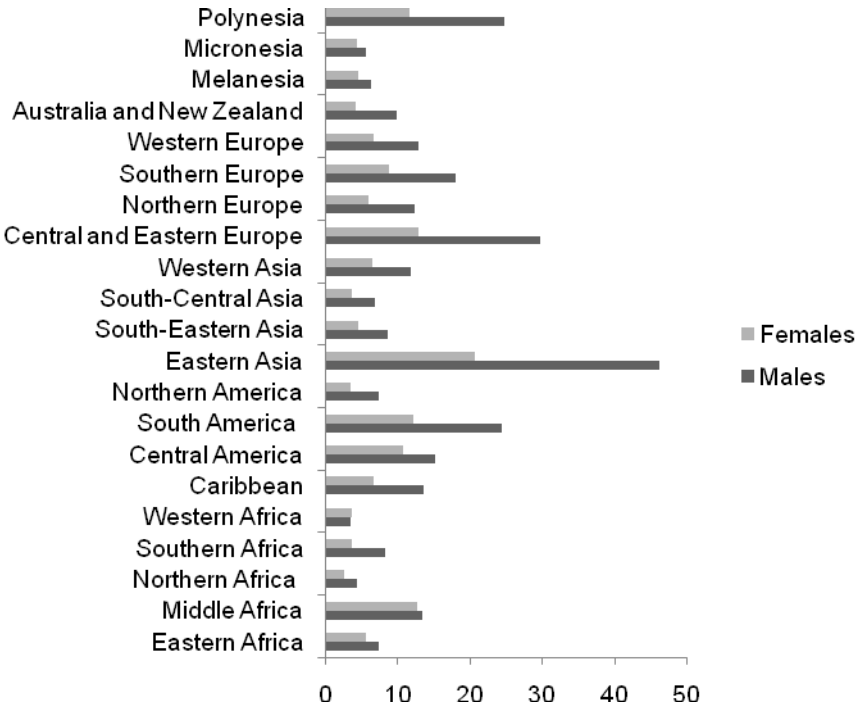

Figure 1. Incidence of the gastric cancer by sex by region (Age-Standardised rates (world reference population)), Source: Globocan-2008 (1).

The incidence rates from gastric cancer in generation migrants tend to move toward to the rates of host country (2-4).

Gastric cancer is the second most common cause of oncological death, with approximately 737 000 deaths annually (1).

In America, there is a gradual increase in mortality rates as we move to the south (figure 2), similarly to what is observed in Europe, with North and West regions presenting the lowest mortality rates, and Southern and Central/Eastern regions the highest (figure 2) (1). In Eastern Asia, the age-standardised mortality rates are among the highest worldwide, with South Eastern, South Central and Western Asian regions presenting mortality rates similar to the observed in Northern and Western Europe (figure 2) (1). Africa is the continent with the lowest age-standardised mortality rates, the lowest being observed in the North and the highest in Middle Africa (figure2) (1).

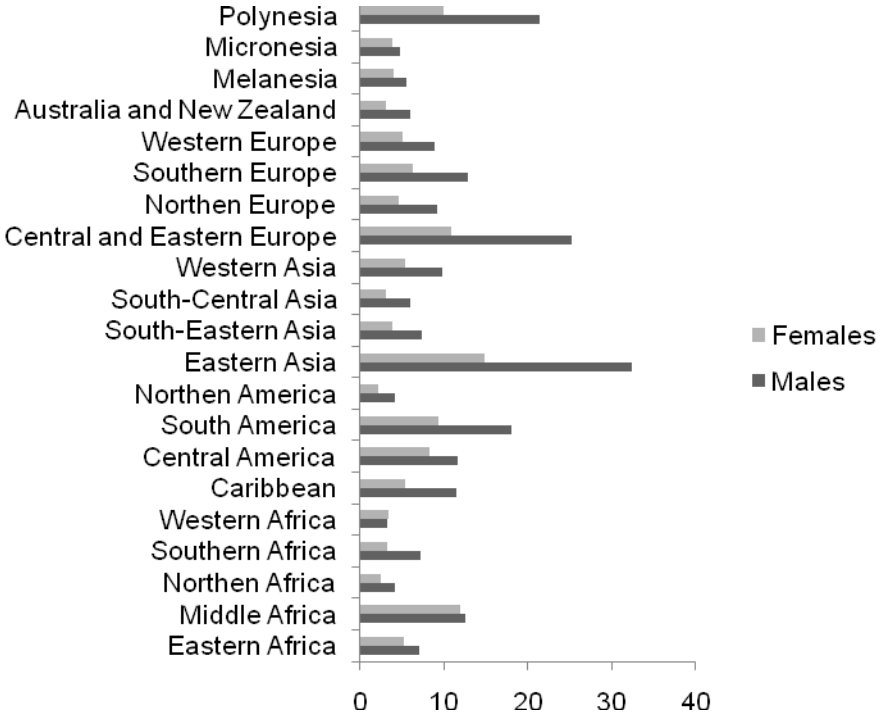

Figure 2. Gastric cancer mortality by region, according to gender (age-standardised rates; world reference population). Source: Globocan-2008 (1).

Since the mid 1980s, incidence rates of gastric cancer have fallen in all high-income countries, and overall rates are now about 15% lower than in 1985 (5-7), with a wide variation in incidence rates across geographical areas (5). There is also a large heterogeneity between countries in the incidence of the two major sites of gastric cancer, namely the proximal (cancer of the cardia) and distal (noncardia cancer) locations (8-11).

1.1. Frequency and trends by cancer location

Despite the overall decline in gastric cancer, most published papers showed an increasing (9, 10, 12-16) or stable incidence in gastric cardia cancer (8, 17-21), in the past 30 years. In the last decade's population-based studies in western countries like United States, Australia, New Zealand and several countries of Europe, reported an increasing incidence of gastric cardia cancer (22), while the incidence of tumours in the non-cardia localization is decreasing (23). An increase in incidence was observed in Northern Europe (Denmark), Southern Europe (Italy, Varese), Eastern Europe (Slovakia) and Western Europe (England and Wales, Scotland) during 1968-1995. In Central Europe (Switzerland, Basel) and in Northern Europe (Iceland), Western Europe (France, Bas-Rhin and Calvados, Southern Ireland), and in Western Europe (Netherlands, Eindhoven) no rise in incidence was observed (11). In Japan, national data reported significant increases in gastric cardia cancer, in both absolute number and in proportion to other gastric cancers over a 36-year period (24). In China, a statistically significant increasing trend of gastric cardia cancer was observed during in the last 16 years (25).

The gastric cardia represents only the proximal 2-3 cm of the stomach. This small anatomical region can easily be overgrown by tumours that originate from adjacent mucosal sites (26).

The definition of the anatomical cardia region has changed over the years and currently differs between countries. Anatomically the gastric cardia is defined as the part of the stomach adjacent to the orifice of the oesophagus (27), but this orifice can also be defined as the oesophagogastric junction (EGJ). As the EGJ forms the border between oesophagus and stomach, the classification of tumors in this region is inherently complex. While some investigators classify all oesophagogastric tumours as oesophageal carcinomas, others consider them to be gastric carcinomas and yet others regard them as separate entities (27). The EGJ is differently defined by anatomists, physiologists, endoscopists and pathologists.

Physiologists define EGJ as the distal border of the lower oesophageal sphincter (LES) as determined by manometry. This location, however, is difficult to identify by endoscopic techniques and there is a large discrepancy between manometric and endoscopic estimation of the LES. As the distal oesophagus is a dynamic structure with peristaltic activity, which moves during respiration, it is a difficult task for accurate endoscopic judgments of the EGJ. Histologically, the gastric cardia has a distinct pattern of loosely packed mucous glands in which an occasional parietal

cell can be present. Histology, however, needs to be combined with the endoscopic appearance, as cardiac type mucosa can be present in the oesophagus in the setting of columnar lined oesophagus (27).

Adenocarcinomas of the EGJ, in China, are generally referred to as gastric cardia adenocarcinomas. Therefore, adenocarcinomas arising from the EGJ/cardia have been referred to as oesophageal cancers for the past several decades. As most of the adenocarcinomas arising from the cardia are diagnosed at an advanced stage, it is very difficult to accurately define whether these tumours have a primary oesophageal or gastric origin (28). Imprecise clinical and pathological definitions of adenocarcinomas of the lower oesophagus, EGJ, and gastric cardia may be one of the reasons underlying inconsistencies across studies (27). In populations where both oesophageal and gastric adenocarcinomas are common, tumours described as cardia cancers undoubtedly include a mixture of neoplasms arising from the lower oesophagus as well as the gastric cardia and distal stomach (26).

2. Aetiological epidemiology of gastric cardia cancer

2.1. Environmental factors

2.1.1. Lifestyles

The geographic variation, time trend, and the migratory effect on gastric cancer incidence suggest that environmental factors are the major determinants of this disease.

A protective effect from diets rich in fresh fruit and vegetables has been suggested (29, 30). The World Cancer Research Fund (WCRF) published a report in which a substantial amount of evidence is available for vegetables and fruit that protect against stomach cancer (29). Similar results were documented in published cohort and case-control studies evaluating the association between fruit and vegetables consumption and the occurrence of gastric cardia cancer (30-33).

Vitamin C and other anti-oxidant nutrients have attracted a lot of attention as the potential mediators of a dietary influence on gastric cancer risk. Vitamin C seems a promising candidate since its is a free radical scavenger, it reduces the formation of potentially carcinogenic N-nitroso compounds (34). Data from the EPIC-EUROGAST show a negative correlation between gastric cancer risk and serum vitamin C (35). A Cochrane Collaboration review, which included a number of high-quality randomized trials, has concluded that there is no evidence that dietary supplementation with anti-

oxidants, including vitamin C, reduces gastric cancer risk (36). Dietary vitamin C has been shown inversely associated with both gastric cancer subsites (37, 38).

Salt and nitrite are other dietary components that have been implicated in gastric cancer risk. Pickled and smoked foods may also contain potential carcinogens such as N-nitroso compounds and benzapyrene. Carcinogenic N-nitroso compunds can be formed directly from nitrite and nitrate in the diet, or by the metabolic action of anaerobic bacteria colonizing the stomach (39). Meta-analyses have shown a positive association between gastric cancer risk and both salt and dietary nitrate/nitrite intake (29).

Smoking is an independent risk factor for gastric cancer, which is involved in neoplastic transformation of gastric mucosa. It was found to nearly double the risk of transition from atrophic gastritis to dysplasia in a high-risk population (40). A systematic review and meta-analysis of cohort studies presented an overall summary RR estimate for current smokers in the highest categories of consumption compared to never smokers of 1.66 (95% CI: 1.46-1.88) (41), and prospective studies have demonstrated a significant dose-dependent association between tobacco smoke and gastric cancer (42, 43). In the EPIC-EUROGAST study it was estimated that 17.6% of the gastric cancer cases occurring in this European population were attributable to cigarette smoking (41, 44). Published studies yielded similar conclusions for cardia cancer, supporting that smoking is a risk factor also for this gastric cancer subtype (41, 45).

2.1.2. Helicobacter pylori infection

The discovery of *H. pylori* in 1983 has proved to be pivotal in our understanding of the aetiology of gastric cancer (46). In 1994, the World Health Organization and the International Agency for research on Cancer consensus group stated that there was sufficient evidence to classify *H. pylori* as a Class I human carcinogen (47).

2.1.2.1. Bacterial properties and pathogenic mechanisms

H. pylori is a gram-negative bacillus and when observed *in vivo* is a spiral-shaped or curved rod, a few micrometers long and actively motile. The bacteria can be found in a horse-shoe like U-form and a round, or coccoid forms, in older cultures. The four to six unipolar sheathed flagella are important for bacterial motility (48, 49).

A number of bacterial factors have been suggested to be responsible for successful infection by *H. pylori*: high urease production, which increases the pH and activates the host immune defence; flagella, which facilitate the movement within the mucus layer; adherence to gastric epithelium by different adhesins using hemagglutinins, laminin and Lewis b antigens as receptors (48, 50).

There is accumulated evidence that acid secretory capacity is important in determining the distribution, and natural history of *H. pylori* infection (51, 52). In hosts with low secretory capacity (genetically determined or by pharmacologic inhibition) the organisms are capable of colonising a wider niche than would be possible in the presence of high volumes of acid (51, 52). Colonization of a wider niche including the corpus mucosa leads to corpus gastritis with resultant functional inhibition of acid secretion (51). This inhibition is mediated by *H. pylori* induced inflammatory cytokines (such as IL-1β and TNF-α) and the net effect is the establishment of a more aggressive gastritis that accelerates the development of gastric atrophy. The infected subjects under long term proton pump inhibitors have a higher risk of developing gastric atrophy, a precursor lesion of gastric neoplasia (53). Gastric atrophy seems to be a morphological change hard to reverse, although this issue is still open to debate.

2.1.2.2. Strains variation and the cag pathogenicity island

Early investigations of the differential pathogenic properties of *H. pylori* strains indicated that pathogenicity was associated with the ability of these more virulent strains to induce morphological changes, vacuolization, and successive degeneration of in vitro-cultured cells (54). This activity was then linked to the presence of a protein with a molecular mass of approximately 140 kDa that was named *CagA* (for "cytotoxin-associated gene A") and a highly immunogenic 95-kDa protein that was named VacA (VacA vacuolating cytotoxin) (55).

The *CagA* protein is a highly immunogenic protein encoded by the *cagA* gene (55). The *cagA* gene is present in approximately 50 to 70% of *H. pylori* strains (56-58) and is a marker for the presence of a genomic PAI (cag pathogenicity island) of about 40 kb that, depending on the strain analyzed, encodes between 27 and 31 proteins (55, 59, 60). *H. pylori's CagA* protein is now regarded as having direct oncogenic potential (61). The *CagA* is delivered into gastric epithelial cells by the bacterial type IV secretion system. Once injected into gastric epithelial cells, *CagA* undergoes tyrosine phosphorylation by SRC family Kinases. Phosphorilated *CagA* specifically binds and

activates SHP2 (protein-tyrosine phosphatase), that acts as a human oncoprotein. SHP2 transmits positive signals for cell growth and motility and deregulation of SHP2 by *CagA* is thought to be an important mechanism by which *CagA*-positive *H. pylori* strains may promote gastric carcinogenesis (61). *H. pylori* is capable of subverting cell physiology towards several proneoplastic process through *CagA* and other proteins (*e.g.* activation of growth factor receptors, increased proliferation, sustained angiogenesis and cell dissociation and tissue invasion) (61). *H. pylori* and its citotoxins mediate these proneoplastic mechanisms and it is very likely that the inflammatory process unleashed by the presence of the bacteria on the gastric mucosa also contributes to the neoplastic impel (61). Many of the mediators and products of inflammation are mitogenic and mutagenic (62). Release of pro-inflamatory cytokines, reactive oxygen species and upregulation of cyclooxygenase-2 (Cox-2) all contribute to an intra-gastric environment conductive to neoplastic transformation (62). The mechanisms involve direct DNA damage, inhibition of apoptosis, subversion of immunity, and stimulation of angiogenesis (62). In addition, chronic inflammation in the gastrointestinal tract is also known to affect proliferation, adhesion and cellular transformation (62). However, the clinical outcome of *H. pylori* infection is varied and includes gastric cancer and non-neoplastic conditions (peptic ulcer, non-ulcer dyspepsia and simple asymptomatic gastritis).

The VacA protein plays an important role in the pathogenesis of both peptic ulceration and gastric cancer (63). The activities of VacA include membrane channel formation, disruption of endosomal and lysosomal activity, effects on integrin receptor-induced cell signaling, interference with cytoskeleton-dependent cell functions, induction of apoptosis, and immune modulation (63). The VacA protein is produced as a 140-kDa protoxin that is cleaved into the 95-kDa mature form when secreted. Although all strains carry a functional *vacA* gene, there is considerable variation in vacuolating activities among strains. This is due to the sequence heterogeneity within the *vacA* gene at the signal region (s) and the middle region (m) (63). The s region of the gene, which encodes the signal peptide, occurs as either an s1 or s2 type, whereas the m region, which contains the p58 cell binding domain, exists as an m1 or m2 type. Vacuolating activity is high in s1/m1 genotypes, intermediate in s1/m2 genotypes, and absent in s2/m2 genotypes (63, 64). In line with this, *vacA* s1/m1 genotypes are more frequently associated with peptic ulceration and gastric carcinoma (64). The vacA s2/m1 strain, however, is noncytotoxic (65, 66).

The genes *cagA* and *vacA* were the most intensively studied over the years and have been used as the most common markers for strains with enhanced virulence.

2.1.2.3. Diagnostic methods

Currently there are several methods for detection of *H. pylori* infection and can be grouped into 2 categories, depending on the technique used to collect the biological material used: invasive tests (require endoscopy) and non-endoscopy tests (67, 68). The ^{13}C-urea breath test (^{13}C UBT), is a technique used to detect *H. pylori* colonization in the stomach, without an endoscopical examination. The high urease activity of *H. pylori* turns urea into ammonia and produce ^{13}C-labeled CO_2 which can be measured with biochemical tests kits in market. Other non-endoscopy technique is based on the collection of saliva, serum, or stool samples that need processing before the test can be performed (*e.g.* enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), Western blot, complement fixation, latex agglutination assays) (68, 69). A large number of enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) tests to detect anti-*H. pylori* Immunoglobulins (*e.g.* IgG, IgM and IgA) are available in the market. Comparisons between sensitivity and specificity of these tests are published regularly (70-72). The median sensitivity and specificity in 36 commercially available kits were 92% and 83% respectively. The immunoblotting techniques are useful method to detect infections caused by more virulent strains (*e.g.*: *VacA* and *CagA* positive) (73-77).

The invasive tests which require biopsy material are: rapid urease test (RUT–CLO-test), direct Gram stain, culture (in non-selective or selective agar), histology (by haematoxylin and eosin, Warthin Starry Silver, modified Giemsa stain or acridine-orange stains), and immunohistochemistry (by immunoperoxidase or immuno-fluorescent techniques using anti-*H. pylori* monoclonal or polyclonal antibodies) (67).

For specific detection of *H. pylori* in environmental samples, stool samples, gastric juice and biopsies, and for detection of *H. pylori* genes, polymerase chain reaction techniques are also used. The most frequently genes that are used for PCR-techniques to distinguish between *H. pylori* and other microorganisms are the adhesin encoding gene *hap*, the urease encoding gene *ureA* and 16S ribosomal RNA gene (78).

2.1.2.4. Epidemiology of infection

The prevalence of *H. pylori* infection varies across geographical regions. In developed countries, the improvement of sanitation and hygiene has been responsible

for the decrease in transmission during the last few decades, and the epidemiology of the bacterium at present follows a birth cohort model (older people infected decades ago still frequently carry the bacterium while children rarely do) (79). As the acquisition of *H. pylori* occurs predominantly in childhood and, if untreated, persists throughout life, a lower seroprevalence of *H. pylori* in older age-groups is expected in the future.

In general, infection is acquired during childhood, and so the prevalence gradually increases, at a higher rate in developing than in developed countries, to reach the maximum in middle age (80). The overall estimate prevalence of infection in middle-aged adults is 74% in developing countries and 58% in developed countries (80). In 2002, the proportion of gastric cancers worldwide attributable to *H. pylori* infection was 63.4%, corresponding to 592 000 cases. Regarding developed and developing countries the estimated numbers of stomach cancer cases attributable to *H. pylori* infection was 61.4% and 64.4%, respectively (80).

From the many risk factors for infection that have been investigated, age and lower socioeconomic status (SES) are the ones considered most important (81). Socioeconomic deprivation in childhood is associated with a high prevalence of *H. pylori* colonisation. While the incidence of *H. pylori* may be declining, it remains common in poor families (82, 83). Low socioeconomic status, poor sanitary indications, and crowded families in childhood were related to high prevalence of *H. pylori* infection (83).

2.1.2.5. *Helicobacter pylori* infection and gastric cardia cancer

Numerous cohort and case-control studies were published demonstrating an association between serological evidence of *H. pylori* infection and increased risk of gastric cancer, which have been summarized in meta-analyses (84-91).

The first meta-analysis on this subject was published in 1998 and included 19 epidemiological studies (cohort and case-control studies) that diagnosed *H. pylori* infection by serology. The researchers concluded that *H. pylori* infection is a risk factor for gastric cancer. They suggested that the heterogeneity of reported results was caused by differences in the selection of controls, patient age and stage of gastric cancer (figure3) (90).

In the following year, a meta-analysis based in prospective studies (10 nested case-control studies) was published, suggesting that gastric cancer was 2 or 3 times more frequent in those chronically infected by *H. pylori* (figure 3) (92). In the same

year, Eslick *et al.* published a meta-analysis performed with 42 studies (8 cohort studies and 34 case-control studies), which confirmed the positive association between *H. pylori* infection and gastric carcinoma. According to the authors, an effort was made to perform an analysis as inclusive as possible and the quality of studies included was explored as a source of heterogeneity. The results suggested that the majority of studies classified as excellent found positive association and the very poor to moderate studies gave mixed results (figure 3) (88).

In 2001, the *Helicobacter* and Cancer Collaborative Group published a study based in a collaborative reanalysis using individual subject data from 12 nested case-control studies intending to clarify the magnitude of the association. The inclusion of data from published and unpublished studies was the main difference in relation to previous meta-analyses. The results support the idea that when *H. pylori* status is assessed close to cancer diagnosis, the magnitude of the association may be underestimated (figure 3) (91).

In the same year, a meta-analysis conducted only with studies published in Chinese populations (11 case-control studies conducted in China) was published. The results showed that *H. pylori* infection is a risk factor for gastric carcinoma, increasing the risk to 3-fold more in Chinese population infected (figure 3) (87).

The most recent meta-analysis on this topic was published in 2007, and it has reviewed systematically the relationship between *H. pylori* infection and the occurrence of early gastric cancer (EGC) and in the advanced gastric cancer (AGC). In the fifteen studies included, the prevalence of *H. pylori* infection in EGC and in non-neoplasm controls was 87.3% (2,377/2,722) and 61.4% (8,588/13,976) respectively, corresponding to a summary OR of 3.38 (95% CI, 2.15–5.33), but the results from the individual studies were substantially heterogeneous (I^2=83.5%, p<0.00001). Sensitivity analyses were conducted by separating studies with factors that could have possible impact on heterogeneity (*e.g.*: *H. pylori* detection methods, different publication years, geography, and different sample sizes); and the prevalence of infection in EGC and controls remained essentially unchanged. The OR was around 3 in all the strata and the heterogeneity was not eliminated. The prevalence of *H. pylori* in EGC group was 87.6% (1,780/2,032), significantly higher than the 77.3% (874/1,130) in the advanced gastric cancer (AGC) group, with an OR of 2.13 (95% CI; 1.75-2.59). No significant heterogeneity was observed (p=0.87) (figure 3) (84).

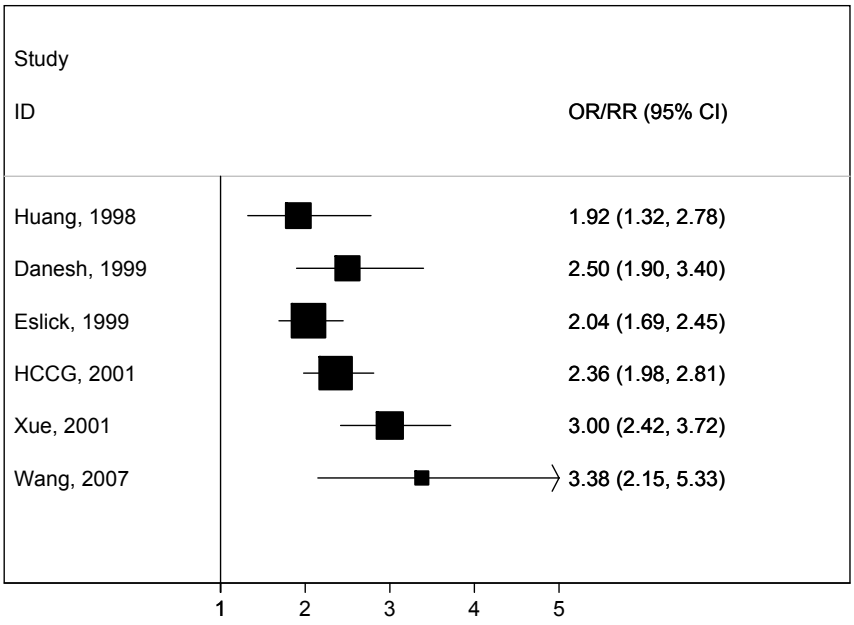

Figure 3. Meta-analyses assessing the risk of gastric cancer in the presence of *H. pylori* infection.

In 2003 two meta-analyses were published by Huang *et al*., aiming to clarify the relationship between infection with *CagA* positive strains and the occurrence of gastric cancer, exploring possible sources of heterogeneity that may explain the conflicting results between individual studies (85, 93). These two meta-analyses, published with an interval of six months between them, produced similar results for the association between *H. pylori* infection and gastric cancer (figure 4). However, the second paper to be published includes more three studies and explores extensively possible sources of heterogeneity (93). In both studies the results showed that patients harbouring *CagA* positive strains had an increased risk of gastric cancer over and above the risk associated with *H. pylori* alone (figure 4). Among *H. pylori* infected populations, infection with cagA-positive strains further increased the risk for gastric cancer by 1.64-fold (95% CI: 1.21-2.24) (93).

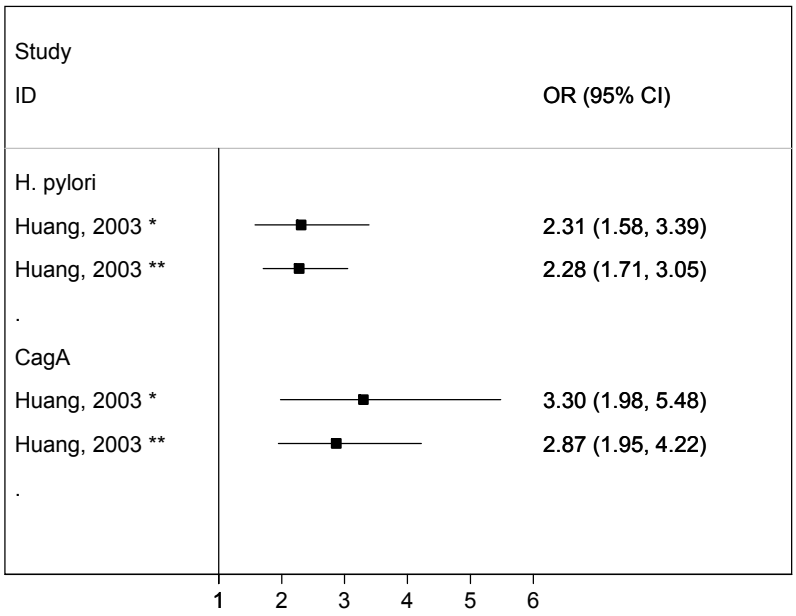

Figure 4. Meta-analyses assessing the risk of gastric cancer in the presence of *CagA* positive strains of *H. pylori* infection. * First meta-analysis to be published. ** Second meta-analysis to be published.

Four of the meta-analyses aforementioned addressed the relation between infection and gastric cancer by anatomical region (85, 90, 91, 93). Two meta-analyses reported summary measures for the association between: 1) *H. pylori* infection and cardia cancer; 2) *H. pylori* infection and non-cardia cancer (90, 91). Two meta-analyses reported summary measures for the association between: 1) CagA infection and cardia cancer; 2) CagA infection and non-cardia cancer (85, 93). The results showed that *H. pylori* infection is a risk factor for non-cardia cancer, but not for gastric cardia cancer (90, 91). Similar results were obtained in populations infected by CagA strains (85, 93).

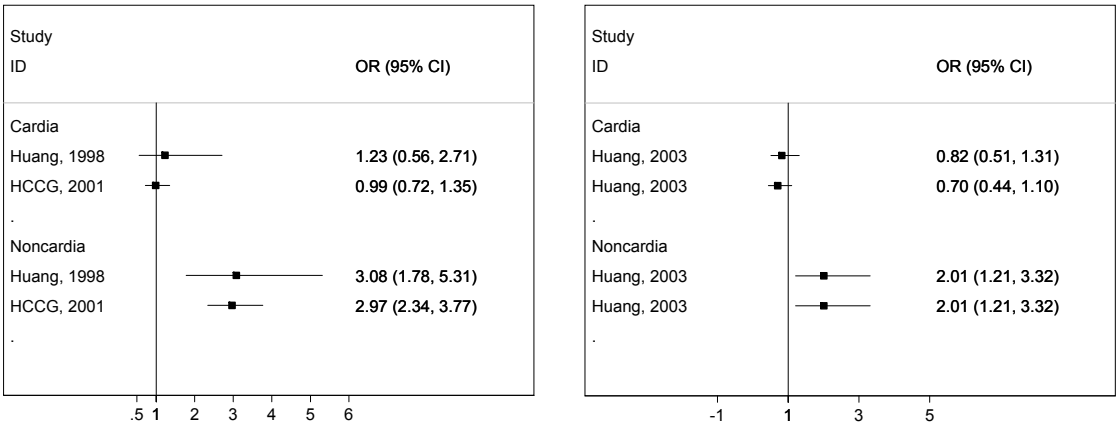

Figure 5. Meta-analysis assessing the risk of gastric cardia and non-cardia cancer in the presence of *H. pylori* infection (left) and according to infection with *CagA* positive strains (right).

Despite these meta-analyses concluded that there is no positive association between infection and cardia cancer, there is a large heterogeneity across studies which is very complex and depends to a large extent on the geographical location. Western countries appear to have a null or even negative association (94-100), while in Eastern countries (e.g.: China, Japan, Iran, and Korea) the association seems to be positive (101-106).

The heterogeneity of the results from individual studies leading to the overall null association between infection and cardia cancer found in these meta-analyses requires a consideration based mainly on three facts: 1) populations with different characteristics (with different incidences of gastric cancer, prevalence of infection, prevalence of infection by virulent strains) were combined in analysis (26). When data of the study of Helicobacter and Cancer Collaborative Group were reanalyzed a positive association between infection and gastric cardia cancer was reported in stratum of Asian studies (populations with similar characteristics) (26). The appropriateness of combining Western and Asian studies was called into question by formal statistical testing (the heterogeneity test had found differences between the results from Western and Asian studies) (26). 2) the type of study design should be consistent with the characteristics of the populations under study. It is known that the estimates from case-control studies conducted in populations with high prevalence of infection are conservative. In case-control studies, in which the search for infection was performed after de diagnosis of gastric cancer, the magnitude of the association

probably was underestimated due to loss of infection in cases. It is possible that *H. pylori* infection may disappear spontaneously over time, especially when severe gastric atrophy or intestinal metaplasia develops in patients with gastric cancer after longstanding *H. pylori* infection (107). There is evidence suggesting that *CagA* antibodies remain in blood stream for a longer period of time after the disappearance of *H. pylori* IgG surface antibodies (75) and relying on *H. pylori* IgG antibodies alone might misclassify a significant proportion of patients who once had the infection. This could lead to an underestimate of the risk for gastric cancer associated with *H. pylori* infection. 3) the information on the classification of cases as belonging to cardia region is lacking in some studies and, when present, the classification criteria adopted differ between studies. In populations where both oesophageal and gastric adenocarcinomas are common, tumours classified as cardia cancers, probably include a mixture of neoplasms arising from the lower oesophagus as well as the true gastric cardia region (26). Diverse subject populations with high prevalence of gastrooesophageal reflux disease may affect the perceived *H. pylori* cardia cancer relationship in the four meta-analyses mentioned above. In 2001, Dawsey *et al*., had proposed that both gastrooesophageal reflux disease and *H. pylori* infection have been suggested as potential risk factors for gastric cardia cancer. In their opinion, gastrooesophageal disease is probably associated with most oesophageal adenocarcinomas and some true gastric cardia cancers whereas chronic *H. pylori* exposure appears to be a predisposing factor for most gastric non-cardia adenocarcinomas and some gastric cardia cancers (26).

A nested case-control study conducted in a Norwegian population has helped elucidate the aetiology of cardia cancer (108). The study set out to examine the association between the state of the gastric mucosa and the risk of subsequently developing cardia versus non-cardia gastric cancer. The researchers found that cardia cancer was negatively associated with *H. pylori* (OR= 0.27, 95% CI 0.12-0.59), but a positive association was observed between atrophic gastritis and cardia cancer in those with infection (OR=3.33, 95% CI: 1.06-10.5). These findings suggest two aetiologically distinct types of cardia gastric cancer. One associated with atrophic gastritis induced by *H. pylori* infection, similar to non-cardia gastric cancer, and the other resembling oesophageal adenocarcinoma, associated with damage of acid/bile-induced reflux. The work of Hansen *et al.* suggests that serological markers of gastric atrophy may provide the key to determining gastric versus oesophageal origin of cardia cancer (109). In 2008, the same research team published a case-control study, performed in northwest Iran, in which they extended her investigation of the aetiology

of cardia cancer by examining the association of both serologic evidence of gastric atrophy and reflux symptoms with adenocarcinoma of the oesophagus, cardia, and non-cardia region of the stomach. They had concluded that there are not only two distinct aetiologies of cardia cancers but that the structural and functional state of the stomach associated with them was profoundly different. One type is associated with a non-atrophic healthy gastric mucosa producing sufficient acid and pepsin to damage the mucosa of the gastro-oesophageal junction and lead to columnar intestinal metaplasia and intestinal subtype cancer. The other is associated with atrophic gastritis of sufficient severity and extent to involve the proximal stomach leading to the development of intestinal or diffuse subtype cancer from the atrophic gastric mucosa (105).

2.2. Host factors

2.2.1. Response to *H. pylori* infection determined by host genetic polymorphisms

Individual differences in the host response to *H. pylori* infection, determined by host genetic polymorphisms, might, in part, explain why some individuals are more likely to develop the gastric cancer phenotype than others. *H. pylori* cause damage by initiating chronic inflammation in the gastric mucosa. This inflammation is mediated by an array of pro and anti-inflammatory cytokines. Genetic polymorphisms directly influence inter-individual variation in the magnitude of cytokine response and this clearly contributes to an individual's ultimate clinical outcome (108). It was speculated that the most relevant candidate genes implicated in the host response to infection would be ones whose products were involved in handling the *H. pylori* attack (innate and adaptive immune responses) and ones that mediated the resulting inflammation (108). The most relevant and consistent genetic factors uncovered thus far are in the interleukin-1 and tumor necrosis factor-α gene clusters. Interleukin-1 beta (IL–1β) is a pro-inflammatory cytokine and also a potent inhibitor of gastric acid secretion. The *IL–1* gene was therefore a potential candidate for host genetic polymorphisms that may influence gastric cancer risk. Individuals with pro-inflammatory *IL–1* gene cluster polymorphisms (*IL-1B* encoding IL-1β and *IL-1RN* encoding its naturally occurring receptor antagonist) are at increased risk of developing mucosal atrophy and hypochlorhydria in response to *H. pylori* infection, and this is reflected in a 2- to 3-fold increase in the risk of gastric cancer(110, 111).

Numerous studies were published demonstrating an association between Interleukin-1 (IL-1) gene cluster polymorphisms and increased risk of gastric cancer, which have been summarized in meta-analyses (112-115). Camargo *et al.*, had published, in 2006, the first meta-analysis on the association between Interleukin-1beta (IL1B) and/or interleukin-1 receptor antagonist (IL1RN) gene polymorphisms and gastric. The authors concluded that IL1B-511T and IL1RN*2 polymorphisms were associated with gastric cancer risk in Caucasians, but not in Asians. No significant association of IL1B+3954T and gastric cancer risk was detected (112). In the same year, Kamangar *et al.* published a meta-analysis where the overall associations between IL-1B or IL-1RN proinflammatory polymorphisms and gastric were null (113). However, the contradiction between the two articles was clarified in a subsequent publication, which stated that: 1) Kamangar *et al*. combined Central/South American studies with those from European/North American populations and contrasted them to Asian studies. Camargo *et al.* examined European/North American populations separately. Asian, Central American, and South American populations have an *IL1B-511T* allele frequency of >50%, in contrast to about 33% in Europeans/North Americans. Therefore, grouping populations that vary in the relevant allele frequencies may unsure the association (116); 2) the two meta-analyses used different genetic models (116); 3) in three European studies; *Kamangar et al*. noted overlapping samples, a duplication that Camargo *et al.* overlooked (116).

In 2009, Al-Moundhri *et al.*, had published a meta-analysis that was the first report on the combined analysis of polymorphisms GSTM1/G1 (glutathione S-transferase (GST) M1 and G1) and IL-1B/IL-1RN genes in gastric adenocarcinoma. The author's suggested that the individual variation in both the cellular inflammatory modulator IL-1RN and the antioxidative property of GSTM1 may predispose individuals to an increased risk of gastric cancer (117).

Individuals with the *IL-1B*-31*C or *IL-1B*-511*T and *IL-1RN**2/*2 genotypes are at an increased risk of developing hypochlorhydria and gastric atrophy in response to *H. pylori* infection (108). The IL1RN*22 genotype seems to consistently increase the risk of gastric precancerous lesions, supporting a role for this polymorphism in the early stages of gastric carcinogenesis (118).

Figueiredo *et al.*, showed an interaction between host and bacterium in the pathogenesis of gastric cancer, for each combination of bacterial/host genotype, the odds ratio were greatest in those with both high-risk genotypes (the combined effects of pro-inflammatory *IL-1* genotypes and bacterial virulence factors, such as, *CagA* genotypes) (119).

TNF–α is another pro-inflammatory cytokine whose expression is up regulated in the gastric mucosa in response to *H. pylori* infection. IL-10 is an anti-inflammatory cytokine that suppresses expression of pro-inflammatory cytokines including IL-1β, TNF–α and interferon – gamma (IFN-γ) (39). Pro-inflammatory genotypes of tumour necrosis factor-α (TNF–α) and interleukin-10 (IL–10) were described as independent risk factors for non-cardia gastric cancer (110).

The IFNGR1-56C/T polymorphisms, in the gene encoding the interferon gamma receptor 1 (IFNGR1), are associated with increased susceptibility to *H. pylori* infection and were also shown to be a relevant host susceptibility factor for gastric cancer development (120).

Approximately 10% of gastric cancer cases show familial clustering but only 1 to 3% of gastric carcinomas arise as a result of inherited gastric cancer predisposition syndromes (121). An increased risk of gastric cancer is associated with recognized dominantly inherited cancer predisposition syndromes, such as familial adenomatous polyposis, hereditary non-polyposis colon cancer and Peutz-Jeghers syndrome (122, 123). Hereditary Gastric Cancer is a genetic disease with a germline gene defect, demonstrated by co-segregation of germline E-cadherin (CDH1) mutations with early onset diffuse gastric cancer in families with an autosomal dominant pattern of inheritance (HDGC) (121). Hereditary Diffuse Gastric Cancer is also inherited as a dominant trait, and in around a third of affected kindred is caused by inactivating mutations in the *CDH1* gene, which encodes the epithelial cell adhesion protein E-cadherin (122, 123). E-cadherin is a transmembrane calcium-dependent cell-adhesion molecule involved in cell-junction formation and the maintenance of epithelial integrity (121).

Gao *et al*., in 2008, published a meta-analysis on tumour invasion-related gene polymorphisms (namely the most widely-studied polymorphism CDH1-160C>A polymorphism) and gastric cancer susceptibility. The results showed an inverse association with gastric cancer among Asians (OR=0.76; 95% CI: 0.55-1.05) and a positive association among Caucasians (OR=1.40; 95% CI: 0.95-2.04). The authors had concluded that genetic polymorphisms in tumour invasion could be candidate biomarkers of gastric cancer risk, however, differences between populations and stages of cancer at diagnosis need to be considered and may explain some of the inconsistencies found in previous studies (124).

3. Aims

The aim of the present dissertation was to examine the relationship between infection with *H. pylori* and the occurrence of gastric cardia cancer, through the accomplishment of the following specific objectives:

1) to quantify the association between infection and gastric cardia cancer through meta-analysis, and to provide an explanation for the expected heterogeneity of results.

2) to compare gastric cardia and non-cardia cancers regarding the frequency of *H. pylori* infection, the histological characteristics of the non-neoplastic gastric mucosa, and the tumor histological type.

The methods, results and discussion of the investigations conducted are presented in the articles included in this dissertation:

Paper I: *Helicobacter pylori* infection and gastric cardia cancer: a systematic review and meta-analysis.

Paper II: Is cardia cancer etiologically different from distal stomach cancer?

Helicobacter pylori infection and gastric cardia cancer: systematic review and meta-analysis

ABSTRACT

Objective: *Helicobacter pylori* infection is the most important risk factor for gastric cancer, but no association with cardia cancer has been recognised. However, a heterogeneous distribution of aetiologically distinct types of cardia cancer may contribute to explain conflicting findings between studies in high- and low-risk settings. We aimed to quantify the association between *H. pylori* infection and gastric cardia cancer through meta-analysis, and to provide an explanation for the expected heterogeneity of results.

Methods: We systematically reviewed published studies addressing the association between *H. pylori* infection and gastric cardia cancer (up to June 2009), and extracted relative risk (RR) estimates for the association with cardia and non-cardia cancers. Summary RR estimates and 95% confidence intervals (95%CI) were computed using random-effects models. Subgroup analyses were conducted, namely according to gastric cancer risk settings.

Results: Thirty-four articles were considered for meta-analysis. For cardia cancer, summary RR was 1.08 (95%CI:0.83-1.40;I^2=52.8%), higher in high-risk (RR=1.98;95%CI:1.38-2.83;I^2=18.4%) than in low-risk settings (RR=0.78;95%CI:0.63-0.97;I^2=11.6%). For non-cardia cancer, RR estimates were similar in high- (RR=3.02;95%CI:1.92-4.74;I^2=90.7%) and low-risk settings (RR=2.56;95%CI:1.99-3.29;I^2=46.6%). These observations were consistent across different inclusion criteria and when accounting for the virulence of the infecting strains.

Conclusions: In high-risk settings a positive association between *H. pylori* infection and gastric cancer was observed both for cardia and non-cardia cancers. The results support the hypothesis of a heterogeneous distribution of etiologically distinct types of cardia cancer.

INTRODUCTION

Gastric cancer incidence and mortality have fallen dramatically over the past decades in all developed countries [1]. Evidence of a decrease in cardia cancer frequency along the years, however, is not so abundant [2-4], and most published papers show increasing [5-9] or stable incidence [10-15] in the past 30 years.

Helicobacter pylori infection is known to increase the risk of non-cardia gastric cancer [16-19] but apparently there is no positive association between infection and gastric cardia adenocarcinomas [19-22]. However, the latter relation was evaluated in fewer and smaller studies [21-23] and the evidence is heterogeneous, depending to a large extent on the geographical location where the investigations took place [21]. Studies conducted in Western countries tend to show a neutral or even negative association [24-27] while in Eastern populations with high gastric cancer incidence, namely China, Korea and Japan, there is evidence of a higher risk of cardia cancer among the infected [21, 22, 28-31].

A positive association between gastric cardia cancer and gastric atrophy has been observed, despite no relation between *H. pylori* infection and cardia cancer [32, 33]. However, this may be explained by the coexistence of two aetiologically distinct types of cardia cancer, as recently proposed [34], one associated with *H. pylori*-induced atrophic gastritis, etiologically similar to non-cardia cancer, more frequent in populations with high frequency of gastric cancer, and the other arising in non-atrophic gastric mucosa, associated with acid/bile-induced damage to the distal oesophagus, resembling oesophageal adenocarcinoma [35], and likely to have a higher relative frequency in settings with low overall gastric cancer risk. This hypothesis is according to the available evidence on the relation between *H. pylori* infection and gastric cancer and may provide an explanation for the heterogeneous findings reported so far.

We systematically reviewed the published evidence on the association between *H. pylori* infection and gastric cancer, aiming to quantify the association between infection and gastric cardia cancer through meta-analysis, across settings with different gastric cancer risk.

MATERIALS AND METHODS

Literature search

We identified cohort, nested case-control, case-cohort and case-control studies presenting data on the association between *H. pylori* infection and gastric cardia cancer through the following search strategy:

1) PubMed® search (http://www.ncbi.nlm.nih.gov/entrez) using the expression *((“systematic review” OR meta analysis OR “combined analysis” OR “pooled analysis”) AND Helicobacter pylori AND (gastric OR stomach) AND cancer)*, from inception to June 30, 2009, aiming to identify published systematic reviews and meta-analyses addressing the association between *H. pylori* infection and gastric cancer. The reference lists of the identified systematic reviews and meta-analyses were searched to identify original research reports on this topic.

2) PubMed® search using the expression *(gastric OR stomach OR cardia) AND cancer AND Helicobacter pylori* between March 1, 2003 (corresponding to the publication date of the most recent original research article [36] included in the most recent systematic review [20], among those identified in the previous step of the search strategy) and June 30, 2009, aiming to identify published articles not included in the previous step of the search strategy.

We evaluated full papers published in English, Spanish, Portuguese, French and Italian. English abstracts of full papers written in other languages were considered when providing the necessary information.

The searching, elaboration of references lists, and selection of articles for systematic review was accomplished independently by two researchers (MP, BP) and discrepancies were discussed until consensus or resolved involving a third researcher (NL).

Data extraction

Data extraction was performed independently by two researchers (MP, BP), following *a priori* defined protocol, and discrepancies resolved by consensus or involving a third researcher (NL).

From each study we collected information on the following items: country/region of origin, study design (cohort, nested case-control, case-cohort, population- or hospital-based case-control studies; follow-up time, when applicable), sample characteristics

(number, age and gender of the participants, anatomic location of gastric adenocarcinomas, and prevalence of *H. pylori* infection across participants' subgroups), criteria used to define gastric cardia cancer, assessment of infection status (methods and criteria), relative risk (RR) estimates for the association between *H. pylori* infection and gastric cancer (according to tumour topography) with the respective precision estimates, or the necessary information to compute the RR and variance estimates, and confounding control.

When similar information from the same study was provided in more than one report only one was selected for systematic review, according to the following criteria (applied consecutively): 1) availability of adjusted RR estimates for cardia and non-cardia cancers; 2) longer follow-up period (applicable to cohort, nested case-control and case-cohort analyses); 3) larger sample size; 4) availability of RR estimates for cardia cancer according to *H. pylori* infection status and according to the virulence of the *H. pylori* infecting strains.

When the risk of gastric cancer according to *H. pylori* infection and CagA status was reported in different articles referring to the same study, both were considered eligible for systematic review, but not included in the same meta-analyses.

Meta-analysis

Meta-analyses were conducted to obtain summary measures for the association between: 1) *H. pylori* infection and cardia cancer; 2) *H. pylori* infection and non-cardia cancer; 3) CagA infection and cardia cancer; 4) CagA infection and non-cardia cancer.

Relative risks (cumulative incidence ratios or incidence density ratios), hazard ratios and odds ratios were treated the same and are referred to as relative risks (RR).

When a study provided RR estimates defining infection with CagA using different criteria, we preferred the ones referring to the comparison of those infected with CagA strains (positive status for both *H. pylori* infection and *H. pylori* CagA [HP positive/CagA positive]) with non-infected subjects (negative status for *H. pylori* infection and CagA [HP negative/CagA negative]). We also considered eligible for meta-analysis: 1) four studies [22, 31, 37, 38] comparing subjects with positive results for infection with CagA regardless of overall *H. pylori* infection status (HP negative or positive/CagA positive) with non-infected subjects (HP negative/CagA negative), 2) one study [39] comparing subjects infected with CagA (HP positive/CagA positive) with those with negative results for infection with CagA regardless of overall *H. pylori* infection status (HP positive or negative/CagA negative). Three studies [40-42] were excluded from the meta-analyses

on the association between infection and cancer according to the virulence of the *H. pylori* strains because: 1) two studies compared subjects with positive results for infection with *H. pylori* CagA strains (HP positive/CagA positive) with those with negative results for infection with CagA strains (HP positive/CagA negative) [41], one of which was included in the systematic review but excluded from the meta-analyses [40] since it was not comparable with the other studies included, and 2) one study compared subjects infected with positive CagA (positive status for infection with CagA or VacA or UreA strains) with those with negative results for infection with CagA strains (negative status for infection with CagA, VacA and UreA strains) [42].

When a study provided crude and adjusted RRs, the adjusted estimates were selected for the analyses. Lin *et al* [43] provided adjusted estimates for the comparison between cardia and gastric antrum cancers and between *corpus* and antrum cancers, and we computed the crude odds ratios for the risk of cardia and non-cardia cancers in comparison with controls to be used in meta-analysis. Lee *et al* [44] and Brenner *et al* [24] provided the crude and adjusted odds ratios for the risk of non-cardia cancer but only crude odds ratios for the cardia cancer, and only the crude estimates were used for meta-analyses. Kikuchi *et al.* [45] provided crude estimates for the association between *H. pylori* infection and cardia cancer and adjusted estimates for the association between CagA infection and cardia cancer. The crude and adjusted estimates were used for meta-analyses of *H. pylori* infection and CagA infection, respectively.

When a study provided different RR estimates regarding confounding control, we selected the one adjusting for the largest number of variables. For studies that presented risk estimates for different follow-up times (applicable to cohort, nested case-control and cohort analyses), we opted for the longest follow-up period reported.

Data referring to “cardia”, “upper third” or “proximal” stomach cancers were taken as equivalent to cancer of the gastric cardia, and “distal” or “non-cardia” stomach cancers were taken as equivalent to cancers not located in the cardia region.

Combined RR estimates and corresponding 95%CI were computed with STATA®, version 9.0, using the DerSimonian-Laird random effects method. Heterogeneity was quantified using the I^2 statistic [46]. Stratified analyses were performed according to the risk of gastric cancer in the settings where the studies were conducted, and according to study design.

The epidemiologic knowledge of the gastric cancer incidence was used to define high- and low-risk settings according to the risk of gastric cancer [47-49], for stratified meta-analyses. China, Japan and Korea were classified as high-risk settings. Australia, Finland, Germany, Norway, Sweden and USA were classified as low-risk settings. A

multicenter prospective study including 10 European countries (Denmark, France, Germany, Greece, Italy, Netherland, Norway, Spain, Sweden and United Kingdom) was also considered as conducted in low-risk settings. The gastric cancer incidence (age-standardized, world reference population) was above 41/100.000 inhabitants in regions classified as high-risk and was below 19/100.000 inhabitants in the low-risk ones [47, 50].

RESULTS

Systematic Review

We identified 59 articles reporting information on the association between *H. pylori* infection and gastric cardia cancer and, after exclusion of 15 duplicates [22, 34, 39, 51-62] and 9 studies [63-71] not providing RR and no suitable information to compute RR (Annex 1), 35 studies were considered eligible, as described in the systematic review flow-chart (Figure 1).

The 35 selected reports referred to studies conducted predominantly in Asian (n=17, 5 in China, 8 in Japan, 3 in Korea, and 1 Iran) and European countries (n=11, 3 in Finland, 2 in Germany, 1 in Norway, 4 in Sweden, and 1 in 10 different European countries (Denmark, France, Germany, Greece, Italy, Netherland, Norway, Spain, Sweden and United Kingdom)). Five studies were from the United States, 1 of which evaluated participants from 6 races/ethnicities, including Chinese, Filipino, Japanese, Korean, Native Hawaiian or White [25]. One study was conducted in Australia, and 1 in Brazil (Annex 2).

Regarding the method used to assess *H. pylori* infection status, 30 studies used serologic tests (enzime immunoassay [EIA], enzime-linked immunosorbent assay [ELISA], or immunobloting [IB]), [23-27, 29, 31, 32, 36-38, 41-43, 45, 72-86] 2 used non-serologic tests (microbial culture, immunohistochemistry, rapid urease test, Giemsa stain, Wartin-Stary stain, or carbolfuchsin stain) [30, 44], and a combination of results from different methods, either serologic or non-serologic, was used in 3 studies [28, 40, 87].

Meta-analyses

The meta-analyses were conducted over results presented in 34 articles. Thirty studies provided RR estimates for the association between infection and cardia cancer (only crude RRs were available from 6 studies [24, 38, 43-45, 79], from which 29 also provided RR estimates for the risk of non-cardia cancer (only crude RRs were available from 6 studies [24, 38, 43-45, 79, 85]. Fourteen articles provided RR estimates for the relation between infection with CagA-positive strains and cardia cancer (only crude RRs were available from 4 studies [24, 37, 38, 85], from which 13 also addressed the risk of non-cardia gastric cancer (only crude RRs were available from 4 studies [24, 37, 38, 85] (Figure 1).

HP positive *vs*. HP negative

For cardia cancer, the summary RR was 1.08 (95%CI: 0.83-1.40; I^2=452.8%; 30 studies). Stratifying the analysis by gastric cancer incidence in the setting where the studies were conducted, the summary RR was 0.78 (95%CI: 0.63-0.97; I^2=11.6%; 16 studies) in the low-risk settings, but a significant positive association was observed in the high-risk regions (summary RR=1.98; (95%CI: 1.38-2.83; I^2=18.4%; 14 studies) (Figure 2). Similar results were obtained for the adjusted RR estimates (Table 1).

Within the group of studies describing the criteria used to classify cases as belonging to the cardia region, non statistically significant positive associations were observed between infection and cardia cancer in the subgroups of studies that described the tumour as: 1) centred within 1 cm proximal and 2-3 cm distal to the origin of the gastroesophageal junction, and 2) located in the upper third of the stomach (Table 1).

Within the group of studies from countries with high incidence of gastric cancer, positive associations were also observed between infection and cardia cancer in the subgroups of cohort/nested case-control and hospital-based case-control studies (Table 1).

For non-cardia gastric cancer the results were much more heterogeneous, and the summary RR was 2.81 (95%CI: 2.14-3.68; I^2=84.4%; 29 studies), similar in both low- and high-risk settings (Figure 3), and for the adjusted RR estimates (Table1).

Statistically significant positive associations were observed between infection and cardia cancer in the subgroups of studies which described the tumour as: 1) centred within 1 cm proximal and 2-3 cm distal to the origin of the gastroesophageal junction, 2) in the upper third of the stomach, and 3) involving cardioesophageal junction (Table 1).

No meaningful differences were observed in the summary estimates according to study design.

CagA positive *vs*. CagA negative

For cardia cancer, the summary RR was 0.85 (95%CI: 0.55–1.33; I^2=73.0%; 14 studies), with point estimates lower in low- (summary RR=0.74; 95%CI: 0.51-1.08; I^2=37.3%; 10 studies) than in high gastric cancer incidence settings (summary RR=1.47; 95%CI: 0.44-4.87; I^2=86.3%; 4 studies) (Figure 4). Similar results were obtained for adjusted RR estimates (Table 1).

For non-cardia cancer, the summary RR was 3.63 (95%CI: 1.93-6.84; I^2=91.4%; 13 studies), with a positive association being observed both in low- (summary RR=4.59; 95%CI: 2.78-7.57; I^2=76.4%; 9 studies) and high-risk settings (summary RR=2.08;

95%CI: 0.40-10.69; I^2=95.7%; 4 studies) (Figure 5). Similar results were obtained for adjusted RR estimates (Table 1).

A sensitivity analyses including the three studies [40-42] using different criteria to define the risk of gastric cancer according to the virulence of the *H. pylori* infecting strains yielded summary RRs of 0.89 (95%CI: 0.60-1.31; I^2=66.8%; 17 studies) and 3.98 (95%CI: 2.22-7.14; I^2=90.6%; 16 studies), respectively for cardia and non-cardia cancers (results not tabulated). Similar results were obtained for adjusted RR estimates (not tabulated).

The small number of studies precludes a sound interpretation of the summary estimates according to study design in low- and high-risk settings (Table 1).

Selection bias

The visual inspection of the funnel plots suggests an asymmetric distribution for gastric cardia cancer and gastric non-cardia cancer; however the corresponding statistical tests for asymmetry show no evidence of publication bias (Figure 6).

DISCUSSION

No overall association was found between *H. pylori* infection and cardia cancer, while a 2.8-fold increased risk was observed for non-cardia cancer. However, when stratifying the data according to the gastric cancer incidence in each population, positive associations were observed in high-risk settings for both cardia (summary RR=1.98, 95%CI: 1.38-2.83) and non-cardia (summary RR=3.02, 95%CI: 1.92-4.74) cancers, and similar results were obtained for infection with CagA strains.

The conclusions reached by systematic reviews and meta-analyses depend on the comprehensiveness of the search strategy and on the criteria for study inclusion and selection of data for quantitative synthesis, in addition to the quality of the evidence being reviewed. These issues have implications in the validity of our findings and deserve further discussion.

We reviewed the original studies addressing the relation between *H. pylori* infection and gastric cancer included in several previous systematic reviews and conducted a comprehensive PubMed® search to identify the most recent reports on this topic. Furthermore, we considered eligible for our meta-analyses all the studies providing the necessary information to quantify the relation between infection and cardia cancer, even if that was not a specific aim of the original studies, which contributed for the identification of a much larger number of reports than those included in the previous meta-analyses and to overcome selection bias. However, some selection bias could have been introduced by having considered English abstracts of publications written in others languages, especially for studies conducted in Asia. Still, funnel plot analysis confirms that our sample of studies assessing the relation between infection and cardia cancer is unbiased, both for low- and high-risk settings. On the other hand, there is the suggestion of underrepresentation of studies yielding stronger association between infection and non-cardia cancer, especially in the high-risk regions. This pattern is probably explained by methodological difficulties in assessing this relation in these settings due to the high prevalence of infection in the general populations [88], which contributes for conservative estimates regarding the differences between cardia and non-cardia cancers in their association with infection.

Sensitivity analysis excluding the studies according to different inclusion criteria yielded results leading to the same conclusions. Also, the conclusions remained unchanged when the analyses were stratified by factors that could have a possible impact on heterogeneity, or if Hawaii was classified as high-risk setting (data not shown).

Another possible source of heterogeneity could be the mixture of RRs both unadjusted and adjusted with different covariates, but no significant differences in the results were found when including only adjusted estimates.

The clinical and pathological interpretation of the anatomical limits of gastric cardia region has changed over the years and currently differs between countries [89]. Imprecise clinical and pathological delimitation of adenocarcinomas of the lower oesophagus, EGJ, and gastric cardia may be one of the reasons underlying inconsistent results across studies. Seventeen (44.7%) of the 38 studies included in meta-analysis, described the definition used to classify cases as belonging to the cardia region and the conclusions remained unchanged when analysis was stratified by presence/absence of gastric cardia definition in studies. When the analysis was stratified by different criteria used to classify cardia cases we found a positive association between infection and cardia cancer in two of four criteria, although the small sample size precludes a sound interpretation of this stratified analysis.

In conclusion, the present study is the most comprehensive assessment of the association between *H. pylori* infection and gastric cardia cancer, adding to previous knowledge an update in understanding the role of low and high-gastric cancer risk settings on this subject. Our results support the hypothesis of different aetiologies for gastric cardia cancer.

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Table 1: Association between *H. pylori* infection or infection with CagA positive strains and cardia and non-cardia gastric cancer, according to different inclusion criteria.

	Cardia cancer						Non-cardia cancer					
	H. pylori			CagA			*H. pylori*			CagA		
	n	RR (95% CI)	I^2 (%)	n	RR (95% CI)	I^2 (%)	n	RR (95% CI)	I^2 (%)	n	RR (95% CI)	I^2 (%)
All studies												
Using crude and adjusted estimates	30	1.08 (0.83-1.40)	52.8	14	0.85 (0.55-1.33)	73.0	29*	2.81 (2.14-3.68)	84.4	13†	3.63 (1.93-6.84)	91.4
Using only adjusted estimates	24	0.90 (0.73-1.12)	14.1	10	0.95 (0.60-1.50)	71.5	23*	2.48 (1.93-3.18)	66.6	9†	4.71 (2.56-8.67)	87.2
Studies providing information for both cardia and non-cardia cancers												
Using crude and adjusted estimates	27	1.10 (0.84-1.44)	46.2	12	1.06 (0.59-1.90)	84.8	28	2.76 (2.10-3.66)	84.7	11	3.36 (1.68-6.72)	92.5
Using only adjusted estimates	22	0.96 (0.77-1.19)	5.3	8	1.05 (0.61-1.83)	73.2	22	2.44 (1.89-3.16)	67.2	8	4.37 (2.30-8.31)	87.9
Studies that used serologic methods to assess *H. pylori* infection												
Using crude and adjusted estimates	24	1.10 (0.84-1.45)	52.0	12	0.95 (0.61-1.50)	73.7	23*	2.78 (2.02-3.84)	86.3	11‡	3.42 (1.67-7.01)	92.5
Using only adjusted estimates	20	0.93 (0.75-1.15)	13.4	10	1.00 (0.63-1.62)	72.5	19*	2.51 (1.88-3.35)	69.4	8†	4.37 (2.30-8.31)	87.9
Description of criteria to classify cases as cardia cancers§												
Studies reporting criteria	12	1.02 (0.63-1.65)	56.8	8	0.95 (0.52-1.74)	78.9	11*	4.11 (2.65-6.37)	72.2	7†	4.10 (1.39-12.10)	95.2
Studies not reporting criteria	18	1.14 (0.83-1.55)	46.4	6	0.72 (0.41-1.27)	42.3	18	2.28 (1.59-3.25)	88.1	6	3.27 (2.07-5.17)	53.6
Criteria used to classify cases as belonging to the cardia region§												
Centred within 1 cm proximal and 2-3 cm distal to the EGJ	5	1.18 (0.70-1.99)	42.1	4	1.35 (0.83-2.19)	57.6	4*	3.72 (1.58-8.72)	72.7	3†	6.54 (0.80-53.21)	94.4
Coded according ICD-O	3	0.63 (0.22-1.83)	53.1	1	0.34 (0.17-0.68)	---	3	4.51 (2.83-7.19)	26.0	1	0.20 (0.11-0.37)	---
In the upper third of the stomach	3	2.06 (0.61-6.91)	40.1	1	6.20 (1.20-32.02)	---	3	5.49 (1.15-26.14)	89.4	1	19.40 (7.21-52.19)	---
Envolving cardioesophageal junction	1	0.28 (0.09-0.87)	68.6	2	0.41 (0.18-0.95)	0.0	1	3.32 (1.72-6.41)	---	2	4.76 (1.76-12.86)	72.6
Gastric cancer incidence												
High-risk populations												
Using crude and adjusted estimates	14	1.98 (1.38-2.83)	18.4	4	1.47 (0.44-4.87)	86.3	14	3.02 (1.92-4.74)	90.7	4	2.08 (0.40-10.69)	95.7
Using only adjusted estimates	11	1.59 (1.03-2.45)	0.0	2	2.52 (0.82-7.72)	54.9	11	3.00 (1.80-5.04)	83.8	2	5.29 (0.45-61.71)	95.5
Low-risk populations												

Table 1 (continuation): Association between *H. pylori* infection or infection with CagA positive strains and cardia and non-cardia gastric cancer, according to different inclusion criteria.

	Cardia cancer						Non-cardia cancer					
	H. pylori			CagA			*H. pylori*			CagA		
	n	RR (95% CI)	I^2 (%)	n	RR (95% CI)	I^2 (%)	n	RR (95% CI)	I^2 (%)	n	RR (95% CI)	I^2 (%)
Using crude and adjusted estimates	16	0.78 (0.63-0.97)	11.6	10	0.74 (0.51-1.08)	37.3	15*	2.56 (1.99-3.29)	46.6	9†	4.59 (2.79-7.57)	76.4
Using only adjusted estimates	14	0.80 (0.63-1.02)	18.9	8	0.74 (0.52-1.06)	30.5	13*	2.50 (1.88-3.32)	52.7	7†	4.67 (2.46-8.86)	81.0
High-risk populations§												
Cohort/nested case-control	4	1.22 (0.60-2.48)	0.0	1	1.75 (1.33-2.31)	---	4	2.61 (0.98-6.95)	88.6	1	1.58 (1.13-2.22)	---
Case-control (PB)	1	1.16 (0.47-2.86)	---	2	0.70 (0.10-4.93)	63.1	1	1.01 (0.58-1.77)	---	2	0.81 (0.05-14.14)	94.0
Case-control (HB)	9	2.54 (1.75-3.54)	0.0	1	6.20 (1.20-32.02)	---	9	3.60 (2.23-5.79)	84.8	1	19.40 (7.21-52.19)	---
Low-risk populations§												
Cohort/nested case-control	6	0.62 (0.37-1.06)	39.2	3	0.72 (0.39-1.36)	0.0	6	3.47 (2.13-5.66)	55.8	3	5.82 (3.44-9.84)	0.0
Case-control (PB)	6	0.86 (0.66-1.11)	2.0	6	0.67 (0.40-1.14)	56.5	5*	2.42 (1.46-4.00)	0.0	5†	3.70 (1.79-7.64)	85.2
Case-control (HB)	4	0.90 (0.54-1.48)	0.0	1	1.48 (0.47-4.65)	---	4	2.42 (1.46-4.01)	43.4	1	6.80 (3.11-14.86)	---

* One study did not report RR estimates for the association between *H. pylori* infection and non-cardia cancer; † One study did not report RR estimates for the association between CagA infection and non-cardia cancer; ‡ Two studies did not report RR estimates for the association between CagA infection and non-cardia cancer; § The analyses included studies that provided only crude estimates and the studies that provided adjusted estimates; The epidemiologic knowledge of the gastric cancer incidence was used to define high- and low-risk populations (the gastric cancer incidence (age-standardized, world reference population) was above 41/100.000 inhabitants in regions classified as high-risk and was below 19/100.000 inhabitants in the low-risk ones); EGJ – Oesophagogastric junction; ICD-O – International Classification of Diseases for Oncology; PB – Population-based; HB – Hospital-based.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Parsonnet, 1991 [51]	USA	Nested case-control Population-based (Mean follow-up: 14.2 years)	**Gastric cardia cancer:** n = 4 *H. pylori* positive*: n = 1 (25%) **Gastric non-cardia cancer:** n = 64 *H. pylori* positive*: n = NS Antrum adenocarcinomas: n = 34 *H. pylori* positive*: n = NS Body adenocarcinomas: n = 30 *H. pylori* positive*: n = NS	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 91% Specificity: 98%	NS	**Matched by:** - Age at serum donation - Sex - Race - Date of serum donation - Site at which the multiphase health check-up was performed	Adenocarcinomas of the antrum or *pylorus* Matched OR = 7.0 (0.9–56.9)* Adenocarcinomas of the body or *fundus* Matched OR = 4.7 (1.3–16.2)*	Duplicate study. The sample was the same used in [86]. It was impossible to calculate the relative risk estimated for gastric cardia cancer.
Estevens, 1993 [63]	Portugal	Case-control Hospital-based	**Gastric cardia cancer:** n = not specified *H. pylori* positive *: 67% **Gastric non-cardia cancer:** n = not specified *H. pylori* positive *: 70%	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: not specified Specificity: not specified	NS	**Matched by:** - Age - Sex	-----	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia because it is matched study.
Hansson, 1993 [53]	Sweden	Case-control Hospital-based	**Gastric cardia cancer:** n = 19 *H. pylori* positive*: n = 13 (68%) **Gastric non-cardia cancer:** n = 93 *H. pylori* positive*: n = 77 (83%)	**EIA** IgG antibodies against whole-cell antigens Sensitivity: 98.7% Specificity: 100%	NS	**Matched by:** - Age period - Gender - Hospital of admission (frequency matched) **Adjusted by:** - Occupation - Access to refrigerator - Vegetables - Citrus - Coffee liquor consumption - Cigarette smoking	All cardia cancer Crude OR = 1.38 (0.44-4.77)* All non-cardia cancer Crude OR = 3.06 (1.49-6.31)*	Duplicate study. The sample was the same used in [39]. The excluded paper only presented the crude odds ratio.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Lin, 1993 [52]	China, Taiwan	Case-control Population-based	**Gastric cardia cancer:** n = 25 *H. pylori* positive*: n = 17 (68%) **Gastric non-cardia cancer:** n = 118 *H. pylori* positive*: n = 73 (62%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	NS	NS	All cardia cancer Crude OR = 1.78 (0.76–4.17)* All non-cardia cancer Crude OR = 1.36 (0.91–2.02)*	Duplicate study. The sample was the same used in [43]. The excluded paper only presented the crude odds ratio.
Blaser, 1995 [64]	Hawaii	Nested case-control Population-based (Mean follow-up: 7.59 ± 1.00 years)	**Gastric cardia cancer:** n = 2 *H. pylori* positive †: n = 2 (100%) **Gastric non-cardia cancer:** n = 101 *H. pylori* positive †: n = 88 (87%)	**ELISA** IgG antibodies against whole-cell and CagA antigens Sensitivity: 94.4% Specificity: 92.5%	NS	**Matched by:** - Age at examination - Date of serum collection	All non-cardia cancer Matched OR = 1.80 (0.90–3.18)†	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer, because it is matched study.
Kikuchi, 1995 [54]	Japan	Case-control Hospital-based	**Gastric cardia cancer:** n = 35 *H. pylori* positive *: n = 30 (86%) **Gastric non-cardia cancer:** n = 70 *H. pylori* positive *: n = 64 (91%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: not specified Specificity: not specified	Proximal cancer was cancer where the lesion existed in the proximal third of the stomach	**Matched by:** - Sex - Age (± 4 years)	Both hospital and screening controls were used to calculate the OR All cardia cancer Adjusted OR = 11.3 (2.6–68.8)* All non-cardia cancer Adjusted OR = 14.8 (4.8–53.9)*	Duplicate study. The sample was the same used in [45]. The excluded paper only presented the crude odds ratio.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Aromaa, 1996 [65]	Finland	Nested case-control Population-based (Mean follow-up: 9.5 years)	**Gastric cardia cancer:** n = 9 *H. pylori* positive§: n = 0 (0%) *H. pylori* positive*: n = 0 (0%) **Gastric non-cardia cancer:** n = 75 *H. pylori* positive§: n = 75 (100%) *H. pylori* positive*: n = 73 (97%)	**EIA** IgG and IgA antibodies against whole-cell antigens **IgG** Sensitivity: 93.7% Specificity: 93.9% Cut-off: ≥ 700 (expressed as reciprocals) **IgA** Sensitivity: 73.1% Specificity: 95.1% Cut-off: ≥ 70 (expressed as reciprocals)	NS	**Matched by:** - Municipality - Age - Sex - Duration of storage of serum samples - Smoking - Occupation - Serum concentration of: α-tocopherol, ß-carotene, retinol, selenium	IgA Crude OR = 1.37 (0.64-2.95)§ Matched OR = 2.76 (1.11–6.87)§ IgG Crude OR = 7.54 (1.74-32.78)* Matched OR = 1.75 (0.80–3.81)*	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer because it is a matched study.
Webb, 1996 [55]	China	Nested case-control Population-based (Mean follow-up: 2.4 years)	**Gastric cardia cancer:** n = 27 *H. pylori* positive*: n = 12 (44%) **Gastric non-cardia cancer:** n = 52 *H. pylori* positive*: n = 30 (58%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	The site of tumour was coded according to the International Classification of Diseases for Oncology (ICD-O) (WHO, 1976)	**Matched by:** - Age - Month and year of sample collection - Neighbourhood of residence **Adjusted by:** - Education - Cigarette smoking - Alcohol consumption - History of peptic ulcer - Blood group - Consumption of: bok choi, cured meats, pickled vegetables (for gastric non-cardia cancers)	All cardia cancer Matched OR = 0.65 (0.25-1.59)* All non-cardia cancer Matched OR =1.10 (0.57-2.14)* Adjusted OR =1.17 (0.54-2.54)*	Duplicate study. The sample was the same used in [84]. The excluded paper has lower follow-up period.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Erkisi, 1997 [67]	Turkey	Case-control Hospital-based	**Gastric cardia cancer**: n = 23 H. pylori positive*: n = 6 (23%) **Gastric non-cardia cancer:** n = 180 H. pylori positive*: n = 112 (62%) **Corpus:** n = 62 H. pylori positive*: n = 29 (47%) **Antrum:** n = 95 H. pylori positive*: n = 70 (74%) **Pylorus:** n = 23 H. pylori positive*: n = 13 (57%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 99% Specificity: 100% Stained by Wartin-Stary technique	NS	**Matched by:** - Sex - Age - Hospital admission (for hospital controls) (Cases and controls related with cases were not matched)	-----	The study did not provided RR estimates for the association between H. pylori infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer because it is matched study.
Martin-de-Argila, 1997 [66]	Spain	Case-control Hospital-based	**Gastric cardia cancer:** n = 5 *H. pylori* positive*: n = 2 (40%) **Gastric non-cardia cancer:** n = 13 *H. pylori* positive*: n = 13 (100%)	**ELISA** IgG antibodies against whole-cell antigens Cut-off: 10 U/ml Sensitivity: 96% Specificity: 93%	NS	**Matched by:** - Age - Geographic area	-----	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer because it is matched study.
Simán, 1997 [56]	Sweden	Nested case-control	**Gastric cardia cancer:** n = 16 No prior gastric surgery: n = 13 *H. pylori* positive*: n = 6 (46%) **Gastric non-cardia cancer:** n = 40 No prior gastric surgery: n = 33 *H. pylori* positive*: n = 29 (89%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 98% Specificity: 81%	NS	**Matched by:** - Gender - Date of birth (± 6 months) - date of enrolment (± 6 months) **Adjusted by:** - Occupation - Tobacco	All cardia cancer Adjusted OR = 0.92 (0.23–3.7)* All non-cardia cancer Adjusted OR = 11.1 (2.4–71.8)*	Duplicate study. The sample was the same used in [74]. The sample size of the excluded study was smaller than the sample considered in the included study.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Whiting, 1998 [68]	England	Case-control Hospital based	**Gastric cardia cancer: n = 13** **H. pylori positive*: n = 5 (38%)** **Gastric non-cardia cancer: n = 73** **H. pylori positive*: n = 62 (85%)**	**ELISA** **IgG antibodies against whole-cell antigens** **Sensitivity: NS** **Specificity: NS**	NS	**Matched by:** **- Sex** **- Age**	-----	The study did not provided RR estimates for the association between H. pylori infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer because it is matched study.
Brenner, 2000 [57]	Germany, Saarland	Case-control Population-based	**Gastric cardia cancer:** n = 10 *H. pylori* positive¶: n = 3 (3%) *H. pylori* positive** n = 1 (1%) **Gastric non-cardia cancer:** n = 58 *H. pylori* positive¶: n = 19 (33%) *H. pylori* positive**: n = 30 (52%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 94% (median) Specificity: 79% (median) **Immunoblotting** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS	NS	**Matched by:** - Age - Gender **Adjusted by:** - Sex - Age - Education (models 1 and 2) - Family history of gastric carcinoma (model 2)	All cardia cancer Crude OR = 0.65 (0.16–2.57)¶ Crude OR = 0.17 (0.02–1.35)** All non-cardia cancer Adjusted OR = 2.3 (0.9–5.6)¶ Adjusted OR = 4.6 (2.0–10.8)**	Duplicate study. The sample was the same used in [24]. The exclude paper not provided RR estimates for the association between *H. pylori* infection with CagA strains and cardia cancer.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Limburg, 2001 [22]	China	Nested case-control Population-based (Cohort: mean follow up of 6.13 years)	**Gastric cardia cancer:** n = 99 (with adequate serum) Median age (yrs) = 55 *H. pylori* positive*: n = 62 (63%) *H. pylori* positive§: n = 39 (39%) *H. pylori* positive¶¶: n = 69 (70%) Time gastric cancer diagnosis Mean (yrs ± SD) = 2.94 ± 1.56 Median (yrs) = 2.84 Range (yrs) = (0.93–5.24) **Gastric non-cardia cancer:** n = 82 (with adequate serum) Median age (yrs) = 60 *H. pylori* positive*: n = 51 (62%) *H. pylori* positive§: n = 30 (37%) *H. pylori* positive¶¶: n = 59 (72%) Time gastric cancer diagnosis Mean (yrs ± SD) = 2.08 ± 1.48 Median (yrs) = 1.81 Range (yrs) = (0.04–5.13)	**ELISA** IgG antibodies against whole-cell and CagA antigens **Whole-cell** Mean: 1.83% SD: 0.26% CV: 14.2% **CagA** Mean: 0.15% SD: 0.04% CV: 26.7%	Defined as the most proximal 3 cm of the stomach	**Matched by:** - Sex - Age **Adjusted by:** - Sex - Age	All cardia cancer Adjusted OR =1.58 (0.95–2.62)* ELISA Adjusted OR = 1.79 (1.05–3.06)§ Immunoblotting Adjusted OR =1.87 (1.10–3.17)¶¶ All non-cardia cancer Adjusted OR =1.68 (0.96–2.95)* ELISA Adjusted OR =1.84 (1.01–3.34)§ Immunoblotting Adjusted OR =2.29 (1.26–4.14)¶¶	Duplicate study. The sample was the same used in [31]. The excluded paper has lower follow-up period.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Louw, 2001 [69]	South Africa	Case-control Hospital-based	**Gastric cardia cancer:** n = 16 *H. pylori* positive††: n = 13 (81%) **Gastric non-cardia cancer:** n = 32 *H. pylori* positive††: n = 23 (71%)	**Rapid urease test** **Histological assessment** **Modified Giemsa staining** **Microbial culture** **ELISA** IgG antibodies against whole-cell antigens Sensitivity: 97% Specificity: 100% **PCR** VacA and CagA Sensitivity: NS Specificity: NS	NS	**Matched by:** - Age (within 5 years) - Gender - Ethnicity	-----	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer because it is matched study.
Brenner, 2002 [58]	Germany, Saarland	Case-control Hospital based	**Gastric cardia cancer:** n = 11 *H. pylori* positive*: n = 4 (36%) *H. pylori* positive¶: n = 3 (27%) *H. pylori* positive**: n = 1 (9%) **Gastric non-cardia cancer:** n = 59 *H. pylori* positive*: n = 49 (83%) *H. pylori* positive¶: n = 20 (34%) *H. pylori* positive*: n = 29 (49%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS **Western blot** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS	NS	**Matched by:** - Sex - Age **Adjusted by:** - Age - Sex - Education - Family history of gastric cancer - Alcohol	All cardia cancer Crude OR = 0.57 (0.15–2.18)¶ Crude OR = 0.33 (0.04–2.61)** All non-cardia cancer Adjusted OR = 2.5 (1.1–5.8)¶ Adjusted OR = 5.5 (2.4–12.4)**	Duplicate study. The sample was the same used in [24]. The exclude study had presented a smaller sample size than the included study.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Enroth, 2002 [59]	Sweden	Case-control Hospital based	**Gastric cardia cancer:** n = 8 *H. pylori* positive‡‡: n = 2 (25%) *H. pylori* positive§§: n = 1 (13%) *H. pylori* positive*: n = 3 (50%) *H. pylori* positiveıı: n = 4 (67%) *H. pylori* positive††: n = 5 (62%) **Non-cardia cancer:** n = 64 *H. pylori* positive‡‡: n = 27 (42%) *H. pylori* positive§§: n = 29 (45%) *H. pylori* positive*: n = 40 (63%) *H. pylori* positiveıı: n = 47 (73%) *H. pylori* positive††: n = 52(81%)	**Microbial culture** **Immunohistochemistry** **ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS **Immunoblotting** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS	NS	**Matched by:** - Age (within 10-years age bands) - Sex - Hospital **Adjusted by:** - Age - Sex - Hospital	All cardia cancer Crude OR = 0.52 (0.10–2.64)‡‡ Crude OR = 0.22 (0.03–1.85)§§ Crude OR = 0.83 (0.19–3.53)* Crude OR = 0.60 (0.15–2.43)ıı Crude OR = 0.87 (0.20–3.70)†† All non-cardia cancer Crude OR = 1.15 (0.67–1.98)‡‡ Crude OR = 1.30 (0.76–2.24)§§ Crude OR = 2.30 (1.33–4.00)* Crude OR = 1.65 (0.91–3.30)** Crude OR = 2.26 (1.16–4.40)††	Duplicate study. The sample was the same used in [87]. The exclude study did not report adjusted OR estimates.
Held, 2004 [39]	Sweden	Case-control Hospital-based	**Gastric cardia cancer:** n = 18 *H. pylori* positive*: n = 13 (72%) *H. pylori* positive¶¶: n = 11 (61%) *H. pylori* positive§: n = NS *H. pylori* positive††: n = NS *H. pylori* positive**: n = NS **Gastric non-cardia cancer:** n = 82 *H. pylori* positive*: n = 68 (83%) *H. pylori* positive¶¶: n = 75 (75%) *H. pylori* positive§: n = NS *H. pylori* positive††: n = NS *H. pylori* positive**: n = NS	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 86% Specificity: 83% **Immunoblotting** IgG antibodies against CagA antigens Sensitivity: 82% Specificity: 94%	Cardia cancer was defined as a tumour with its centre located within 1 cm proximal and 2 cm distal of the gastroesophageal junction	**Matched by:** - Age - Gender - Hospital **Adjusted by:** - Age - Gender - Socio-economic status - Smoking status - Availability of refrigerator (20 years before the interview) - Intake of: fruit, vegetables, coffee, and hard liquor	All cardia cancer Matched OR = 1.9 (0.6-5.6)* Matched OR = 1.3 (0.5-3.6)¶¶ All non-cardia cancer Adjusted OR = 4.5 (2.0–10.3)* Adjusted OR = 15.8 (5.4–46.6)¶¶	Duplicate study. The sample was the same used in [73] The excluded paper has lower follow-up period.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Watabe, 2005 [70]	Japan	Cohort Population-based (Cohort: mean follow up of 4.7 years)	**All participants:** n = 6983 *H. pylori* positive*: n = 3216 (46%) **Gastric cardia cancer:** n = 2 *H. pylori* positive*: n = ns **Gastric non-cardia cancer:** n = 41 *H. pylori* positive*: n = 24 (59%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 95% Specificity: 83%	NS	----	-----	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the crude RR estimates for gastric cardia cancer.
Gonzaléz, 2006 [60]	Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden United Kingdom	Nested case-control	**Gastric cardia cancer:** n = 47 *H. pylori* positive*: n = 22 (47%) **Gastric non-cardia cancer:** n = 113 *H. pylori* positive*: n = 12 (11%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: > 90% Specificity: > 90% Cut-off: 100 EU	NS	**Matched by:** - Sex - Age group (± 2.5 years) - Center - Date of collection of blood (± 45 days)	All cardia cancer Crude OR = 0.43 (0.24–0.77)* All non-cardia cancer Crude OR = 0.06 (0.03–0.11)*	Duplicate study. The sample was the same used in [32]. The excluded paper only presented the prevalence of infection in cases and controls.
Gonzaléz, 2006 [61]	Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden United Kingdom	Nested case-control	**Gastric cardia cancer:** n = 47 *H. pylori* positive*: n = 22 (47%) **Gastric non-cardia cancer:** n = 113 *H. pylori* positive*: n = 12 (11%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: > 90% Specificity: > 90% Cut-off: 100 EU	NS	**Matched by:** - Sex - Age group (± 2.5 years) - Center - Date of collection of blood (± 45 days)	All cardia cancer Crude OR = 0.43 (0.24–0.77)* All non-cardia cancer Crude OR = 0.06 (0.03–0.11)*	Duplicate study. The sample was the same used in [32]. The excluded paper only presented the prevalence of infection in cases and controls.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Hansen, 2007 [34]	Norway	Nested case-control (Cohort: mean follow up of 11.9 years)	**Gastric cardia cancer:** n = 44 *H. pylori* positive*: n = 19 (43%) **Gastric non-cardia cancer:** n = 129 *H. pylori* positive*: n = 113 (90%)	**EIA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	Cardia cancers were defined as tumours whose centre was judged to be within 2 cm distal to the gastro-oesophageal junction	**Matched by:** - Gender - Date of birth - Date of serum sampling - Serum source **Adjusted by:** - Gender - Date of birth - Date of serum sampling - Serum source	All cardia cancer OR = 0.27(0.12-0.59)* All non-cardia cancer: OR = 4.75 (2.56-8.81)*	Duplicate study. The sample was the same used in [76]. The excluded paper had presented a smaller sample size than the included study.
Suzuki, 2007 [71]	Japan	Nested case-control Population based (Cohort: mean follow up of 2.3 years)	**Gastric cardia cancer:** n = 22 *H. pylori* positive*: n = 18 (82%) *H. pylori* positive††: n = 17 (77%) **Gastric non-cardia cancer:** n = 299 *H. pylori* positive*: n = 67 (89%) *H. pylori* positive††: n = 232 (78%)	**EIA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS Cut-off: ≥16.5 units/ml **ELISA** IgG antibodies against CagA antigens Sensitivity: 93.7% Specificity: 100% Cut-off: ≥15 units/ml	NS	**Matched by:** - Age - Gender - City - Time of serum storage - Type of serum storage - Radiation dose - Smoking	IgG levels < 15 U/ml Adjusted RR = 2.2 (1.3–3.9)* IgG levels [15-23] U/ml Adjusted RR = 3.9 (2.1–7.0)†† IgG levels ≥23 U/ml Adjusted RR = 2.0 (1.3–3.2)††	The study did not provided RR estimates for the association between *H. pylori* infection and gastric cardia cancer. It was impossible to calculate the relative risk estimated for gastric cardia cancer.

Annex 1. Characteristics of the studies identified by systematic review and excluded because were duplicate reports or not provided relative risks and no suitable information to compute relative risks.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics	Assessment of infection status	Cardia/proximal definition	Control for confounding	Risk estimate (95% confidence interval [CI])	Reason(s) for exclusion
Ren, 2008 [62]	China	Nested case-control Population-based (Cohort: mean follow up of 8 years)	**Cardia cancer diagnosed:** n = 1,089 **Gastric cardia cancer:** n = 196 (were randomly sample from 1,089 cases) *H. pylori* positive*: n = 161 (82%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	NS	**Unmatched** **Adjusted by:** - NS	All cardia cancer All follow-up time Crude OR = 1.85 (1.14-3.00)* Adjusted OR =2.00 (1.21–3.31)* Follow-up ≤ 5 years Adjusted OR =1.78 (0.88–3.60)* Follow-up [6-10] years Adjusted OR =1.66 (0.80–3.44)* Follow-up > 10 years Adjusted OR =2.23 (1.05–4.74)*	Duplicate study. The sample was the same used in [31]. The excluded paper only has available adjusted RR estimate for cardia cancer.

* *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens; † *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens and/or *H. pylori* CagA status positive; § *H. pylori* positive indicates seropositivity to anti-*H. pylori* IgA antibodies; ¶ *H. pylori* positive indicates seropositivity to IgG antibodies against surface/ whole-cell antigens and *H. pylori* CagA status negative; ** *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens and *H. pylori* CagA status positive; †† *H. pylori* positive indicates that all cases and controls were at least one test positive from among the four tests used (positive at RUT test, or H&M and Giemsa staining, or microbial culture, or EIA test); ‡‡ *H. pylori* positive indicates the presence of *H. pylori* colonies on the culture plates; §§ *H. pylori* positive indicates that patients were classified positive on the presence of stained *H. pylori* by immunohistochemical staining method; ıı *H. pylori* positive indicates detection of one reaction band of 116 kDa (CagA) and/or 89 kDa (VacA) and/or 35 kDa (major antigens), and/or two other reaction bands (minor antigens, 30 kDa, 26.5 kDa, 19.5 kDa), by immunobloting test; NS – not specified.

Annex 2. Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Talley, 1991 [27]	USA	Case-control Hospital-based	**Total gastric cancer:** n = 69 Min. age (yrs) = ns Median age (yrs) = 63 Max. age (yrs) = NS Male (n) = 36 (52%) *H. pylori* positive*: n = 36 (52%) **Gastric cardia cancer:** n = 32 Median age (yrs) = 62.5 Male (n) = 21 (66%) *H. pylori* positive*: n = 12 (38%) **Gastric non-cardia cancer:** n = 37 Median age (yrs) = 64 Male (n) = 15 (41%) *H. pylori* positive*: n = 24 (65%)	**Total controls:** n = 252 Min. age (yrs) = ns Median age (yrs) = 61 Max. age (yrs) = ns Male (n) = 126 (50%) *H. pylori* positive*: n = 96 (38%) (Controls included 76 health asymptomatic volunteers with no current history of gastrointestinal disease and 176 patients with non-malignant disorders)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 96% Specificity: 94%	NS	**HP$^+$ *vs.* HP$^-$** Crude OR = 0.98 (0.46-2.08)* Adjusted OR = 0.94 (0.34-2.61)* (99% CI)	**HP$^+$ *vs.* HP$^-$** Crude OR = 3.00 (1.46-6.17)* Adjusted OR = 2.67 (1.01-7.06)* (99% CI)	**Adjusted by:** - Age - Gender - Race - Occupation - Geographic - Residence. - Smoking status - Age of the sera collection
Lin, 1993 [43]	China	Case-control Population-based	**Total gastric cancer:** n = 148 Min. age (yrs) = 24 Mean age (yrs) = 58.9 ± 14.5 Max. age (yrs) = 87 Male (n) = 91 (55%) *H. pylori* positive*: n = 92 (62%) **Gastric cardia cancer:** n = 26 *H. pylori* positive*: n = 17 (65%) **Gastric non-cardia cancer:** n = 114 *H. pylori* positive*: n = 71 (62%) Corpus: n = 28 *H. pylori* positive*: n = 17 (61%) Antrum: n = 86 *H. pylori* positive*: n = 54 (63%)	**Total controls:** n = 92 Min. age (yrs) = 22 Mean age (yrs) = 52.1 ± 10.6 Max. age (yrs) = 77 Male (n) = 54 (59%) *H. pylori* positive*: n = 57 (62%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 96% Specificity: 93%	NS	**HP$^+$ *vs.* HP$^-$** Crude OR = 1.16 (0.47–2.86)* **HP$^+$ *vs.* HP$^-$** Adjusted OR = 1.17 (0.46-2.96)* (The reference group used to calculate the adjusted estimate was a group of gastric antrum cancer cases)	**HP$^+$ *vs.* HP$^-$** All non-cardia cancers Crude OR = 1.01 (0.58-1.79)* **HP $^+$ *vs.* HP $^-$** Gastric corpus cancer Adjusted OR=0.92 (0.38-2.22)* (The reference group used to calculate the adjusted estimate was a group of gastric antrum cancer cases)	**Adjusted by:** - Age

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Parsonnet, 1993 [86]	USA	Case-control Hospital-based	**Total gastric cancer:** n = 128 Min. age (yrs) = NS Mean age (yrs) = 53.6 Max. age (yrs) = NS Male (n) = 92 (72%) *H. pylori* positive*: n = 101 (79%) **Gastric cardia cancer:** n = 30 *H. pylori* positive*: n = 17 (56.7%) **Gastric non-cardia cancer:** n = 98 *H. pylori* positive*: n = 84 (85.7%)	**Total controls:** n = 128 Min. age (yrs) = NS Mean age (yrs) = 53.6 Max. age (yrs) = NS Male (n) = 92 (78%) *H. pylori* positive*: n = 81 (63%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	NS	**HP$^+$ *vs.* HP$^-$** Crude OR = 0.65 (0.23–1.86)* Matched OR = 0.8 (0.30–2.00)*	**HP$^+$ *vs.* HP$^-$** Crude OR =3.64 (1.81–7.31)* Matched OR = 3.6 (1.7–7.4)*	**Matched by:** - Birth cohort - Gender - Race - Anatomical site - Date of serum collection
Fukuda, 1995 [29]	Japan	Case-control Hospital-based	**Total gastric cancer:** n = 282 Min. age (yrs) = 23 Mean age (yrs) = 57.1 Max. age (yrs) = 83 Male (n) = 177 (63%) *H. pylori* positive*: n = 215 (76%) **Gastric cardia cancer:** n = 52 *H. pylori* positive*: n = NS **Gastric non-cardia cancer:** n = 230 *H. pylori* positive*: n = NS	**Total controls:** n = 767 Min. age (yrs) = 25 Mean age (yrs) = 53.4 Max. age (yrs) = 84 Male (n) = 351 (46%) *H. pylori* positive*: n = 567 (74%) Controls for cardia: n = 112 *H. pylori* positive*: n = NS Controls for non-cardia: n = 655 *H. pylori* positive*: n = NS (Controls were out-patients with no cancerous lesions detected clinically in any organ, a visit within the previous 3 to 6 months and no history of hospitalization in the hospital)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS Cut-off: 2.2 U/ml	The tumours were classified as cardia gastric cancer when the main tumour was located in the upper third of the stomach	**HP$^+$ *vs.* HP$^-$** Matched OR = 0.86 (0.38–1.92)* Model 1 **HP$^+$ *vs.* HP$^-$** Adjusted OR = 1.11 (0.42–2.97)* Model 2 **HP$^+$ *vs.* HP$^-$** Adjusted OR = 0.96 (0.28–3.30)*	**HP$^+$ *vs.* HP$^-$** Matched OR=1.09 (0.73–1.62)* Model 1 **HP$^+$ *vs.* HP$^-$** Adjusted OR=1.41 (0.87-2.29)* Model 2 **HP$^+$ *vs.* HP$^-$** Adjusted OR=1.88 (1.07–3.31)*	**Matched by:** - Sex - Age (± 3 years) - Date of blood sampling **Adjusted by:** - Sex - Age - Date of blood sampling - Low level of pepsinogen I (model 1) - Interaction between *H. pylori* and low PG I (model1) - Interaction between *H. pylori* and low PG I/II (model2)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Rudi, 1995 [75]	Germany	Case-control Hospital-based	**Total gastric cancer:** n = 111 Min. age (yrs) = 26 Mean age (yrs) = 60 Max. age (yrs) = 83 Male (n) = 82 (74%) *H. pylori* positive*: n = 65 (59%) **Gastric cardia cancer:** n = 36 Min. age (yrs) = 45 Mean age (yrs) = 60.5 Max. age (yrs) = 79 Male (n) = 30 (83%) *H. pylori* positive*: n = 20 (56%) **Gastric non-cardia cancer:** n = 70 Min. age (yrs) = 26 Mean age (yrs) = 63.0 Max. age (yrs) = 83 Male (n) = 48 (69%) *H. pylori* positive*: n = 40 (57%)	**Total controls:** n = 111 Min. age (yrs) = 27 Mean age (yrs) = 61.0 Max. age (yrs) = 82 Male (n) = 72 (65%) *H. pylori* positive*: n = 57 (51%) Controls for cardia: n = 36 *H. pylori* positive*: n = 18 (50%) Controls for non-cardia: n = 70 *H. pylori* positive*: n = 36 (51%) (All controls were patients with colorectal carcinomas)	**ELISA** IgG antibodies against whole-cell and CagA antigens Sensitivity: 96% Specificity: 95% Cut-off: 10 U/ml	NS	**HP$^+$ *vs.* HP$^-$** Matched OR = 1.25 (0.49–3.16)*	**HP$^+$ *vs.* HP$^-$** Matched OR =1.26 (0.65–2.46)*	**Matched by:** - Age
Shibata, 1996 [28]	Japan	Case-control Hospital-based	**Total gastric cancer:** n = 50 Min. age (yrs) = ns Mean age (yrs) = 62.0 Max. age (yrs) = ns Male (n) = 37 (74%) *H. pylori* positive*: n = 36 (72%) *H. pylori* positive†: n = 45 (90%) **Gastric cardia cancer:** n = 5 *H. pylori* positive*: n = 3 (60%) *H. pylori* positive†: n = 4 (80%) **Gastric non-cardia cancer:** n = 45 *H. pylori* positive*: n = 33 (73%) *H. pylori* positive†: n = 41 (83%)	**Total controls:** n = 50 Min. age (yrs) = ns Mean age (yrs) = 61.8 Max. age (yrs) = ns Male (n) = 37 (74%) *H. pylori* positive*: n = ns *H. pylori* positive†: n = 35 (70%) (Controls were referred to the hospital because of radiologic abnormalities of the stomach or duodenum found during health check or because of gastrointestinal complaints without radiologic abnormalities, who were without any history of gastric surgery or of systematic disease)	**Microbial culture** (colonies were tested for Gram staining, oxidase, catalase and urease tests) **ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	Three anatomical parts of the stomach were considered according to the classification scheme of the Japanese Research Society for Gastric Cancer.	**HP$^+$ *vs.* HP$^-$** Matched OR=1.71 (0.18–16.65)†	**HP$^+$ *vs.* HP$^-$** Matched OR=4.39 (1.33–14.46)†	**Matched by:** - Age

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Kim, 1997 [30]	Korea	Case-control Hospital-based	**Total gastric cancer:** n = 160 Min. age (yrs) = 28 Mean age (yrs) = 57.3 Max. age (yrs) = 89 Male (n) = 104 (88%) *H. pylori* positive‡: n = 96 (60%) **Gastric cardia cancer:** n = 12 Min. Age (yrs) = 34 Mean age (yrs) = 57 Max. age (yrs) = 73 *H. pylori* positive‡: n = 8 (67%) **Gastric non-cardia cancer:** n = 148 Min. Age (yrs) = 28 Mean age (yrs) = 56.6 Max. age (yrs) = 89 *H. pylori* positive‡: n = 88 (59%) Gastric body cancers: n = 56 *H. pylori* positive‡: n = 37 (66%) Gastric angle cancers: n = 14 *H. pylori* positive‡: n = 8 (57%) Gastric antrum cancers: n = 78 *H. pylori* positive‡: n = 43 (55%)	**Total controls:** n = 160 Min. age (yrs) = 21 Mean age (yrs) = 56.9 Max. age (yrs) = 82 Male (n) = 99 (62%) *H. pylori* positive‡: n = 83 (52%) Controls for gastric cardia: n = 12 *H. pylori* positive‡: n = 7 (58%) Controls for gastric non-cardia: n = 148 *H. pylori* positive‡: n = 76 (51%) Controls for gastric body: n = 56 *H. pylori* positive‡: n = 30 (54%) Controls for gastric angle: n = 14 *H. pylori* positive‡: n = 8 (57%) Controls for gastric antrum: n = 78 *H. pylori* positive‡: n = 38 (49%)	**Rapid urease test** **Histology** (Wright-Giemsa stain)	NS	**HP$^+$ *vs.* HP$^-$** Matched OR = 1.43 (0.27–7.52)‡	All non-cardia cancer **HP$^+$ *vs.* HP$^-$** Matched OR = 1.36 (0.87-2.14)‡ Gastric body cancer **HP$^+$ *vs.* HP$^-$** Matched OR=1.69 (0.79–3.62)‡ Gastric angle cancer **HP$^+$ *vs.* HP$^-$** Matched OR=1.00 (0.224–4.47)‡ Gastric antrum cancer **HP$^+$ *vs.* HP$^-$** Matched OR=1.29 (0.689–2.43)‡	**Matched by:** - Age

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Chow, 1998 [23]	USA	Case-control Population-based	**Total gastric cancer:** n = 196 Min. age (yrs) =30 Mean age (yrs) = NS Max. age (yrs) =79 Male (n) = NS *H. pylori* positive*: n =71 (36%) *H. pylori* positive§: n = 33 (17%) **Gastric cardia cancer:** n = 129 *H. pylori* positive*: n = 38 (29%) *H. pylori* positive§: n = 12 (9%) **Gastric non-cardia cancer:** n = 67 *H. pylori* positive*: n = 33 (49%) *H. pylori* positive§: n = 21 (31%)	**Total controls:** n = 224 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = ns *H. pylori* positive*: n = 86 (63%) *H. pylori* positive§: n = 46 (21%)	**ELISA** IgG antibodies against whole-cell and CagA antigens Sensitivity: NS Specificity: NS	NS	**HP^+ *vs.* HP^-** Adjusted = 0.70 (0.40–1.10)* **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 0.4 (0.2–0.8)§	**HP^+ *vs.* HP^-** Adjusted OR =1.3 (0.7– 2.3)* **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 1.4 (0.7–2.8)§	**Matched by:** - Age - Sex - Race **Adjusted by:** - Age - Sex - Race - Geographic centre - Education
Lee, 1998 [44]	Korea	Case-control Population-based	**Total gastric cancer:** n =175 Min. age (yrs) = NS Mean age (yrs) = 54.4 Max. age (yrs) = NS Male (n) =118 (67.4%) *H. pylori* positiveıı: n = 138 (78.9%) **Gastric cardia cancer:** n = 17 *H. pylori* positiveıı: n = 13 (77%) **Gastric non-cardia cancer:** n =156 *H. pylori* positiveıı: n = 123 (79%)	**Total controls:** n = 113 Min. age (yrs) = NS Mean age (yrs) = 40.5 Max. age (yrs) = NS Male (n) = 81 (72%) *H. pylori* positiveıı: n = 47 (41.6%)	**CLO test** To detect urease in gastric mucosal biopsies Sensitivity: NS Specificity: NS	NS	**HP^+ *vs.* HP^-** Crude OR = 4.56 (1.40–14.87)ıı	**HP^+ *vs.* HP^-** Crude OR = 5.2 (3.1–8.8)ıı Adjusted OR = 5.2 p<0.025 (Adjusted OR was calculated by the Mantel-Haenszel method; the p value was determined by X^2 test)	**Adjusted by:** - Age - Tumour site

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Queiroz, 1998 [40]	Brazil	Case-control Hospital-based	**Total gastric cancer:** n = 119 Min. age (yrs) = 32 Mean age (yrs ± SD) = NS Max. age (yrs) = 96 Male (n) = 84 (71%) *H. pylori* positive¶: n =119 (100%) *H. pylori* positive§: n =113 (95%) **Gastric cardia cancer:** n = 15 *H. pylori* positive¶: n = 15 (100%) *H. pylori* positive§: n =11 (73%) **Gastric non-cardia cancer:** n=104 *H. pylori* positive¶: n = 104 (100%) *H. pylori* positive§: n =102 (98%)	**Total controls:** n = 119 Min. age (yrs) = 32 Mean age (yrs ± SD) = 61.3 ± 13.6 Max. age (yrs) = 96 Male (n) = 84 (71%) *H. pylori* positive¶: n =119 (100%) *H. pylori* positive§: n =79 (66%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 95.4% Specificity: 100% **Histology** **Urease test** **Carbolfuchsin stain** **Microbial culture** **PCR** (for UreA and CagA genes)	NS	**$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Matched OR = 1.3 (0.1–4.1)§	**$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Matched OR = 25.9 (5.8–75.3)§	**Matched by:** - Sex - Age
Komoto, 1998 [83]	Japan	Case-control Hospital-based	**Total gastric cancer:** n = 141 Carcinomas: 105 Min. age (yrs) = NS Mean age (yrs) = 64.9 ± 1.2 Max. age (yrs) = NS Male (n) = 82 *H. pylori* positive*: n = 98 (93%) **Gastric cardia cancer:** n = 14 *H. pylori* positive*: n = 13 (93%) **Gastric non-cardia cancer:** n = 91 *H. pylori* positive*: n = 85 (93%)	**Total controls:** n = 105 Min. age (yrs) = NS Mean age (yrs ± SD) = 62.4 ± 1.1 Max. age (yrs) = NS Male (n) = 75 (71.43%) *H. pylori* positive*: n = 75 (71%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS Cut-off: ≥1.0 (ratio) **Giemsa staining**	Cardia was defined as the area in the stomach within 20 mm distance from the esophagogastric junction	**HP^+ *vs.* HP^-** Matched OR = 5.20 (0.65–41.68)*	**HP^+ *vs.* HP^-** Matched OR = 5.67 (2.25–14.44)*	**Matched by:** - Sex - Age

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Hansen, 1999 [76]	Norway	Nested case-control (Cohort: mean follow up of 12.0 years)	**Study population:** n = 101,601 **Total gastric cancer:** n = 208 Min. age (yrs) = NS Median age (yrs) = 45.5 Max. age (yrs) = NS Male (n) = 155 (75%) *H. pylori* positive*: n = 166 (80%) **Gastric cardia cancer:** n = 45 *H. pylori* positive*: n = NS (Adenocarcinomas of the cardia and esophagogastric junction were grouped together) **Gastric non-cardia cancer:** n =132 *H. pylori* positive*: n = NS Gastric *fundus* cancers: n = 9 *H. pylori* positive*: n = NS Gastric body cancers: n = 37 *H. pylori* positive*: n = NS Gastric antrum cancers: n = 55 *H. pylori* positive*: n = NS	**Total controls:** n = 983 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 809 (86%) *H. pylori* positive*: n = 619 (66%) Controls for cardia cancer: n = 228 *H. pylori* positive*: n = NS Controls for non-cardia: n = 614 *H. pylori* positive*: n = NS Controls for *fundus*: n = 43 *H. pylori* positive*: n = NS Controls for body: n = 181 *H. pylori* positive*: n = NS Controls for antrum: n = 250 *H. pylori* positive*: n = NS	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS Cut-off: - 250 U/l - 500 U/l	The gastric adenocarcinoma cases were subsite-classified in accordance to the International Classification of Diseases for Oncology	IgG levels ≥ 250 U/l **HP$^+$ *vs.* HP$^-$** Matched OR = 0.40 (0.20–0.77)* IgG levels ≥ 500 U/l **HP$^+$ *vs.* HP$^-$** Matched OR = 0.58 (0.29–1.13)* Model 1 **HP$^+$ *vs.* HP$^-$** Matched OR = 0.41 (0.21-0.81)* Adjusted OR = 0.33 (0.16–0.68)* Model 2 **HP$^+$ *vs.* HP$^-$** Matched OR = 0.40 (0.20-0.81)* Adjusted OR =0.32 (0.15–0.67)*	IgG levels ≥ 250 U/l **HP$^+$ *vs.* HP$^-$** Matched OR=5.15 (2.83–9.37)* IgG levels ≥ 500 U/l **HP$^+$ *vs.* HP$^-$** Matched OR=2.32 (1.52–3.55)* Model 1 **HP$^+$ *vs.* HP$^-$** Matched OR = 5.17 (2.83-9.44)* Adjusted OR = 4.66 (2.53–8.58)* Model 2 **HP$^+$ *vs.* HP$^-$** Matched OR= 6.75 (3.32-13.70)* Adjusted OR = 5.85 (2.85–12.0)*	**Matched by:** - Sex - Date of birth (±13 months) - Date of serum sample (± 7 months) - Serum source **Adjusted by:** - Crowding in 1960 (models 1 and 2) - Education (models 1 and 2) - Occupation (model 2) - Smoking (model 2)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Kikuchi, 1999 [45]	Japan	Case-control Hospital-based	**Total gastric cancer:** n = 103 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = 39 Male (n) = 47 (46%) *H. pylori* positive**: n = 2 (2%) *H. pylori* positive††: n = 34 (33%) *H. pylori* positive§: n = 58 (56%) *H. pylori* positive‡‡: n = 92 (89%) **Gastric cardia cancer:** n = 10 *H. pylori* positive**: n = 0 (0%) *H. pylori* positive††: n = 2 (20%) *H. pylori* positive§: n = 6 (60%) *H. pylori* positive‡‡: n = 8 (80%) **Gastric non-cardia cancer:** n = 75 *H. pylori* positive**: n = 0 (0%) *H. pylori* positive††: n = 27 (36%) *H. pylori* positive§: n = 43 (57%) *H. pylori* positive‡‡: n = 73 (93%)	**Total controls:** n = 201 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = 42 Male (n) = 88 (44%) *H. pylori* positive**: n = 12 (6%) *H. pylori* positive††: n = 29 (14%) *H. pylori* positive§: n = 50 (25%) *H. pylori* positive‡‡: n = 79 (39%) Hospital controls: n = 100 *H. pylori* positive**: n = 6 (6%) *H. pylori* positive††: n = 18 (18%) *H. pylori* positive§: n = 23 (23%) *H. pylori* positive‡‡: n = 41 (41%) Screening controls: n = 101 *H. pylori* positive**: n = 6 (6%) *H. pylori* positive††: n = 11 (11%) *H. pylori* positive§: n = 27 (27%) *H. pylori* positive‡‡: n = 38 (38%)	**ELISA** IgG antibodies against whole-cell and CagA antigens Sensitivity: NS Specificity: NS	Defined as cancer where the main lesion was within the proximal third of the stomach	**$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Crude OR = 1.48 (0.30-7.33)†† Adjusted OR = 3.3 (0.4–24.8)†† **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 4.53 (1.23-16.70)§ Adjusted OR = 6.20 (1.20–32.0)§ **$HP^+/(CagA^-$ or $CagA^+)$ *vs.* $HP^-/CagA^-$** Crude OR =6.18 (1.28-29.85)‡‡ (The hospital and screening controls were used to calculate the all crude and adjusted odds ratio)	**$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Crude OR = 3.34 (1.81-6.17)†† Adjusted OR = 21.3 (7.5–60.6)†† **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 4.06 (2.32-7.09)§ Adjusted OR = 19.4 (7.2–52.1)§ **$HP^+/(CagA^-$ or $CagA^+)$ *vs.* $HP^-/CagA^-$** Crude OR =21.62 (8.36-55.93)‡‡ (The hospital and screening controls were used to calculate the all crude and adjusted odds ratio)	**Adjusted by:** - Sex - Age

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Yamaoka, 1999 [41]	Japan	Case-control Hospital-based	**Total gastric cancer:** n = 110 Min. age (yrs) = 42 Mean age (yrs) = 64.5 Max. age (yrs) = 84 Male (n) =80 (73%) *H. pylori* positive*: n = 90 (82%) *H. pylori* positive§: n = 75 (68%) *H. pylori* positive§: n = 66 (73%) *H. pylori* positive§§: n = 74 (82%) **Gastric cardia cancer:** n = 23 *H. pylori* positive*: n = 15 (65%) *H. pylori* positive§: n = 12 (80%) *H. pylori* positive§: n = 10 (67%) *H. pylori* positive§§: n = 14 (93%) **Gastric non-cardia cancer:** n = 87 *H. pylori* positive*: n = 75 (86%) *H. pylori* positive§: n = 63 (84%) *H. pylori* positive§: n = 56 (75%) *H. pylori* positive§§: n = 60 (80%)	**Controls (group 1):** n = 110 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 80 (73%) *H. pylori* positive*: n = 74 (67%) Controls for cardia cancer: n = 23 *H. pylori* positive*: n = 15 (65%) Controls for non-cardia: n = 87 *H. pylori* positive*: n = 59 (68%) **Controls (group 2)**: n = 90 *H. pylori* positive*: n = 90 (100%) *H. pylori* positive§: n = 75 (83%) *H. pylori* positive§: n = 64 (71%) *H. pylori* positive§§: n = 73 (81%) Controls for cardia cancer: n = 15 *H. pylori* positive§: n = 12 (80%) *H. pylori* positive§: n = 10 (67%) *H. pylori* positive§§: n = 12 (80%) Controls for non-cardia: n = 75 *H. pylori* positive§: n = 61 (81%) *H. pylori* positive§: n = 54 (72%) *H. pylori* positive§§: n = 61 (81%) (First, each cancer patient was sex and aged matched with asymptomatic controls to assess the role of *H. pylori*	**EIA** IgG antibodies against whole-cell antigens Sensitivity: 100% Specificity: 96% **ELISA** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS **Immunoblotting** IgG antibodies against CagA and VacA antigens Sensitivity: NS Specificity: NS	NS	**HP^+ *vs.* HP^-** Matched OR = 1.00 (0.29–3.36)* ELISA **$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Matched OR = 1.0 (0.17–5.99)§ Immunoblotting **$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Matched OR = 1.0 (0.22–4.57)§ **$HP^+/VacA^+$ *vs.* $HP^+/VacA^-$** Matched OR = 3.50 (0.32–38.26)§§	**HP^+ *vs.* HP^-** Matched OR = 2.97 (1.39–6.33)* ELISA **$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Matched OR = 1.20 (0.52–2.81)§ Immunoblotting **$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Matched OR = 1.15 (0.55–2.37)§ **$HP^+/VacA^+$ *vs.* $HP^+/VacA^-$** Matched OR = 0.92 (0.41–2.07)§§	**Matched by:** - Sex - Age

	infection (group 1); a second control (group 2) compared CagA antibody and VacA antibody status in *H. pylori* positive cases and required the addition of *H. pylori* antibody positive age and sex matched cases)	

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Yuan, 1999 [84]	China	Nested case-control Population-based (Cohort: mean follow up of 5.2 years)	**Total gastric cancer:** n = 188 Min. age (yrs) = 45 Mean age (yrs) = 63.4 Max. age (yrs) = 64 Male (n) = 188 (100%) All follow-up *H. pylori* positive*: n = 168 (89%) Follow-up time < 5 years: n = 97 *H. pylori* positive*: n = 83 (86%) Follow-up time ≥ 5 years: n = 91 *H. pylori* positive*: n = 85 (93%) **Gastric cardia cancer:** n = 43 All follow-up *H. pylori* positive*: n = 39 (91%) Follow-up time < 5 years: n = 24 *H. pylori* positive*: n = 21 (88%) Follow-up time ≥ 5years: n = 19 *H. pylori* positive*: n = 18 (95%) **Gastric non-cardia cancer:** n = 114 All follow-up *H. pylori* positive*: n = 100 (96%) Follow-up time < 5 years: n = 61 *H. pylori* positive*: n = 51 (84%) Follow-up time ≥ 5years: n = 53 *H. pylori* positive*: n = 49 (92%)	**Total controls:** n = 548 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 548 (100%) All follow-up *H. pylori* positive*: n = 451 (82%) Follow-up time < 5 years: n = 275 *H. pylori* positive*: n = 231 (84%) Follow-up time ≥ 5years: n = 273 *H. pylori* positive*: n = 220 (81%) **Controls for cardia:** n = 124 All follow-up *H. pylori* positive*: n = 108 (87%) Follow-up time < 5 years: n = 67 *H. pylori* positive*: n = 60 (90%) Follow-up time ≥ 5years: n = 57 *H. pylori* positive*: n = 48 (84%) **Controls for non-cardia:** n = 331 All follow-up *H. pylori* positive*: n = 272 (%) Follow-up time < 5 years: n = 172 *H. pylori* positive*: n = 141 (82%) Follow-up time ≥ 5years: n = 159 *H. pylori* positive*: n = 131 (82%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 94% Specificity: 87% **Microbial culture and smear** (biopsy materials)	NS	Follow-up < 5 years **HP^+ *vs.* HP^-** Matched OR = 0.75 (0.17–3.25)* Follow-up ≥ 5 years **HP^+ *vs.* HP^-** Matched OR = 4.32 (0.45–41.80)*	Follow-up < 5 years **HP^+ *vs.* HP^-** Matched OR = 1.10 (0.50–2.39)* Follow-up ≥ 5 years **HP^+ *vs.* HP^-** Matched OR = 2.67 (0.88–8.11)*	**Matched by:** - Age - Month and year of sample collection - Neighbourhood of residence

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Yamagata, 2000 [78]	Japan	Cohort Population -based (Mean follow up of 4.9 years)	**Cohort:** n = 2602 Min. age (yrs) = 40 Mean age (yrs) = 58 Max. age (yrs) = 58.4 Male (n) = 1070 (41%) **Total gastric cancer:** n = 67 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 48 (72%) *H. pylori* positive*: n = 51 (76%) Male (all follow-up time): *H. pylori* positive*: n = 40 (83%) Male (follow-up ≤ 5 years): *H. pylori* positive*: n = NS Male (follow-up [6-9] years): *H. pylori* positive*: n = NS **Gastric cardia cancer:** n = 10 (data only given for male) *H. pylori* positive*: n = NS **Gastric non-cardia cancer:** n = 40 (data only given for male) *H. pylori* positive*: n = NS	**Cohort:** n = 2602 Min. age (yrs) = 40 Mean age (yrs) = 58 Max. age (yrs) = NS Male (n) = 1070 (41%) **Subcohort pairs:** n = 2535 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 1022 (40%) *H. pylori* positive*: n = 1670 (64%) **Subcohort pairs for cardia:** n = NS *H. pylori* positive*: n = NS **Subcohort pairs for non-cardia:** n = NS *H. pylori* positive*: n = NS	**EIA** IgG antibodies against whole-cell antigens Sensitivity: [95.0-98.7]% Specificity: [96.4-100]%	NS	All follow-up time **HP^+ *vs.* HP^-** Adjusted RR = 1.29 (0.28-6.09)* (The RR estimates were reported only for males)	All follow-up time **HP^+ *vs.* HP^-** Adjusted RR = 3.66 (1.12-11.92)* (The RR estimates were reported only for males)	**Matched by:** - NS **Adjusted by:** - Age

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Enroth, 2000 [87]	Sweden	Case-control Hospital-based	**Total gastric cancer:** n = 72 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = 72.7 Male (n) = 52 (72%) *H. pylori* positive‖‖‖: n = 57 (79%) **Gastric cardia cancer:** n = 8 *H. pylori* positive‖‖‖: n = 5 (63%) **Gastric non-cardia cancer:** n = 64 *H. pylori* positive‖‖‖: n = 52 (81%)	**Total controls:** n = 324 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = 70.9 Male (n) = 210 (65%) *H. pylori* positive‖‖‖: n = 213 (66%)	**Microbial culture** **Immunohistochemical staining** **ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS **Western blotting** IgG antibodies against CagA and VacA antigens Sensitivity: NS Specificity: NS	NS	**HP^+ *vs.* HP^-** Adjusted OR = 0.7 (0.2–3.2)‖‖‖	**HP^+ *vs.* HP^-** Adjusted OR = 2.4 (1.2–4.8)‖‖‖	**Matched by:** - Sex - Age **Adjusted by:** - Sex - Age - Hospital

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Ekstrom, 2001 [73]	Sweden	Case-control Population-based	**Total gastric cancer:** n = 254 Min. age (yrs) = 40 Mean age (yrs) = 68.3 Max. age (yrs) = 79 Male (n) = 187 (67%) *H. pylori* positive*: n = 181 (71%) *H. pylori* positive§: n = 228 (90%) *H. pylori* positive§: n = 234 (92%) **Gastric cardia cancer:** n = 48 *H. pylori* positive*: n = 25 (52%) *H. pylori* positive§: n = 32 (67%) *H. pylori* positive§: n = 35 (73%) **Gastric non-cardia cancer:** n = 206 *H. pylori* positive*: n = 156 (76%) *H. pylori* positive§: n = 196 (95%) *H. pylori* positive§: n = 199 (97%)	**Total controls:** n = 238 Min. age (yrs) = 40 Mean age (yrs) = 67.2 Max. age (yrs) = 79 Male (n) = 159 (67%) *H. pylori* positive*: n = 131 (55%) *H. pylori* positive§: n = 132 (56%) *H. pylori* positive§: n = 141 (59%) (Controls were randomly selected from the complete and continuously update population register)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: > 98% Specificity: [96-100]% **Immunoblotting** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS	Cardia cancer was defined as adenocarcinoma centred within 1 cm proximal and 2 cm distal to the origin of the longitudinal gastric folds	Model 1 **HP^+ *vs.* HP^-** Adjusted OR = 0.8 (0.4-1.8)* **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 1.6 (0.8-3.6)§ **$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Adjusted OR = 1.8 (0.80–4.30)§	Model 2 **HP^+ *vs.* HP^-** Adjusted OR = 2.2 (1.4–3.6)* **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 21.7 (9.6–48.7)§ **$HP^+/CagA^+$ *vs.* $HP^+/CagA^-$** Adjusted OR = 27.7 (11.0–69.7)§	**Matched by:** - Age (model 1 and 2) - Sex (model 1 and 2) **Adjusted by:** - Age (model 1 and 2) - Sex (model 1 and 2) - Age at access to refrigerator (model 1 and 2) - Meals/day (model 1 and 2) - Geographic risk area (model 1 and 2) - Total fruit intake (model 1 and 2) - Total vegetable intake (model 1 and 2) - Cigarette smoking (model 1 and 2) - Body mass index (model 1) - SES (model 2) - Siblings (model 2) - Salt intake (model 2)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Wu, 2003 [36]	USA	Case-control Population-based	**Total gastric cancers:** n = 214 Min. age (yrs) = 30 Mean age (yrs) = NS Max. age (yrs) = 74 Male (n) = 150 (70%) *H. pylori* positive*: n = 162 (76%) *H. pylori* positive††: n = 95 (44%) *H. pylori* positive**: n = 13 (6%) *H. pylori* positive§: n = 67 (31%) **Gastric cardia cancer:** n = 87 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 71 (82%) *H. pylori* positive*: n = 59 (68%) *H. pylori* positive††: n = 47 (54%) *H. pylori* positive**: n = 6 (43%) *H. pylori* positive§: n = 12 (14%) **Gastric non-cardia cancer:** n = 127 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 79 (62%) *H. pylori* positive*: n = 103 (81%) *H. pylori* positive††: n = 48 (38%) *H. pylori* positive**: n = 7 (6%) *H. pylori* positive§: n = 55 (43%)	**Total controls:** n = 1,356 **Controls:** n = 356 Mean age (yrs) = NS Min. age (yrs) = NS Max. age (yrs) = 69 Male (n) = 261 (73.30%) *H. pylori* positive*: n = 230 (65%) *H. pylori* positive††: n = 143 (40%) *H. pylori* positive**: n = 19 (5%) *H. pylori* positive§: n = 87 (24%)	**ELISA** IgG antibodies against whole-cell and CagA antigens **Whole-cell** Sensitivity: 94% Specificity: 85.5% **CagA** Sensitivity: 95% Specificity: 97%	NS	**HP^+ *vs.* HP^-** Adjusted OR = 1.43 (0.83–2.45)* **$HP^+/CagA^-$ *vs.* HP^-** Adjusted OR = 1.70 (0.97–2.98)†† **$HP^+/CagA^+$ *vs.* HP^-** Adjusted OR = 0.80 (0.36–1.81)§ **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Adjusted OR = 1.96 (1.07–3.59)†† **$HP^-/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 2.19 (0.74–6.43)** **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 0.92 (0.40–2.13)§	**HP^+ *vs.* HP^-** Adjusted OR = 1.85 (1.03–3.32)* **$HP^+/CagA^-$ *vs.* HP^-** Adjusted OR = 1.62 (0.86–3.06)†† **$HP^+/CagA^+$ *vs.* HP^-** Adjusted OR = 2.20 (1.13–4.26)§ **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Adjusted OR = 1.88 (0.92–3.83)†† **$HP^-/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 1.87 (0.57–6.18)** **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Adjusted OR = 2.58 (1.22–5.43)§	**Matched by:** - Gender - Race - Date of birth **Adjusted by:** - Age - Gender - Education - Birth place - Ethnic group - Smoking

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Brenner, 2004 [24]	Germany	Case-control Population-based	**Total gastric cancers:** n = 68 Min. age (yrs) = NS Median age (yrs) = 64 Max. age (yrs) = 80 Male (n) = 41 (60%) *H. pylori* positive*: n = 53 (78%) *H. pylori* positive††: n = 22 (32%) *H. pylori* positive§: n = 31 (46%) *H. pylori* positive**: n = 2 (3%) *H. pylori* positive¶¶: n = 33 (49%) **Gastric cardia cancer:** n = 11 *H. pylori* positive*: n = 5 (45%) *H. pylori* positive††: n = 4 (36%) *H. pylori* positive§: n=1 (9%) *H. pylori* positive**: n = 0 (0%) *H. pylori* positive¶¶: n = 1 (9%) **Gastric non-cardia cancer:** n = 57 *H. pylori* positive*: n = 48 (84%) *H. pylori* positive††: n = 18 (32%) *H. pylori* positive§: n = 30 (53%) *H. pylori* positive**: n = 2 (3%) *H. pylori* positive¶¶: n = 32 (56%)	**Total controls:** n = 360 Min. age = NS Mean age (yrs) = 66 Max. age = 80 Male (n) = 216 (60%) *H. pylori* positive*: n = 227 (63%) *H. pylori* positive††: n =143 (40%) *H. pylori* positive§: n = 84 (23%) *H. pylori* positive**: n = 21 (6%) *H. pylori* positive¶¶: n = 105 (29%) (Controls had colorectal cancer)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS **Western blot** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS Cut-off: > 20 U/ml	NS	**HP^+ *vs.* HP^-** Crude OR = 0.49 (0.15–1.63)* **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Crude OR = 0.25 (0.08-0.77)†† **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 0.11 (0.01-0.81)§ **$(HP^-$ or $HP^+)/CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 0.24 (0.03–1.92)¶¶	**HP^+ *vs.* HP^-** Crude OR = 3.12 (1.48-6.57)* Adjusted OR = 3.7 (1.70–7.90)* **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Crude OR = 1.12 (0.54-2.30)†† Adjusted OR = 2.3 (1.0–5.3)†† **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 3.17 (1.61-6.23)§ Adjusted OR = 5.7 (2.6–12.8)§ **$(HP^-$ or $HP^+)/CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 3.11 (1.76-5.50)¶¶	**Adjusted by:** - Age - Sex

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Kato, 2004 [79]	Japan	Case-control Hospital-based	**Total gastric cancers:** n = 2,503 Min. age (yrs) = 21 Max. age (yrs) = NS Mean age (yrs)= NS Male (n) = NS *H. pylori* positive*: n = 2,072 (83%) **Gastric cardia cancer:** n = 86 *H. pylori* positive*: n = 65 (76%) **Gastric non-cardia cancer:** n = 1,998 *H. pylori* positive*: n = 1,663 (83%)	**Total controls:** n = 6,578 Min. age = 21 Mean age (yrs)= NS Max. age = NS Male (n) = NS *H. pylori* positive*: n = 3,300 (50%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS	NS	**HP^+ *vs.* HP^-** Crude OR = 3.07 (1.88–5.04)*	**HP^+ *vs.* HP^-** Crude OR = 4.93 (4.34–5.60)*	**Unmatched** **Unadjusted**
Shen, 2004 [37]	China	Case-control Population-based	**Total gastric cancers:** n = 165 Min. age (yrs) = 34.72 Mean age (yrs ± SD) = 59.36 ± 9.29 Max. age (yrs) = 81.95 Male (n) = 110 (66.67%) *H. pylori* positive¶¶: n = 29 (18%) **Gastric cardia cancer:** n = 50 *H. pylori* positive¶¶: n = 13 (26%) **Gastric non-cardia cancer:** n = 93 *H. pylori* positive¶¶: n = 15 (16%)	**Total controls:** n= ns Min. age (yrs) = 30.77 Mean age (yrs ± SD)= ns Max. age (yrs) = ns Male (n) = 190 (64.41%) *H. pylori* positive¶¶: n = 110 (37%)	**ELISA** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS Cut-off: 0.3A units	The authors used the International Classification of Diseases for Oncology IX, code = 151	**(HP^- or HP^+)/$CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 0.34 (0.17–0.68)¶¶	**(HP^- or HP^+)/$CagA^+$ *vs.* $HP^-/CagA^-$** Crude OR = 0.20 (0.11–0.37)¶¶	**Adjusted by:** - Sex - Age (Applicable only to the adjusted OR for all types of gastric cancer)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Ye, 2004 [77]	Sweden	Case-control Population -based	**Gastric cardia cancer:** n = 133 Min. age (yrs) = NS Mean age (yrs) = 65 Max. age (yrs) = NS Male (n) = 115 (86%) *H. pylori* positive*: n = 45 (34%) *H. pylori* positive¶¶: n = 80 (60%) *H. pylori* positive††: n = 3 (2%) *H. pylori* positive**: n = 38 (29%) *H. pylori* positive§: n = 42 (32%)	**Total controls:** n = 499 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = 69 Male (n) = 414 (83%) *H. pylori* positive*: n = 198 (40%) *H. pylori* positive¶¶:: n = 293 (59%) *H. pylori* positive††: n = 11 (2%) *H. pylori* positive**: n = 106 (21%) *H. pylori* positive§: n = 187 (37%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 98% Specificity: 85% **Immunoblotting** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS	Cancer of the gastric cardia was defined as a tumour with its centre within 2 cm proximal or 3 cm distal of the gastroesophageal junction and without evidence of Barrett's oesophagus	**HP^+ *vs.* HP^-** Model 1 Adjusted OR = 0.9 (0.60-1.40)* Model 2 Adjusted OR = 0.8 (0.50–1.20)* **(HP^- or HP^+)/$CagA^+$ *vs.* HP^-/$CagA^-$** Model 1 Adjusted OR = 1.2 (0.80–1.80)¶¶ Model 2 Adjusted OR = 1.0 (0.70–1.60)¶¶ **HP^+/$CagA^-$ *vs.* HP^-/$CagA^-$** Model 1 Adjusted OR = 1.3 (0.30–5.00)†† Model 2 Adjusted OR = 0.9 (0.20–3.80)†† **HP^-/$CagA^+$ *vs.* HP^-/$CagA^-$** Model 1 Adjusted OR = 1.4 (0.9–2.4)** Model 2 Adjusted OR = 1.3 (0.80–2.20)** **HP^+/$CagA^+$ *vs.* HP^-/$CagA^-$** Model 1 Adjusted OR = 1.0 (0.60-1.70)§ Model 2 Adjusted OR = 0.8 (0.5–1.4)§	----	**Matched by:** - Age - Sex **Adjusted by:** - Age (model 1 and 2) - Sex (model 1 and 2) - Education (model 2) - Intake of: fruits (model 2), vegetables (model 2) - Body mass index (model 2) - Smoking (model 2)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Kokkola, 2005 [38]	Finland	Case-control Hospital-based	**Total gastric cancers:** n = 143 Min. age (yrs) = 32 Mean age (yrs) = 64 Max. age (yrs) = 89 Male (n) = 77 (55%) *H. pylori* positive*: n = 101 (71%) *H. pylori* positive¶¶: n = 127 (89%) **Gastric cardia cancer:** n = 15 *H. pylori* positive*: n = 4 (27%) *H. pylori* positive¶¶: n = 10 (67%) **Gastric non-cardia cancer:** n = 128 *H. pylori* positive*: n = 97 (76%) *H. pylori* positive¶¶: n = 117 (91%)	**Total controls:** n = 108 Min. age (yrs) = 17 Mean age (yrs) = 61 Max. age (yrs) = 98 Male (n) = 59 (54,63%) *H. pylori* positive*: n = 42 (39%) *H. pylori* positive¶¶: n = 62 (57%) (Patients who underwent endoscopy for some reasons other than gastrointestinal malignancy)	**EIA** IgG and IgA antibodies against whole-cell antigens Sensitivity: 100% Specificity: 93% **IgG** Cut-off: ≥ 700 µg/l **IgA** Cut-off: ≥ 70 µg/l **Immunoblotting** IgG antibodies against CagA antigens Sensitivity: NS Specificity: NS	NS	**HP^+ *vs.* HP^-** Crude OR = 0.57 (0.17-1.91)* **(HP^- or HP^+)/$CagA^+$ *vs.* HP^-/$CagA^-$** Crude OR = 1.48 (0.47-4.64)¶¶	**HP^+ *vs.* HP^-** Crude OR = 3.1 (1.50–6.30)* **(HP^- or HP^+)/$CagA^+$ *vs.* HP^-/$CagA^-$** Crude OR = 6.80 (3.10–14.80)¶¶	**Unmatched** **Unadjusted**
Nomura, 2005 [25]	Hawaii	Case-control Population-based	**Total gastric cancer:** n = 276 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = NS Male (n) = NS *H. pylori* positive‡‡‡: n = 199 (72%) *H. pylori* positive**: n = 138 (50%) **Gastric cardia cancer:** n = 33 *H. pylori* positive‡‡‡: n = 14 (42%) *H. pylori* positive**: n = 4 (12%) **Gastric non-cardia cancer:** n = 243 *H. pylori* positive‡‡‡: n = 185 (76%) *H. pylori* positive**: n = 134 (55%)	**Total controls:** n = 336 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = 70.6 ± 12.7 Male (n) = 228 (68%) *H. pylori* positive¶¶: n = 164 (49%) *H. pylori* positive§: n = 97 (29%)	**ELISA** IgG antibodies against whole-cell and CagA antigens Sensitivity: NS Specificity: NS	Tumours were classified as cardia if the cardioesophageal junction was involved	**HP^- or $CagA^+$ *vs.* HP^- + $CagA^-$** Adjusted OR = 0.97 (0.45-2.09) ‡‡‡ **HP^-/$CagA^+$ *vs.* HP^-/$CagA^-$** Crude OR = 0.34 (0.12-0.99)§ Adjusted OR = 0.40 (0.13-1.18) **	**HP^- or $CagA^+$ *vs.* HP^- + $CagA^-$** Adjusted OR = 3.41 (2.35-4.94) ‡‡‡ **HP^-/$CagA^+$ *vs.* HP^-/$CagA^-$** Crude OR = 3.03 (2.14-4.28)§ Adjusted OR = 3.16 (2.22-4.51) **	**Matched by:** - Sex - Ethnicity - 5-year age groups **Adjusted by:** - Sex - Ethnicity - 5-year age groups

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Shin, 2005 [80]	Korea	Nested case-control Population-based (Cohort: mean follow up of 2.6 years)	**Total gastric cancer:** n = 86 Min. age (yrs) = 40 Mean age (yrs) = 63 Max. age (yrs) = 82 Male (n) = 57 (66.3%) *H. pylori* positive*: n = 72 (84%) **Gastric cardia cancer:** n = 6 *H. pylori* positive*: n = 4 (67%) **Gastric non-cardia cancer:** n = 70 *H. pylori* positive*: n = 60 (86%)	**Total controls:** n = 344 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 228 (66%) *H. pylori* positive*: n = 278 (81%) Controls for cardia: n = 24 *H. pylori* positive*: n = 20 (83%) Controls for non-cardia: n = 280 *H. pylori* positive*: n = 231 (83%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: 100% Specificity: 81.3%	ns	**HP $^{+}$ *vs.* HP $^{-}$** Adjusted OR = 0.88 (0.38-2.28)*	**HP $^{+}$ *vs.* HP $^{-}$** Adjusted OR =1.07 (0.77-1.49)*	**Matched by:** - Age - Gender - Year and site of their recruitment **Adjusted by:** - Education - Alcohol intake - Cumulative dose of smoking
Palli, 2006 [32]	10 European countries: Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom	Nested case-control Population-based (Cohort: mean follow up of 6.10 years)	**Total gastric cancer:** n = 233 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 127 (55%) *H. pylori* positive*: n = 31 (13%) *H. pylori* positive§: n = 195 (84%) *H. pylori* positive¶¶: n = 164 (70%) **Gastric cardia cancer:** n = 54 *H. pylori* positive*: n = 10 (19%) *H. pylori* positive§: n = 35 (65%) *H. pylori* positive¶¶: n = 25 (46%) **Gastric non-cardia cancer:** n = 127 *H. pylori* positive*: n = 15 (12%) *H. pylori* positive§: n = 115 (91%) *H. pylori* positive¶¶: n = 100 (79%)	**Total controls:** n = 910 Min. age (yrs) = NS Mean age (yrs)= NS Max. age (yrs) = NS Male (n) = 490 (54%) *H. pylori* positive*: n = 205 (23%) *H. pylori* positive§: n = 625 (69%) *H. pylori* positive¶¶: n = 420 (46%) Controls for cardia cases: n = NS *H. pylori* positive*: n = NS *H. pylori* positive§: n = NS *H. pylori* positive¶¶: n = NS Controls for non-cardia cases: n = NS *H. pylori* positive*: n = NS *H. pylori* positive§: n= NS *H. pylori* positive¶¶: n = NS	**ELISA** IgG antibodies against whole-cell and CagA antigens **Whole-cell** Sensitivity: > 90% Specificity: > 90% Cut-off: > 100 EU **CagA** Sensitivity: NS Specificity: NS Cut-off: > 30 EU	NS	**HP^{+} *vs.* HP^{-}** Adjusted OR = 0.8 (0.3-2.1)* **HP^{+}/CagA^{+} *vs.* HP^{-} /CagA^{-}** Adjusted OR = 0.8 (0.4–1.8)§ **(HP^{-} or HP^{+})/CagA^{+} *vs.* HP^{-}/CagA^{-}** Adjusted OR = 0.8 (0.4–1.9)¶¶	**HP^{+} *vs.* HP^{-}** Adjusted OR = 1.6 (0.7-3.8)* **HP^{+}/CagA^{+} *vs.* HP^{-} /CagA^{-}** Adjusted OR = 4.7 (2.5–9.0)§ **(HP^{-} or HP^{+})/CagA^{+} *vs.* HP^{-}/CagA^{-}** Adjusted OR = 6.5 (3.3–12.6)¶¶	**Matched by:** - Centre - Gender - Age - Blood donation date **Adjusted by:** - Education - Smoking - Weight - Vegetables - Fruit - Red meat intake - Preserved meat intake

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Kamangar, 2006 [26]	Finland	Nested case–control Population -based (Cohort: follow-up mean time not specified)	**Total gastric cancer:** n = 234 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 234 (100%) *H. pylori* positive*: n = 184 (79%) *H. pylori* positive††: n = 62 (26%) *H. pylori* positive§: n = 135 (58%) *H. pylori* positive¶¶: n = 197 (84%) **Gastric cardia cancer:** n = 61 Mean age (yrs ± SD) = 59.4 ± 4.7 Male (n) = 61 (100%) *H. pylori* positive*: n = 35 (57%) *H. pylori* positive††: n = 11 (18%) *H. pylori* positive§: n = 25 (41%) *H. pylori* positive¶¶: n = 36 (59%) **Gastric non-cardia cancer:** n = 173 Mean age (yrs ± SD) = 58.8 ± 5.0 Male (n) = 173 (100%) *H. pylori* positive*: n =149 (86%) *H. pylori* positive††: n = 51 (29%) *H. pylori* positive§: n =110 (64%) *H. pylori* positive¶¶: n = 161 (93%)	**Total controls:** n = 234 Male (n) = 234 (100%) *H. pylori* positive*: n = 166 (71%) *H. pylori* positive††: n = 68 (29%) *H. pylori* positive§: n = 108 (46%) *H. pylori* positive¶¶: n = 176 (75%) **Controls for cardia:** n = 61 Mean age (yrs ± SD)= 59.5 ± 4.8 Male (n) = 61 (100%) *H. pylori* positive*: n = 44 (72%) *H. pylori* positive††: n = 24 (39%) *H. pylori* positive§: n = 22 (36%) *H. pylori* positive¶¶: n = 46 (75%) **Controls for non-cardia:** n = 173 Mean age (yrs ± SD)= 58.8 ± 5.0 Male (n) = 173 (100%) *H. pylori* positive*: n = 122 (71%) *H. pylori* positive††: n = 44 (25%) *H. pylori* positive§: n = 86 (50%) *H. pylori* positive¶¶: n = 130 (75%)	**ELISA** IgG antibodies against whole-cell and CagA antigens **Whole-cell** CV: 15% **CagA** CV: 20%	Cases were classified as gastric cardia cancer if they involved the esophago gas-tric junction	**HP^+ *vs.* HP^-** Unconditional logistic model Matched OR = 0.49 (0.28-0.88)* Adjusted OR = 0.47 (0.25-0.89)* Conditional logistic model Adjusted OR = 0.28 (0.09-0.86)* **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Matched OR = 0.34 (0.14–0.85)†† Adjusted OR = 0.21 (0.06–0.81)†† **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Matched OR = 0.81 (0.35-1.85)§ Adjusted OR = 0.43 (0.12–1.52)§ **(HP^- or HP^+)/$CagA^+$ *vs.* $HP^-/CagA^-$** Matched OR = 0.54 (0.27–1.10)¶¶ Adjusted OR = 0.31 (0.11–0.89)¶¶	**HP^+ *vs.* HP^-** Unconditional logistic model Matched OR = 4.50 (2.34-8.65)* Adjusted OR = 5.11 (2.51-10.43)* Conditional logistic model Adjusted OR = 3.32 (1.72-6.42)* **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Matched OR = 5.05 (2.11-12.07)†† Adjusted OR = 6.55 (2.31–18.53)†† **$HP^+/CagA^+$ *vs.* $HP^-/CagA^-$** Matched OR = 5.64 (2.47–12.88)§ Adjusted OR = 8.93 (3.27-24.40)§ **(HP^- or HP^+)/$CagA^+$ *vs.* $HP^-/CagA^-$** Matched OR = 5.43 (2.42–12.16)¶¶ Adjusted OR = 7.92 (3.02–20.90)¶¶	**Matched by:** - Age **Adjusted by:** - Age at enrolment - Education - Area of residence - Mean smoking duration - Mean body mass index - Mean dietary intake: nitrate, fruit, vegetable, starch, and sodium

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Yang, 2006 [85]	China	Case-control Population -based	**Total gastric cancer:** n = 29 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = 58.9 Male (n) = 14 (48%) *H. pylori* positive***: n = 11 (38%) *H. pylori* positive§: n = 23 (79%) **Gastric cardia cancer:** n = 4 *H. pylori* positive***: n = 0 (0%) *H. pylori* positive§: n = 3 (75%) **Gastric non-cardia cancer:** n = 25 *H. pylori* positive***: n = 11 (44%) *H. pylori* positive§: n = 20 (80%)	**Total controls:** n = 25 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs) = 35.9 Male (n) = 6 (24%) *H. pylori* positive***: n = 11 (44%) *H. pylori* positive§: n = 13 (52%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS **Rapid urease test** **Giemsa stain** **Microbial culture** **Immunoblotting** IgG antibodies against CagA and VacA antigens Sensitivity: NS Specificity: NS	NS	**HP$^+$/CagA$^+$ *vs.* HP$^-$ /CagA$^-$** Crude OR = 2.77 (0.25–30.38)§	**HP$^+$ *vs.* HP$^-$** Crude OR = 1.00 (0.33–3.06)*** **HP$^+$/CagA$^+$ *vs.* HP$^-$ /CagA$^-$** Crude OR = 3.69 (1.05–12.96)§	**Unmatched** **Unadjusted**
Knekt, 2006 [72]	Finland	Nested case-control Population -based (Cohort: maxims follow up of 24 years)	**Total gastric cancer:** n = 225 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs ± SD) = 68 ± 14 Male (n) = 140 (62%) *H. pylori* positive†††: n = 182 (81%) *H. pylori* positive*: n = 201 (89%) **Gastric cardia cancer:** n = 32 *H. pylori* positive†††: n = 24 (75%) *H. pylori* positive*: n = 25 (78%) **Gastric non-cardia cancer:** n = 193 *H. pylori* positive †††: n = 158 (82%) *H. pylori* positive*: n = 176 (91%)	**Total controls:** n = 435 Min. age (yrs) = NS Max. age (yrs) = NS Mean age (yrs ± SD) = 68 ± 14 Male (n) = NS *H. pylori* positive†††: n= 277 (64%) *H. pylori* positive*: n = 345 (79%) Controls for cardia cancer: n = 63 *H. pylori* positive†††: n = 47 (75%) *H. pylori* positive*: n = 51 (81%) Controls for non-cardia cancer: n = 372 *H. pylori* positive†††: n = 230 (62%) *H. pylori* positive*: n = 294 (79%)	**EIA** IgG and IgA antibodies against whole-cell antigens **IgG** Sensitivity: 94% Specificity: 93% Cut-off: > 700 µg/l **IgA** Sensitivity: 73% Specificity: 95% Cut-off: > 70 µg/l	NS	IgA **HP$^+$ *vs.* HP$^-$** Matched OR = 1.00 (0.36–2.74)††† IgG **HP$^+$ *vs.* HP$^-$** Matched OR = 0.82 (0.29–2.35)*	IgA **HP$^+$ *vs.* HP$^-$** Matched OR = 3.12 (1.97–4.95)††† IgG **HP$^+$ *vs.* HP$^-$** Matched OR = 2.88 (1.63–5.07)*	**Matched by:** - Sex - Age - Municipality - Duration of storage of serum samples

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Sasazuki, 2006 [82]	Japan	Nested case-control Population-based (Cohort: maximum follow up was 9 years)	**Total gastric cancer:** n = 511 Min. age (yrs) = 49 Mean age (yrs ± SD) = 57.4 ± 0.32 Max. age (yrs) = 69 Male (n) = 342 (67%) *H. pylori* positive*: n = 478 (94%) *H. pylori* positive¶¶: n = 390 (76%) *H. pylori* positive††: n = 115 (23%) *H. pylori* positive**: n = 27 (5%) *H. pylori* positive‡‡‡: n = 505 (99%) *H. pylori* positive§: n = 363 (71%) **Gastric cardia cancer:** n = 39 *H. pylori* positive*: n = 37 (99%) **Gastric non-cardia cancer:** n = 368 *H. pylori* positive*: n = 344 (93%)	**Total controls:** n = 511 Min. age (yrs) = 49 Mean age (yrs ± SD) = 57.4 ± 0.32 Max. age (yrs) = 69 Male (n) = 342 *H. pylori* positive*: n = 383 (75%) *H. pylori* positive¶¶: n = 358 (70%) *H. pylori* positive††: n = 102 (20%) *H. pylori* positive**: n = 77 (15%) *H. pylori* positive‡‡‡: n = 460 (90%) *H. pylori* positive§: n = 281 (55%) Controls for cardia cases: n = 39 *H. pylori* positive*: n = 33 (85%) Controls for non-cardia cases: n = 368 *H. pylori* positive*: n = 274 (74%)	**ELISA** IgG antibodies against whole-cell and CagA antigens **Whole-cell** Sensitivity: 100% Specificity: 80% Cut-off: > 492 nm **CagA** Sensitivity: 100% Specificity: 93.7% Cut-off: NS	The authors combined tumours located in the esophagogastric junction and in the upper third of the stomach into one group for analysis in this study (ICD-O code C160-161)	**HP^+ *vs.* HP^-** Adjusted OR = 3.7 (0.2–68.4)*	**HP^+ *vs.* HP^-** Adjusted OR = 5.1 (3.0–8.6)*	**Matched by:** - Gender - Age - Study area - Blood donation date - Fasting time at blood donation **Adjusted by:** - Smoking - Intake of: fish gut, green/yellow other vegetables, fruit, green tea - Body mass index - Family history of gastric cancer

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Kamangar, 2007 [31]	China	Cohort Maximum follow-up of 16 years	**Total gastric cancers:** n = 925 Min. age = 40 Mean age (yrs ± SD) = 55.75 ± 7.8 Max. age = 69 Males (n) = 579 (63%) *H. pylori* positive ¶¶: n = 749 (81%) *H. pylori* positive§: n = 575 (62%) *H. pylori* positive††: n = 174 (19%) Diagnostic ≤ 5 years: n = 340 *H. pylori* positive¶¶: n = 278 (82%) Diagnostic]5.1-10] years: n = 312 *H. pylori* positive¶¶: n = 246 (79%) Diagnostic > 10 years: n = 273 *H. pylori* positive¶¶: n = 225 (82%) **Gastric cardia cancer:** n = 582 Mean age (yrs ± SD) = 55.5 ± 7.7 Males (n) = 351 (60.3%) *H. pylori* positive¶¶: n = 473 (81%) *H. pylori* positive§: n = 373 (64%) *H. pylori* positive††: n = 100 (17%) Diagnostic ≤ 5 years: n = 216 *H. pylori* positive¶¶: n = 171 (79%) Diagnostic]5.1-10] years: n = 199 *H. pylori* positive¶¶: n = 158 (79%)	**Subcohort pars:** n = 992 Min. age = NS Mean age (yrs ± SD) = 51.9 ± 8.9 Max. age = NS Males (n) = 449 (45.3%) *H. pylori* positive¶¶: n = 727 (73%) *H. pylori* positive§: n = 552 (56%) *H. pylori* positive††: n = 175 (18%)	**ELISA** IgG antibodies against whole-cell and CagA antigens **Whole-cell** CV: 15% Cut-off: ≥ 1.0 OD **CagA** CV: 20% Cut-off: ≥ 0.35 OD	Cancers were defined as cardia cancers if they were in the most proximal 3 cm of the stomach	All follow-up time **HP^+ or $CagA^+$ *vs.* $HP^-/CagA^-$** Crude HR = 1.59 (1.24–2.05)¶¶ Adjusted HR = 1.64 (1.26–2.14)¶¶ **$(HP^+$ or $HP^-)/CagA^+$ *vs.* $HP^-/CagA^-$** Crude HR = 1.64 (1.27-2.13)§ Adjusted HR = 1.75 (1.32–2.30)§ **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Crude HR = 1.43 (1.03–2.00)†† Adjusted HR = 1.35 (0.95–1.92)††	All follow-up time **HP^+ or $CagA^+$ *vs.* $HP^-/CagA^-$** Crude HR = 1.51 (1.12–2.05)¶¶ Adjusted HR = 1.60 (1.15–2.21)¶¶ **$(HP^+$ or $HP^-)/CagA^+$ *vs.* $HP^-/CagA^-$** Crude HR = 1.45 (1.06-1.98)§ Adjusted HR = 1.58 (1.13–2.22)§ **$HP^+/CagA^-$ *vs.* $HP^-/CagA^-$** Crude HR = 1.73 (1.18–2.54)†† Adjusted HR = 1.62 (1.08–2.45)††	**Adjusted by:** - Age - Age squared - Sex

	Diagnostic > 10 years: n = 167 *H. pylori* positive¶¶: n = 144 (86%) **Gastric non-cardia cancer:** n = 343 Mean age (yrs ± SD) = 56.0 ± 7.9 Males (n) = 228 (66.5%) *H. pylori* positive¶¶: n = 276 (80%) *H. pylori* positive§: n = 202 (59%) *H. pylori* positive††: n = 74 (22%) Diagnostic ≤ 5 years: n = 124 *H. pylori* positive¶¶: n = 107 (86%) Diagnostic]5.1-10] years: n = 113 *H. pylori* positive¶¶: n = 88 (78%) Diagnostic ≥ 10 years: n = 106 *H. pylori* positive¶¶: n = 81 (76%)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Simán, 2007 [74]	Sweden	Nested case-control Population-based (Cohort: mean follow-up ranged from 9.2 to 12.6 years)	**Total gastric cancer:** n = 91 Min. age (yrs) = 37.6 Mean age (yrs) = NS Max. age (yrs) = 76 Male (n) = 76 (84%) *H. pylori* positive*: n = 82 (90%) *H. pylori* positive¶¶: n = 86 (95%) *H. pylori* positive§: n = 82 (90%) **Gastric cardia cancer:** n = 24 Mean age (yrs) = 51.4 *H. pylori* positive*: n = 17 (71%) *H. pylori* positive¶¶: n = 20 (83%) *H. pylori* positive§: n = 17 (71%) **Gastric non-cardia cancer:** n = 67 Mean age (yrs) = 50.7 *H. pylori* positive*: n = 65 (97%) *H. pylori* positive¶¶: n = 66 (99%) *H. pylori* positive§: n = 65 (97%)	**Total controls:** n = 338 Min. age (yrs) = 37.6 Mean age (yrs) = NS Max. age (yrs) = 76 Male (n) = 424 (83%) *H. pylori* positive*: n = 201 (59%) *H. pylori* positive¶¶: n = 254 (76%) *H. pylori* positive§: n = 181 (54%) Controls for cardia: n = 88 *H. pylori* positive*: n = 54 (61%) *H. pylori* positive¶¶: n = 61 (69%) *H. pylori* positive§: n = 48 (55%) Controls for non-cardia: n = 250 *H. pylori* positive*: n = 147 (59%) *H. pylori* positive¶¶: n = 193 (77%) *H. pylori* positive§: n = 133 (53%)	**Western blot** IgG antibodies against whole-cell and CagA antigens Sensitivity: 96% Specificity: 95%	A cardia adenocarcinoma was defined as occurring within 3 cm proximal or 2 cm distal to the gastro-oesophageal junction, without Barrett's mucosa	**HP^+ *vs.* HP^-** Matched OR = 1.5 (0.54-4.6)* Adjusted OR = 1.5 (0.51-4.8)* **(HP^- or HP^+)/$CagA^+$ *vs.* HP^-/$CagA^-$** Matched OR = 2.2 (0.65-9.7)¶¶ Adjusted OR = 2.3 (0.66-12)¶¶ **HP^+/$CagA^+$ *vs.* HP^-/$CagA^-$** Matched OR = 2.5 (0.36-∞)§ Adjusted OR = 2.7 (0.38-∞)§	**HP^+ *vs.* HP^-** Matched OR = 22.4 (5.3–93.8)* Adjusted OR = 17.8 (4.2–74.8)* **(HP^- or HP^+)/$CagA^+$ *vs.* HP^-/$CagA^-$** Matched OR = 20.1 (2.7-148)¶¶ Adjusted OR = 16.8 (2.2-130)¶¶ **HP^+/$CagA^+$ *vs.* HP^-/$CagA^-$** Matched OR = 9.6 (1.5-∞)§ Adjusted OR = 9.7 (1.5-∞)§	**Matched by:** - Gender - Date of birth (± 6 months) - Date of enrolment (± 6 months) **Adjusted by:** - Occupation - Smoking

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Mitchell, 2008 [42]	Australia	Nested case-control Population -based (Cohort: mean follow-up of 11.6 years)	**Total gastric cancer:** n = 52 Min. age (yrs) = 42 Mean age (yrs) = NS Max. age (yrs) = 69 Male (n) = 35 (67%) *H. pylori* positive*: n = 33 (63%) *H. pylori* positiveIIII: n = 40 (77%) **Gastric cardia cancer:** n = 18 Min. age (yrs) = 45 Median age (yrs) = 63 Max. age (yrs) = 69 Males (n) = 14 (77.8%) *H. pylori* positive*: n = 6 (33%) *H. pylori* positiveIIII: n = 8 (44%) **Gastric non-cardia cancer:** n = 34 Min. age (yrs) = 42 Median age (yrs)= 62 Max. age (yrs) = 69 Males (n) = 21 (61.8%) *H. pylori* positive*: n = 27 (79%) *H. pylori* positiveIIII: n = 32 (94%)	**Total controls:** n = 203 Min. age (yrs) = 42 Mean age (yrs) = NS Max. age (yrs) = 69 Male (n) = 139 (68%) *H. pylori* positive*: n = 109 (54%) *H. pylori* positiveIIII: n = 112 (55%) Controls to cardia cancer: n = 69 Min. age (yrs) = 43 Median age (yrs)= 62 Max. age (yrs) = 69 Males (n) = 55 (79.7%) *H. pylori* positive*: n = 24 (35%) *H. pylori* positiveIIII: n = 27 (39%) Controls to non-cardia cancer: n = 134 Min. age (yrs) = 42 Median age (yrs) = 62 Max. age (yrs) = 69 Males (n) = 84 (62.7%) *H. pylori* positive*: n = 85 (63%) *H. pylori* positiveIIII: n = 85 (63%)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: >95% Specificity: 95% **Immunoblotting** IgG antibodies against CagA, VacA and UreA antigens Sensitivity: 96% Specificity: 93%	Case patients were identified from notifications to the Victorian Cancer Registry of diagnoses of histologically confirmed cardia gastric adenocarcinoma or lymphoma (International Classification of Diseases 9th revision rubric 1510 or 10th revision rubric, C160)	**HP^+ *vs.* HP^-** Matched OR = 0.9 (0.30-2.70)* **$CagA^+$or $VacA^+$ or $UreA^+$ *vs.* $CagA^-$ /$VacA^-$/$UreA^-$** Matched OR = 1.2 (0.4-3.4)IIII	**HP^+ *vs.* HP^-** Matched OR = 2.3 (0.90-5.80)* **$CagA^+$or $VacA^+$ or $UreA^+$ *vs.* $CagA^-$ /$VacA^-$/$UreA^-$** Matched OR = 10.60 (2.40-47.40) IIII	**Matched by:** - Sex - Age - Country of birth (Australia, UK, Greece, Italy) - Date of blood draw (same calendar quarter)

Annex 2 (continuation). Characteristics of the studies included in the systematic review.

1st author, year of publication [ref]	Country, region	Type of study (follow-up time)	Subjects characteristics		Assessment of infection status	Cardia/ proximal definition	Risk estimate (95% confidence interval [CI])		Control for confounding
			Cases	Controls or subcohort pairs			Cardia cancer	Non-cardia cancer	
Derakhshan, 2008 [81]	Iran	Case-control Hospital-based	**Total gastric cancer:** n = 119 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = 89 (75%) *H. pylori* positive*: n = 106 (89%) **Gastric cardia cancer:** n = 53 Min. age (yrs) = NS Mean age (yrs ± SD) = 63.8 ± 7.1 Max. age (yrs) = NS Male (n) = 37 (70%) *H. pylori* positive*: n = 44 (83%) **Gastric non-cardia cancer:** n = 66 Min. age (yrs) = NS Mean age (yrs ± SD) = 65.9 ± 6.5 Max. age (yrs) = NS Male (n) = 49 (74%) *H. pylori* positive*: n = 62 (94%)	**Total controls:** n = 119 Min. age (yrs) = NS Mean age (yrs) = NS Max. age (yrs) = NS Male (n) = NS *H. pylori* positive*: n = 88 (74%) Controls for cardia cancer: n = 53 Min. age (yrs) = NS Mean age (yrs ± SD) = 63.8 ± 7.1 Max. age (yrs) = NS Male (n) = 37 (70%) *H. pylori* positive*: n = 39 (74%) Controls for non-cardia cancer: n = 66 Min. age (yrs) = NS Mean age (yrs ± SD) = 65.9 ± 6.5 Max. age (yrs) = NS Male (n) = 49 (74%) *H. pylori* positive*: n = 49 (74%) (The participants were dyspeptic.)	**ELISA** IgG antibodies against whole-cell antigens Sensitivity: NS Specificity: NS Cut-off: 30 EIU	Cardia cancer was defined as tumours whose main bulk was within 2 cm distal to the gastro-oesophageal junction	**HP^+ *vs.* HP^-** Matched OR = 1.46 (0.68–3.14)* Adjusted OR = 2.42 (0.84–7.02)*	**HP^+ *vs.* HP^-** Matched OR = 2.22 (1.11–4.46)* Adjusted OR = 1.53 (0.57–4.14)*	**Matched by:** - Age - Sex **Adjusted by:** - Smoking - Gastroesophageal reflux disease symptoms - Ratio pepsinogen I/II

* *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens; † *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens or indicates the presence of *H. pylori* colonies on the culture plates; ‡ *H. pylori* positive indicates rapid urease test and/or histology positives; § *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens and *H. pylori* CagA status positive; ıı *H. pylori* positive indicates rapid urease test positive (also known as the CLO test); ¶ *H. pylori* positive indicates positive culture or positive results to at least two of the tests used (determination the presence of IgG-specific anti-*H. pylori* antibodies by ELISA, histological examination, urease test, carbolfuchsin stained, smear and tissue Polymerase Chain Reaction (PCR)); ** *H. pylori* positive indicates seronegativity to IgG antibodies against surface/whole-cell antigens *H. pylori* CagA status positive; †† *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens and *H. pylori* CagA status negative; ‡‡ *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens and *H. pylori* CagA status negative or positive; §§ *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens and *H. pylori* VacA status positive; ııı *H. pylori* positive indicates seropositivity to IgG antibodies against CagA or VacA or UreA antigens; ¶¶ *H. pylori* positive indicates seropositivity or seronegativity to IgG antibodies against surface/whole-cell antigens and *H. pylori* CagA status positive; *** *H. pylori* positive indicates detection of one reaction band of 116 kDa (CagA) and/or 89 kDa (VacA) and/or 35 kDa (major antigens), and/or two other reaction bands (minor antigens, 30 kDa, 26.5 kDa, 19.5 kDa), by immunobloting test; ††† *H. pylori* positive indicates seropositivity to IgA antibodies against surface/whole-cell antigens; ‡‡‡ *H. pylori* positive indicates seropositivity to IgG antibodies against surface/whole-cell antigens or *H. pylori* CagA status positive; NS – not specified; SD – standard deviation; CV – coefficient of variation.

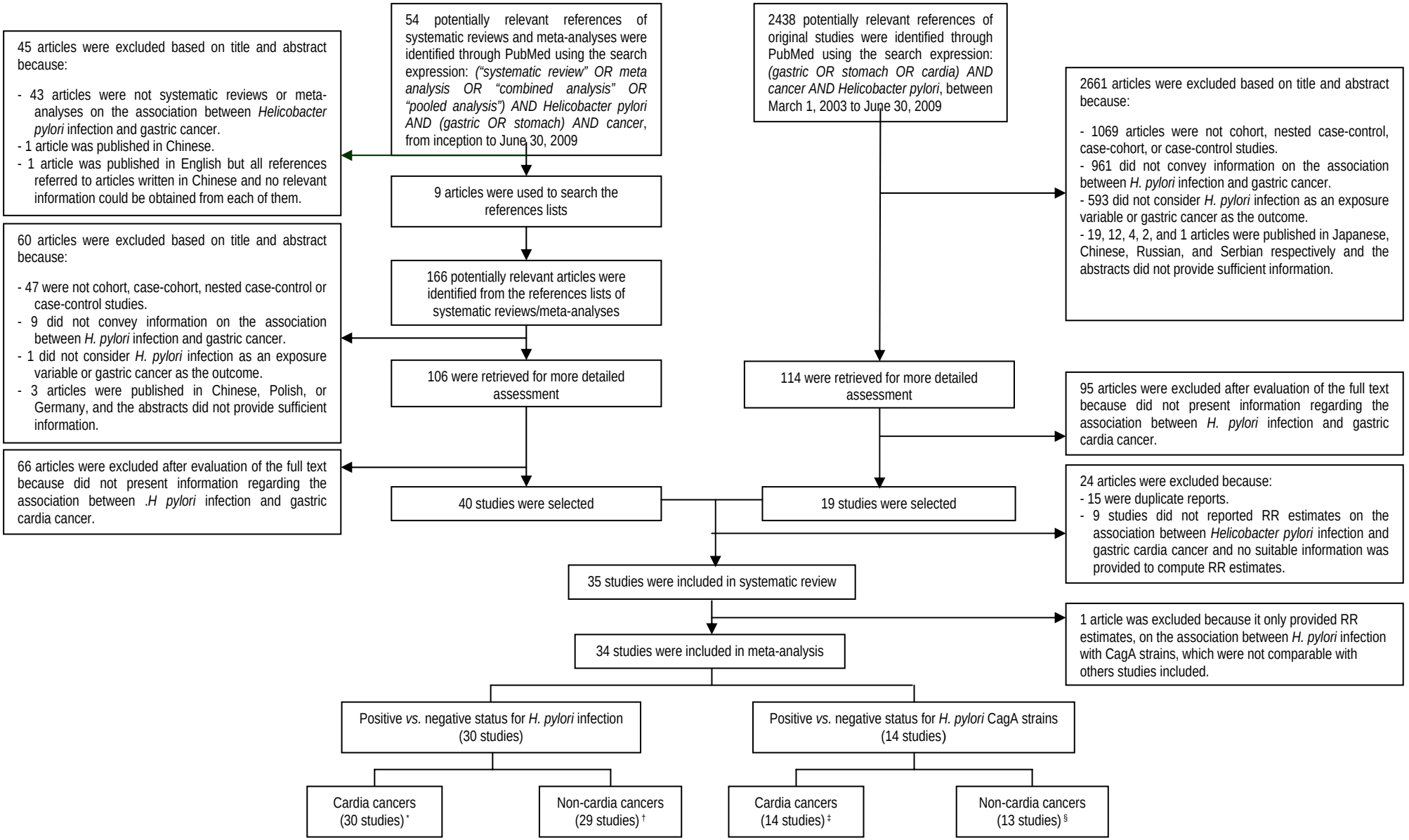

54 potentially relevant references of systematic reviews and meta-analyses were identified through PubMed using the search expression: *("systematic review" OR meta analysis OR "combined analysis" OR "pooled analysis") AND Helicobacter pylori AND (gastric OR stomach) AND cancer*, from inception to June 30, 2009
45 articles were excluded based on title and abstract because:
- 43 articles were not systematic reviews or meta-analyses on the association between *Helicobacter pylori* infection and gastric cancer.
- 1 article was published in Chinese.
- 1 article was published in English but all references referred to articles written in Chinese and no relevant information could be obtained from each of them.
9 articles were used to search the references lists
166 potentially relevant articles were identified from the references lists of systematic reviews/meta-analyses
60 articles were excluded based on title and abstract because:
- 47 were not cohort, case-cohort, nested case-control or case-control studies.
- 9 did not convey information on the association between *H. pylori* infection and gastric cancer.
- 1 did not consider *H. pylori* infection as an exposure variable or gastric cancer as the outcome.
- 3 articles were published in Chinese, Polish, or Germany, and the abstracts did not provide sufficient information.
106 were retrieved for more detailed assessment
66 articles were excluded after evaluation of the full text because did not present information regarding the association between *.H pylori* infection and gastric cardia cancer.
40 studies were selected
2438 potentially relevant references of original studies were identified through PubMed using the search expression: *(gastric OR stomach OR cardia) AND cancer AND Helicobacter pylori*, between March 1, 2003 to June 30, 2009
2661 articles were excluded based on title and abstract because:
- 1069 articles were not cohort, nested case-control, case-cohort, or case-control studies.
- 961 did not convey information on the association between *H. pylori* infection and gastric cancer.
- 593 did not consider *H. pylori* infection as an exposure variable or gastric cancer as the outcome.
- 19, 12, 4, 2, and 1 articles were published in Japanese, Chinese, Russian, and Serbian respectively and the abstracts did not provide sufficient information.
114 were retrieved for more detailed assessment
95 articles were excluded after evaluation of the full text because did not present information regarding the association between *H. pylori* infection and gastric cardia cancer.
19 studies were selected
24 articles were excluded because:
- 15 were duplicate reports.
- 9 studies did not reported RR estimates on the association between *Helicobacter pylori* infection and gastric cardia cancer and no suitable information was provided to compute RR estimates.
35 studies were included in systematic review
1 article was excluded because it only provided RR estimates, on the association between *H. pylori* infection with CagA strains, which were not comparable with others studies included.
34 studies were included in meta-analysis
Positive *vs.* negative status for *H. pylori* infection (30 studies)
Positive *vs.* negative status for *H. pylori* CagA strains (14 studies)
Cardia cancers (30 studies) *
Non-cardia cancers (29 studies) †
Cardia cancers (14 studies) ‡
Non-cardia cancers (13 studies) §

Figure 1. Systematic review flow-chart.

* From 30 studies 24 provided adjusted RR estimates; † From 29 studies 23 provided adjusted RR estimates; ‡ From 14 studies 10 provided adjusted RR estimates; § From 13 studies 9 provided adjusted RR estimates.

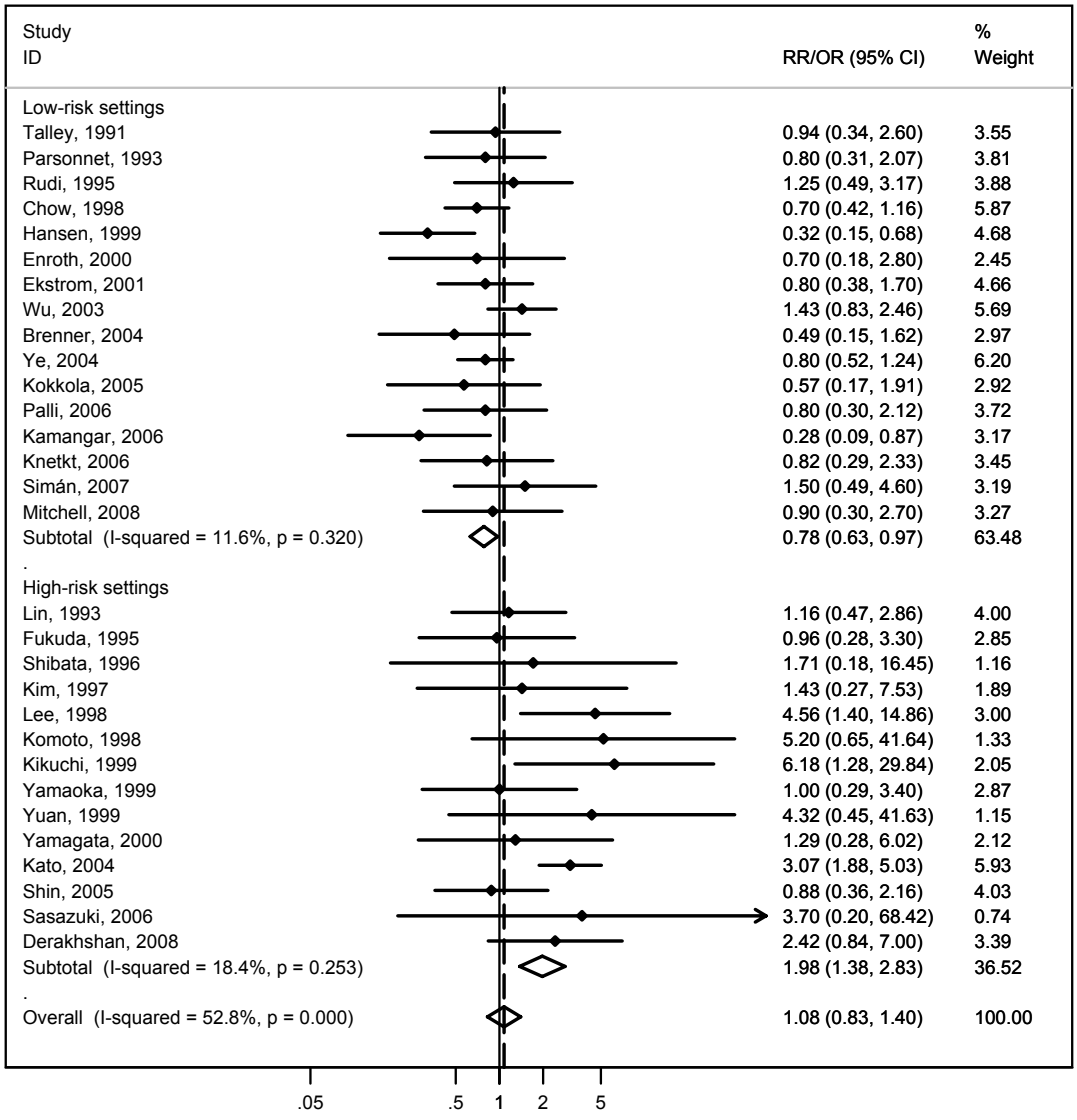


Figure 2. Meta-analyses of cohort, nested case-control, case-cohort, and case-control studies evaluating the association between *Helicobacter pylori* infection and gastric cardia cancer.

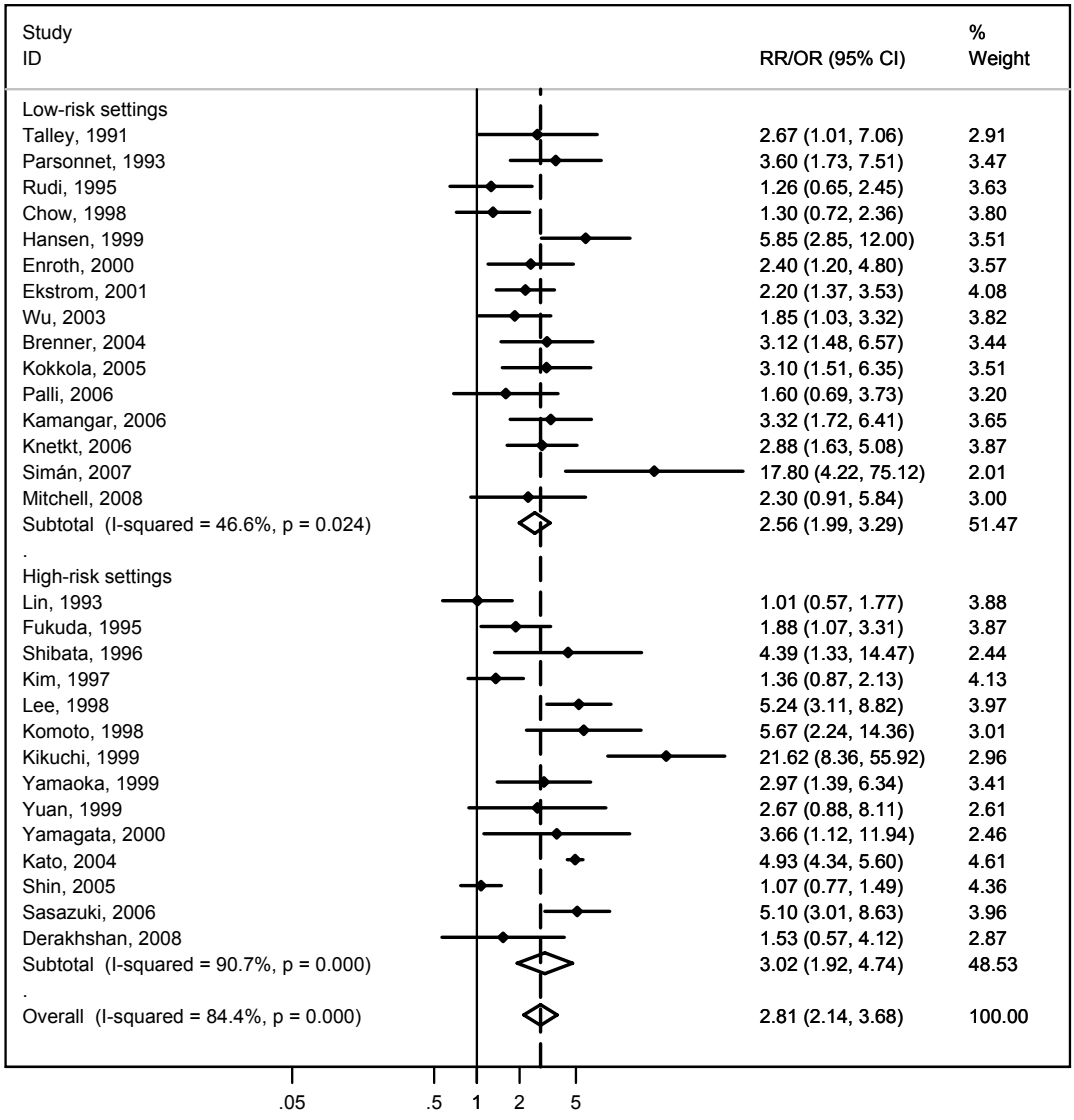


Figure 3. Meta-analyses of cohort, nested case-control, case-cohort, and case-control studies evaluating the association between *Helicobacter pylori* infection and gastric non-cardia cancer.

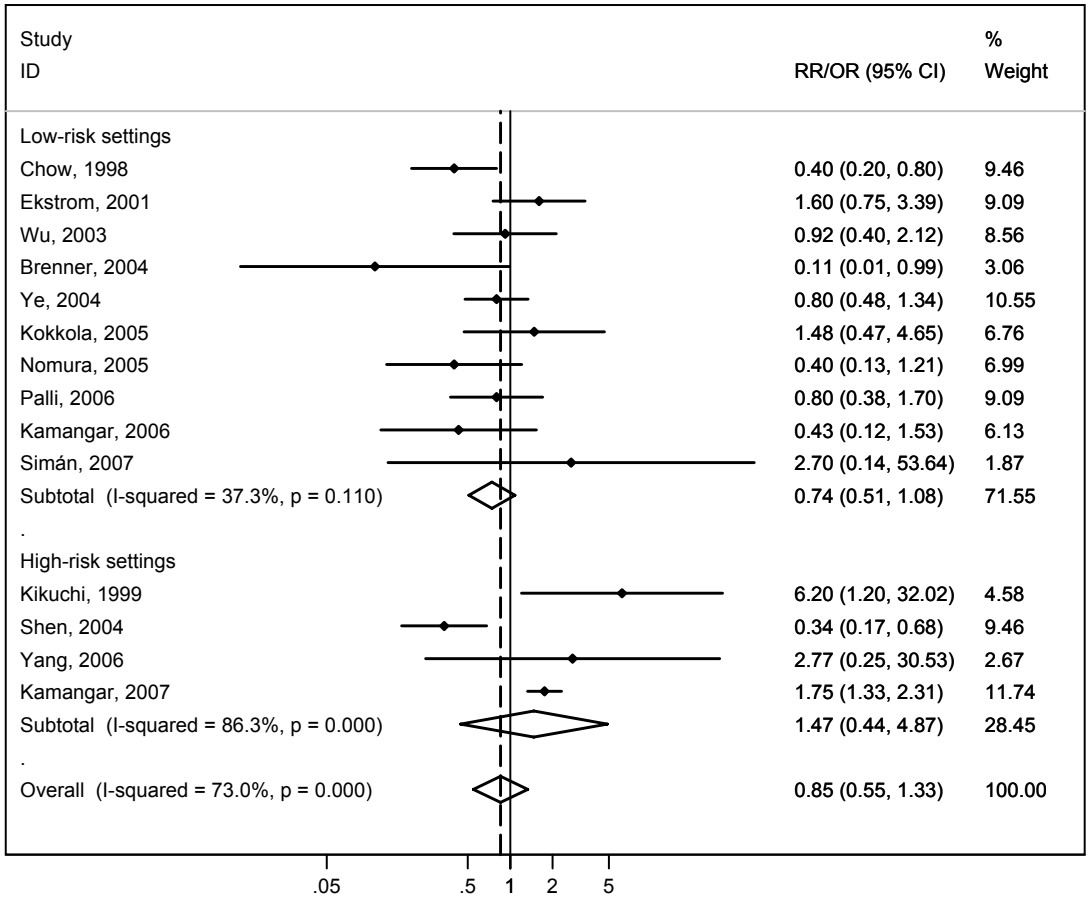


Figure 4. Meta-analyses of cohort, nested case-control, case-cohort, and case-control studies evaluating the association between virulent strains of *Helicobacter pylori* and gastric cardia cancer.

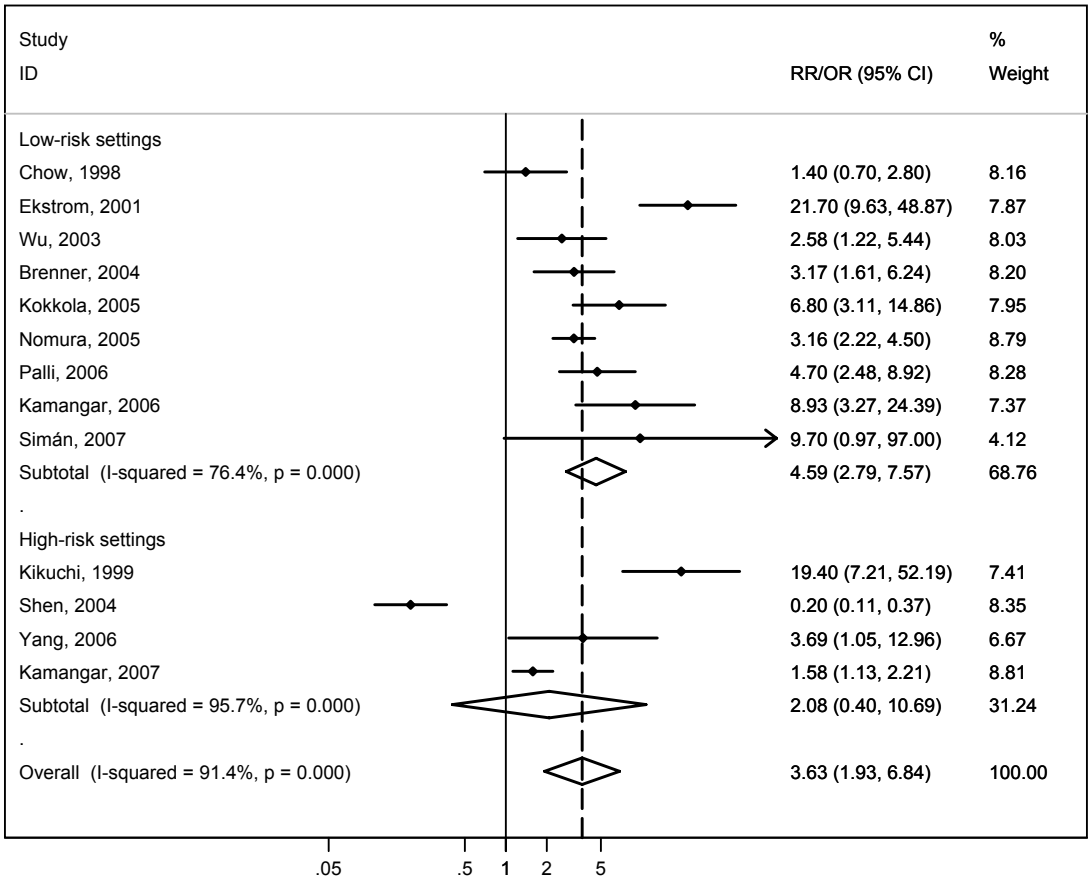
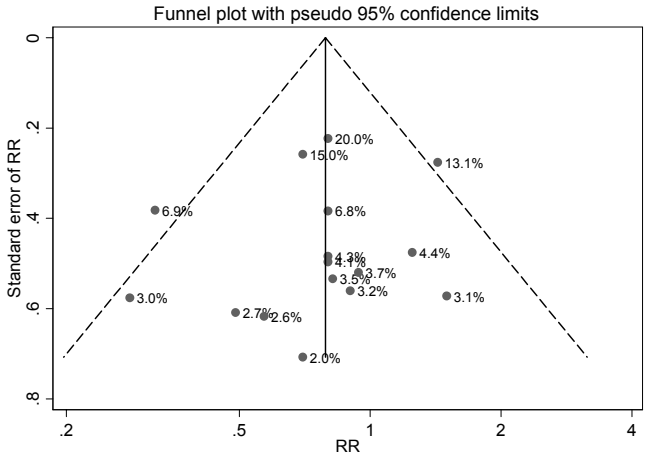
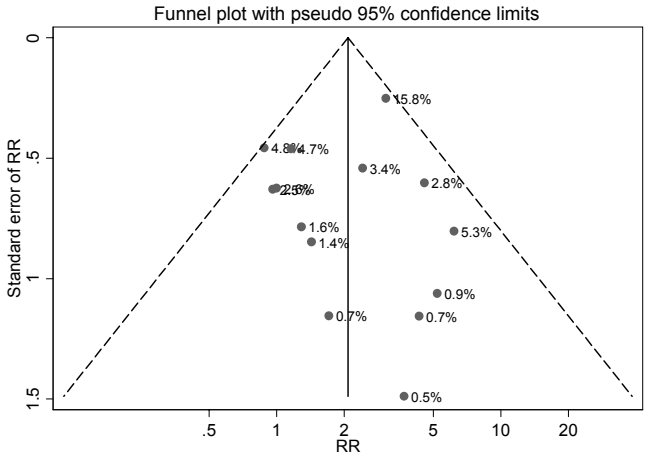
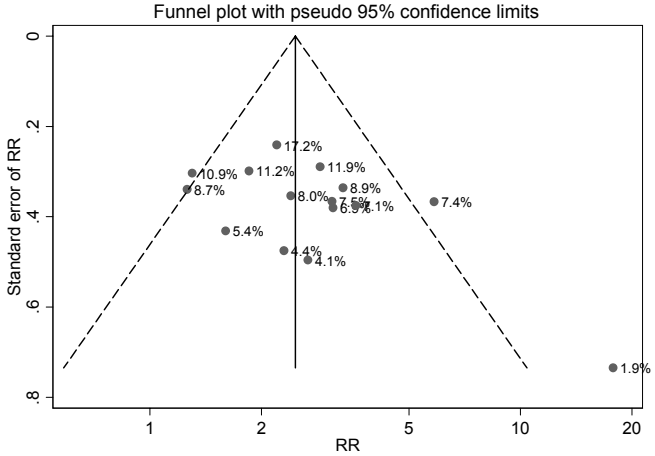


Figure 5. Meta-analyses of cohort, nested case-control, case-cohort, and case-control studies evaluating the association between virulent strains of *Helicobacter pylori* and gastric non-cardia cancer.


Funnel plot with pseudo 95% confidence limits
Standard error of RR
RR
20.0%
15.0%
13.1%
6.9%
6.8%
4.4%
3.0%
3.1%
2.0%


Funnel plot with pseudo 95% confidence limits
Standard error of RR
RR
15.8%
3.4%
2.8%
1.6%
5.3%
1.4%
0.9%
0.7%
0.7%
0.5%


Funnel plot with pseudo 95% confidence limits
Standard error of RR
RR
17.2%
10.9%
11.2%
11.9%
8.7%
8.9%
8.0%
7.4%
5.4%
4.4%
4.1%
1.9%

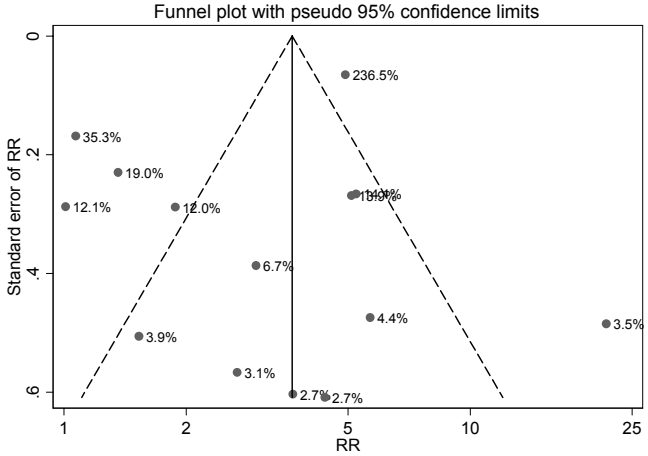

Funnel plot with pseudo 95% confidence limits
Standard error of RR
RR
236.5%
35.3%
19.0%
12.1%
12.0%
6.7%
4.4%
3.5%
3.9%
3.1%
2.7%
2.7%

Figure 6. Funnel plot of cohort, nested case-control, case-cohort, and case-control studies evaluating the association between *Helicobacter pylori* infection and gastric cardia cancer in low-risk settings (top left) and in high-risk settings (top right). Begg adjusted rank correlation test (low-risk settings: p=0.499; high-risk settings: p=0.324), Egger's regression asymmetry test (low-risk settings: p=0.498; high-risk settings: p=0.842). Funnel plot of cohort, nested case-control, case-cohort, and case-control studies evaluating the association between *Helicobacter pylori* infection and gastric non-cardia cancer in low-risk settings (bottom left) and in high-risk settings (bottom right). Begg adjusted rank correlation test (low-risk settings: p=0.198; high-risk settings: p=0.661), Egger's regression asymmetry test (low-risk settings: p=0.082; high-risk settings: p=0.288).

Is cardia cancer etiologically different from distal stomach cancer?

ABSTRACT

Objective: The coexistence of two etiologically distinct types of cardia cancer, with distinctive histological characteristics of the neoplastic and non-neoplastic gastric mucosa may explain the heterogeneous evidence on its association with *H. pylori* infection. We compared gastric cardia and non-cardia cancers regarding the frequency of *H. pylori* infection, the histological characteristics of the non-neoplastic gastric mucosa, and the tumor histological type.

Methods: We evaluated 41 cardia and 229 non-cardia cancer cases undergoing gastrectomy, and 270 community controls. *H. pylori* infection and CagA infection status were assessed by ELISA and Western Blot, respectively. Histological characteristics of the neoplastic and non-neoplastic mucosa were obtained from pathology reports. The association between infection and cancers with different location was quantified in a case-control analysis and cardia and non-cardia cancers were further compared.

Results: No positive relation was found for *H. pylori* infection, but CagA-positive strains were associated with an increased risk of non-cardia cancer (OR=1.97, 95%CI: 1.35-2.87). Thirty-one (75.6%) cardia cancer cases, predominantly of the intestinal type (67.7%), had non-neoplastic atrophic mucosa, as well as 173 (75.6%) non-cardia cancers (54.3% of the intestinal type). Among cases occurring in non-atrophic patients, the proportion of cancers of the Laurén's intestinal type was 70.0% for cardia and 39.3% for non-cardia gastric cancers.

Conclusions: Cardia and non-cardia cancers were similar regarding the relation with infection, histological type and condition of the non-neoplastic mucosa, supporting the predominance of cardia cancers determined by *H. pylori* infection in this Western high-risk setting.

INTRODUCTION

The discovery of *Helicobacter pylori* [1], and its recognition as a human carcinogen [2] proved to be a key point in the understanding of the gastric cancer etiology. Both cohort and case-control studies show an average three-fold increased risk of distal gastric cancer among the infected, but apparently no positive relation between *H. pylori* infection and cardia cancer. The evidence supporting this lack of association, however, is based on contradictory findings [3, 4, 5]. Studies from Western countries support a negative association between *H. pylori* infection and cardia cancer [6, 7, 8, 9, 10, 11, 12], whereas there is a trend towards positive association in studies from Eastern Asia [9, 12, 13, 14, 15].

The gastric cardia is a small anatomical region, which can be overgrown by tumors that originate from adjacent mucosal sites. Tumors described as “cardia cancers” include a mixture of neoplasms arising from the lower esophagus as well as the gastric cardia, to a higher or lesser extent depending on the frequency of esophageal and gastric adenocarcinomas on the populations under study [16]. The observation of a positive association with gastric atrophy [17, 18, 19], despite no overall relation with *H. pylori* infection, supports the coexistence of two etiologically distinct types of cardia cancer [20], one associated with *H. pylori*-induced atrophic gastritis, etiologically similar to non-cardia cancer, more frequent in populations with higher overall gastric cancer incidence; and the other arising in non-atrophic gastric mucosa, associated with acid/bile-induced damage to the distal esophagus, resembling esophageal adenocarcinoma, and likely to have a higher relative frequency in settings with low-gastric cancer risk.

The potential for misclassification of gastric cancer topography brings important challenges to the study of the cardia cancer etiology, as it is usually impossible to determine the origin of these cancers by examining them grossly or microscopically [20]. However, the hypothesis described above provides a basis for a surrogate definition of two subtypes of gastric cardia cancer, and may be useful for a proper assessment of its etiology [20].

This report comprises a case-control study, in which cardia and non-cardia cancers are compared with community controls regarding the frequency of infection with *H. pylori*, taking into account the virulence of the infecting strains, and a case-case analysis for the comparison of cardia and non-cardia cancers regarding the histological characteristics of the neoplastic and non-neoplastic mucosas. The latter comparison is stratified according to the infection status of the cases, aiming to provide evidence on

the frequency of etiologically distinct cases of cardia cancer, either resembling distal stomach cancer or esophageal adenocarcinoma.

METHODS

Cases

From 2001 to 2006, we identified 709 incident cases of gastric adenocarcinoma with no previous cancer diagnosis (except for skin non-melanoma) and not having performed a subtotal gastrectomy for benign conditions. As previously described [21, 22], patients were admitted to the surgery wards of the two largest public hospitals providing care to cancer patients in the North of the country, Hospital de S. João and Instituto Português de Oncologia Francisco Gentil, both in Porto. Cancer diagnosis was based on gastrectomy specimens, endoscopic biopsy material or the evaluation of metastasis.

A blood sample was drawn from 477 cases and serum was kept frozen at -20ºC. In addition, 207 cases were excluded because no gastrectomy specimen was available, precluding the proper assessment of tumor histological type and characterization of gastric lesions in the non-neoplastic mucosa. The remaining 270 cases were not significantly different from those excluded, regarding gender (58.9% *vs.* 60.5% males, p=0.682), age (median: 66 *vs.* 65 years, p=0.542) or education (median number of school years: 4 *vs.* 4 years, p=0.426).

Controls

Controls were part of a representative sample of the adult population of Porto [23, 24]. Participants were recruited by random digit dialing using households as the sampling frame, followed by simple random sampling to select one eligible person among permanent residents in each household that was invited to visit our department for interview and blood collection. For each of the 270 gastric cancer cases, we selected age and gender frequency-matched controls.

Assessment of *H. pylori* infection status

H. pylori infection status was assessed using an anti-*H. pylori* IgG antibody Enzyme Linked Immunoassay (ELISA) (Anti-*Helicobacter pylori* ELISA, EuroImmun®, Lubeck, Germany) on serum samples. Participants were classified according to manufacturer instructions as negative if they had less than 16 RU/ml, as borderline if their antibody concentration was between 16 and 22 RU/ml and as positive if the antibody level was equal or greater than 22 RU/ml. For analysis, subjects having borderline results were considered infected.

Further testing of *H. pylori* infection status was performed by Western Blot (Helico Blot 2.1, Genelabs Diagnostics®, Singapore). The assay was conducted as proposed by the manufacturer, and the results were interpreted following the recommended criteria for CagA seropositivity: presence of the 116kD band (CagA) with one or more of the following bands: 89kD (VacA); 37kD; 35kD; 30kD (UreA) and 19.5kD together.

Assessment of the histological characteristics of the neoplastic and non-neoplastic mucosas of cases

For each case, the anatomic site was classified following image or pathology descriptions. Cardia cancer was defined as a tumor in the cardioesophageal junction, esophagogastric junction or gastroesophageal junction [16]. Information on the tumor's histological type, according to Lauren´s criteria (intestinal, diffuse, or mixed) [25], was obtained for cases submitted to gastrectomy. The presence of gastric lesions in the non-neoplastic mucosa was registered in the surgical specimen pathology reports. In this study, we considered that atrophy was present in individuals having histological evidence of chronic atrophic gastritis regardless of the presence of other gastric lesions.

Statistical analysis

Odds Ratios (OR) and the corresponding 95% confidence intervals (95%CI) were computed by multinomial logistic regression to quantify the association of between *H. pylori* infection and gastric cardia and non-cardia cancers and unconditional logistic regression to compare cardia and non-cardia cancer cases. All analyses were conducted using STATA®, version 9.2.

Ethics

The ethics committees of the involved hospitals approved the study, and all participants provided written informed consent.

RESULTS

Case-control study

No positive association was observed between *H. pylori* infection and gastric cancer, neither for cardia (OR=1.72, 95%CI: 0.50-5.90) or non-cardia cancers (OR=0.93, 95%CI: 0.55-1.58). Infection with CagA-positive strains was associated with increased risk of non-cardia cancer (OR=1.97, 95%CI: 1.35-2.87) but not with cardia cancer (OR=1.69, 95%CI: 0.84-3.42) (Table 1).

Case-case analysis

The frequency of infection with CagA-positive strains was similar in cardia and non-cardia cancers (age- and gender-adjusted OR=0.84, 95%CI: 0.40-1.73).

Approximately three quarters of the cases classified as *H. pylori*-infected had non-neoplastic atrophic mucosa. Nearly half of the cardia and non-cardia cancers were of the intestinal histological type, regardless of the characteristics of the non-neoplastic mucosa (Table 2). Similar results were observed among subjects infected with CagA-positive strains (Table 3).

Among cases classified as *H. pylori*-negative, a similar proportion of both cardia and non-cardia cancers had non-neoplastic atrophic mucosa. All cardia and 30.0% of non-cardia cancers were of the Laurén's intestinal type for cases occurring in non-atrophic patients. The same trend was found for cases presenting non-neoplastic atrophic mucosa (Table 2). Similar results were observed among subjects not *H. pylori*-infected or infected with CagA-negative strains (Table 3).

DISCUSSION

The infection with *H*. *pylori* CagA-positive strains was associated with an increased risk of both cardia and non-cardia cancers. Three-quarters of the gastric cardia cancer cases observed in this high-gastric cancer risk setting had non-neoplastic atrophic mucosa, and most of them were of the intestinal histological type, similarly to what was observed for non-cardia cancers. Among cases occurring in non-infected patients, cardia cancers were predominantly of the intestinal type whereas only one third of non-cardia cancers presented with Laurén's intestinal type, regardless of the characteristics of the non-neoplastic mucosa.

As in other high-risk settings, we found no association between *H. pylori* infection and gastric cancer. The high prevalence of infection observed in the general Portuguese population contributes to weak associations between infection and gastric cancer, namely at distal locations [4, 26]. In the general population the prevalence of infection increases with age [27] and infection tends to persist during adulthood, while cancer cases are more likely to have been infected early in life and to lack evidence of infection as cancer progresses [28]. In high-prevalence settings, the difference between cancer cases and controls regarding the proportion of subjects infected later in life tends to be clearer and the potential for underestimation of the association between infection and cancer in case-control designs is even higher [26]. In our study, however, infection with CagA-positive strains was associated with an increased risk of gastric cancer, in accordance with previous studies reporting that CagA antibodies reflect past infection more accurately than surface antibodies detected by conventional IgG ELISA [29].

The positive association between infection and gastric cardia cancer observed in our study, although divergent from the mainstream opinion that there is no such relation [4, 5, 30], is consistent with the results obtained in samples from populations with high-risk for gastric cancer and prevalence of infection [31], and probably reflects the predominance of the gastric cardia cancer subtype arising from *H pylori*-induced atrophic gastritis. These tumors are expected to be similar to the non-cardia cancers regarding the relation with infection and histological type, which is also supported by our results. Among *H. pylori*-infected cases, a 1:1 ratio of intestinal and diffuse/mixed histological subtypes was observed for both cardia and non-cardia cancers. However, unlike non-cardia cancers, the cardia cases occurring in non-infected patients had a much higher proportion of intestinal versus diffuse/mixed histological subtype. This

predominant intestinal histological subtype is similar to that reported in esophageal adenocarcinoma [32] and supports an etiological resemblance with the latter.

The exclusion of cases not submitted to gastrectomy, as all histological characteristics were assessed in surgical specimens, precludes the generalization of our results to all gastric cancer cases. Since diffuse cancers have a worse prognosis than those of the Laurén's intestinal type [33, 34], this selection bias may have contributed to an oversampling of the latter, among which the presence of an atrophic non-neoplastic mucosa is more likely [35].

The interpretation of these results, although limited by the small sample size is driven by a sound and well documented hypothesis. Our findings support the existence of two etiologically different types of gastric cardia cancer, one of which is positively associated with *H. pylori* infection and therefore more frequent in high-risk settings. These results contribute to explain the heterogeneous results across high and low gastric cancer risk settings regarding the association with *H. pylori* infection.

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Table 1. Association between *H. pylori* infection and CagA strains, and cardia and non-cardia cancers (case-control study).

	Controls	Cardia		Non-cardia	
	n (%)	n (%)	Adjusted * OR (95%CI)	n (%)	Adjusted * OR (95%CI)
***H. pylori* infection status**					
Negative	33 (12.2)	3 (7.3)	1	30 (13.1)	1
Positive	237 (87.8)	38 (92.7)	1.72 (0.50-5.90)	199 (86.9)	0.93 (0.55-1.58)
CagA infection status					
Negative	117 (43.3)	13 (31.7)	1	64 (28.0)	1
Positive	153 (56.7)	28 (68.3)	1.69 (0.84-3.42)	165 (72.0)	1.97 (1.35-2.87)

* age- (categorical: 18-39, 40-54, 55-69, ≥70 years) and gender-adjusted.

Table 2. Comparison between gastric cardia and non-cardia cancers regarding the histological characteristics of the non-neoplastic gastric mucosa and the tumor histological type, according to *Helicobacter pylori* infection status assessed by ELISA (case-case analysis).

	Cardia	Non-cardia	Cardia *vs.* Non-cardia
	n (%)	n (%)	Adjusted * OR (95%CI)
***H. pylori* infected**			
Non-neoplastic gastric mucosa			
Non-atrophic	13 (34.2)	58 (29.2)	1
Atrophic	25 (65.8)	141 (70.8)	0.70 (0.33-1.51)
Tumor histological type			
Cases with non-atrophic non-neoplastic mucosa			
Intestinal	9 (69.2)	29 (50.0)	1
Diffuse/mixed	4 (30.8)	29 (50.0)	0.50 (0.12-2.02)
Cases with atrophic non-neoplastic mucosa			
Intestinal	16 (64.0)	75 (53.2)	1
Diffuse/mixed	9 (36.0)	66 (46.8)	0.70 (0.29-1.71)
Not infected with *H. pylori*			
Non-neoplastic gastric mucosa			
Non-atrophic	1 (33.3)	11 (36.7)	1
Atrophic	2 (66.7)	19 (63.3)	0.90 (0.06-14.40)
Tumor histological type			
Cases with non-atrophic non-neoplastic mucosa			
Intestinal	1 (100.0)	3 (27.3)	1
Diffuse/mixed	0 (0.0)	8 (72.7)	-
Cases with atrophic non-neoplastic mucosa			
Intestinal	2 (100.0)	9 (47.4)	1
Diffuse/mixed	0 (0.0)	10 (52.6)	-

* age- (categorical: 18-39, 40-54, 55-69, ≥70 years) and gender-adjusted.

Table 3. Comparison between gastric cardia and non-cardia cancers regarding the histological characteristics of the non-neoplastic gastric mucosa and the tumor histological type, according to *Helicobacter pylori* infection with CagA-positive strains assessed by Western Blot (case-case analysis).

	Cardia n (%)	Non-cardia n (%)	Cardia *vs.* Non-cardia Adjusted * OR (95%CI)
Infected with CagA positive strains			
Non-neoplastic gastric mucosa			
Non-atrophic	8 (28.6)	56 (33.9)	1
Atrophic	20 (71.4)	109 (66.1)	1.30 (0.52-3.27)
Tumor histological type			
Cases with non-atrophic non-neoplastic mucosa			
Intestinal	4 (50.0)	27 (48.2)	1
Diffuse/mixed	4 (50.0)	29 (51.8)	0.89 (0.18-4.39)
Cases with atrophic non-neoplastic mucosa			
Intestinal	12 (60.0)	57 (52.3)	1
Diffuse/mixed	8 (40.0)	52 (47.7)	0.77 (0.28-2.06)
Not infected or infected with CagA negative strains			
Non-neoplastic gastric mucosa			
Non-atrophic	6 (46.2)	13 (20.3)	1
Atrophic	7 (53.8)	51 (79.7)	0.29 (0.08-1.08)
Tumor histological type			
Cases with non-atrophic non-neoplastic mucosa			
Intestinal	6 (100.0)	5 (38.5)	1
Diffuse/mixed	0 (0.0)	8 (61.5)	-
Cases with atrophic non-neoplastic mucosa			
Intestinal	6 (85.7)	27 (52.9)	1
Diffuse/mixed	1 (14.3)	24 (47.1)	0.15 (0.01-1.58)

* age- (categorical: 18-39, 40-54, 55-69, ≥70 years) and gender-adjusted.

4. General discussion and conclusions

Epidemiological studies conducted so far concluded that *H. pylori* infection is an important risk factor for gastric non-cardia adenocarcinoma but results are not consistent for gastric cardia adenocarcinoma. The latter has been suggested to depend to a large extent on the geographical location of the studies (26). In Western countries, with low gastric cancer incidence and low prevalence of infection (125), the association appears to be null or even negative (125-127), while in Eastern countries, with high gastric cancer incidence and high prevalence of infection (125), a positive association has been observed (126-128).

In 2007, Hansen *et al*. (109) proposed two aetiologies for gastric cardia cancer: one was associated with *H. pylori*-induced atrophic gastritis, and the other associated with non-atrophic gastric mucosa, resembling oesophageal adenocarcinomas. In low-risk populations, seems that the majority of cardia adenocarcinomas share with oesophageal adenocarcinomas the risk factors. Therefore, in low-risk settings the gastroesophageal reflux disease, Barrett's oesophagus, hiatus hernia, tobacco consumption, and dietary habits may have a greater effect on cardia cancer risk than *H. pylori* infection. Furthermore, in countries with a low incidence of gastric cancer, the prevalence of infection is decreasing over the years (129) but Barrett's oesophagus and gastroesophageal reflux disease are frequent (24). In high-risk settings, the prevalence of infection is high since very young ages (80), as well as the frequency of precancerous lesions – atrophic gastritis (80, 130-133). It is speculated that this type of cardia cancer arises when the gastric phenotype is predominantly characterized by atrophic gastritis induced by *H. pylori* infection (109). Therefore, in high-risk settings, the cardia cancer seems to share the same major risk factor with non-cardia cancer, which is *H. pylori* infection.

The meta-analysis included in this dissertation is based in the most comprehensive systematic review on this topic and further expands previous observations. It demonstrates that in high gastric cancer incidence settings the risk of cardia cancer is increased by *H. pylori* infection, but also shows that the magnitude of the association is similar to the observed for non-cardia cancer in the same settings. These results support to the hypothesis of a heterogeneous proportional distribution of two different etiological types of cardia cancer across countries with high and low overall frequency of gastric cancer.

The results from the case-control study conducted in Portugal are consistent with the conclusions from the meta-analysis, and provide the first estimate of the

association between infection and cardia cancer in this setting, and one of the few available from European countries with high prevalence of infection and gastric cancer incidence. Furthermore, it provides complementary information for the understanding of the relation between infection and cardia cancer showing that cardia and non-cardia cancers are similar regarding characteristics that may distinguish etiologically different subtypes of cardia cancer. Unfortunately, no information could be obtained on gastroesophageal reflux symptoms in the cases.

In conclusion, the comprehensive assessment of the association between *H. pylori* infection and gastric cardia cancer provides quantitative evidence of a positive relation in high gastric cancer risk populations. The results obtained in the Portuguese study showed that cardia and non-cardia cancers were similar regarding the relation with infection, histological type and condition of the non-neoplastic gastric mucosa, according to the hypothesis of a predominance of cardia cancers determined by *H. pylori* infection in this Western high-risk setting. Overall, our findings support the existence of two etiologically different types of gastric cardia cancer, one of which is positively associated with *H. pylori* infection and more frequent in settings with a high gastric cancer incidence.

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6. Summary

Relationship between *Helicobacter pylori* infection and gastric cardia cancer

The discovery of *H. pylori* in 1983 has proved to be pivotal in our understanding of the aetiology of gastric cancer. In 1994, the World Health Organization and the International Agency for research on Cancer consensus group stated that there was sufficient evidence to classify *H. pylori* as a Class I human carcinogen. The overall prevalence of *H. pylori* infection in middle-aged adults is estimated in 74% in developing and 58% in developed countries, and worldwide 63.4% of the gastric cancer cases are attributable to infection, corresponding to 592,000 cases.

Epidemiological studies conducted so far concluded that *H. pylori* infection is an important risk factor for gastric non-cardia adenocarcinoma but results are not consistent for gastric cardia adenocarcinoma. The latter depends to a large extent on the geographical location of the studies, with those conducted in Western countries showing predominantly a null or negative association and those from Eastern countries suggesting a positive association. Moreover, two aetiologically distinct types of cardia gastric cancer were recently proposed, one associated with *H. pylori*-induced atrophic gastritis and other occurring in non-atrophic gastric mucosa. The heterogeneous geographic distribution of these two types of cardia cancer may contribute to explain the conflicting findings between studies conducted in populations with different gastric cancer risk.

The aim of the present dissertation was to examine the relationship between infection with *H. pylori* and the occurrence of gastric cardia cancer, through the accomplishment of the following specific objectives:

1) to quantify the association between infection and gastric cardia cancer through meta-analysis, and to provide an explanation for the expected heterogeneity of results. (Paper I)

2) to compare gastric cardia and non-cardia cancers regarding the frequency of *H. pylori* infection, the histological characteristics of the non-neoplastic gastric mucosa, and the tumor histological type. (Paper II)

Paper I: *Helicobacter pylori* infection and gastric cardia cancer: a systematic review and meta-analysis

Published studies addressing the association between *H. pylori* infection and gastric cardia cancer (up to June 2009) were systematically reviewed, and extracted relative risk (RR) estimates for the association with cardia and non-cardia cancers. Summary RR estimates and 95% confidence intervals (95%CI) were computed using random-effects models. Subgroup analyses were conducted, namely according to gastric cancer risk settings.

Thirty-four articles were considered for meta-analysis. For cardia cancer, summary RR was 1.08 (95%CI: 0.83-1.40; I^2=52.8%), higher in high-risk (RR=1.98; 95%CI: 1.38-2.83; I^2=18.4%) than in low-risk settings (RR=0.78; 95%CI: 0.63-0.97; I^2=11.6%). For non-cardia cancer, RR estimates were similar in high-risk settings (RR=3.02; 95%CI: 1.92-4.74; I^2=90.7%) and low-risk settings (RR=2.56; 95%CI: 1.99-3.29; I^2=46.6%). These observations were consistent across different inclusion criteria and when accounting for the virulence of the infecting strains.

Paper II: Is cardia cancer etiologically different from distal stomach cancer?

This report comprises a case-control study and a case-case analysis. In the former, 41 cardia and 229 non-cardia cancers were compared with 270 community controls regarding the frequency of infection with *H. pylori*, taking into account the virulence of the infecting strains. Patients were admitted to the surgery wards of Hospital de S. João and Instituto Português de Oncologia Francisco Gentil, both in Porto. Controls were part of a representative sample of the adult population of Porto. A case-case analysis was performed for the comparison of cardia and non-cardia cancers regarding the histological characteristics of the neoplastic and non-neoplastic mucosas and was stratified according to the infection status of the cases, to provide evidence on the frequency of etiologically distinct cases of cardia cancer. *H. pylori* infection and CagA infection status were assessed by ELISA and Western Blot, respectively. Odds Ratios (OR) and the corresponding 95% confidence intervals (95%CI) were computed by multinomial logistic regression to quantify the association of between *H. pylori* infection and gastric cardia and non-cardia cancers and unconditional logistic regression to compare cardia and non-cardia cancer cases.

A negative association was found for *H. pylori* infection, but CagA-positive strains were associated with an increased risk of non-cardia cancer (OR=1.97, 95%CI: 1.35-2.87). Thirty-one (75.6%) cardia cancer cases, predominantly of the intestinal type (67.7%), had non-neoplastic atrophic mucosa, as well as 173 (75.6%) non-cardia cancers (54.3% of the intestinal type). Among cases occurring in non-atrophic patients, the proportion of cancers of the Laurén's intestinal type was 70.0% for cardia and 39.3% for non-cardia gastric cancers.

Conclusions

The comprehensive assessment of the association between *H. pylori* infection and gastric cardia cancer provides quantitative evidence of a positive relation in high gastric cancer risk populations. The results obtained in the Portuguese study showed that cardia and non-cardia cancers were similar regarding the relation with infection, histological type and condition of the non-neoplastic gastric mucosa, according to the hypothesis of a predominance of cardia cancers determined by *H. pylori* infection in this Western high-risk setting.

Overall, our findings support the existence of two etiologically different types of gastric cardia cancer, one of which is positively associated with *H. pylori* infection and more frequent in settings with a high gastric cancer incidence.

7. Sumário

Relação entre infecção por *Helicobacter pylori* e cancro gástrico do cardia

A descoberta da *H. pylori* em 1983 revelou-se fundamental para a compreensão da etiologia do cancro gástrico. Em 1994, a Organização Mundial de Saúde e a Agência Internacional para investigação em Cancro reconheceram existir evidência suficiente para classificar a *H. pylori* como carcinogénico de classe I. A estimativa global da prevalência de infecção por *H. pylori* em adultos de meia-idade é de 74% em países em desenvolvimento e 58% nos países desenvolvidos e, a nível mundial, 63,4% dos casos de cancro gástrico são atribuíveis à infecção, correspondendo a 592 000 casos.

Os estudos epidemiológicos realizados até ao momento concluíram que a infecção por *H. pylori* é um factor de risco importante para o adenocarcinoma gástrico não-cardia, mas não se demonstrou associação consistente com o adenocarcinoma gástrico do cardia. Contudo, a relação entre a infecção e o cancro do cardia depende em larga medida da localização geográfica dos estudos, com uma associação neutra ou negativa na maioria das investigações conduzidas em países Ocidentais enquanto nas realizadas em países Orientais é sugerida uma associação positiva. Adicionalmente, foram propostos recentemente dois tipos etiologicamente distintos de cancro gástrico do cardia, um associado a gastrite atrófica induzidas pela infecção e o outro ocorrendo na mucosa gástrica sem atrofia. A distribuição heterogénea destes tipos de cancro do cardia poderá contribuir para explicar os resultados discrepantes entre estudos realizados em populações com risco diferente de cancro gástrico.

O objective da presente dissertação foi avaliar a relação entre a infecção por *H. pylori* e a ocorrência de cancro gástrico do cardia, através da realização dos seguintes objectivos específicos:

1) quantificar a associação entre a infecção e o cancro gástrico do cardia através de meta-análise, e fornecer explicações para a esperada heterogeneidade de resultados (Artigo I).

2) comparar cancros gástricos do cardia e não-cardia relativamente à frequência da infecção por *H. pylori*, às características histológicas da mucosa gástrica não neoplásica e ao tipo histológico do tumor (Artigo II).

Artigo I: Infecção por *Helicobacter pylori* e o cancro gástrico do cardia: revisão sistemática e meta-análise

Os estudos publicados sobre a associação entre a infecção por *H. pylori* e o cancro do cardia (até Junho de 2009) foram sistematicamente revistos e as estimativas do risco relativo (RR) para a associação com o cancro do cardia e não-cardia foram extraídas. As estimativas sumárias do RR e intervalos de confiança a 95% (IC95%) foram calculados usando modelos de efeitos aleatórios. Foram realizadas análises estratificadas de acordo com a incidência de cancro gástrico nos locais em que foram conduzidos os estudos.

Trinta e quatro artigos foram considerados para a meta-análise. Para o cancro do cardia o RR sumário foi 1.08 (IC95%: 0.83-1.40; I^2=52.8%), maior nos estudos realizados em países com elevado risco de cancro gástrico (RR=1.98; IC95%: 1.38-2.83; I^2=18.4%) do que nos de baixo risco (RR=0.78; IC95%: 0.63-0.97; I^2=11.6%). Para o cancro não-cardia, as estimativas do RR foram semelhantes nos estudos de países de elevado risco (RR=3.02; IC95%: 1.92-4.74; I^2=90.7%) e nos de baixo risco (RR=2.56; IC95%: 1.99-3.29; I^2=46.6%). Estas observações foram consistentes entre os diferentes critérios de inclusão e quando considerada a virulência das estirpes.

Artigo II: O cancro do cardia é etiologicamente diferente do cancro do estômago distal?

Este artigo compreende um estudo caso-controlo e uma análise caso-caso. No primeiro, foram comparados 41 cancros gástricos do cardia e 229 cancros não-cardia com 270 controlos comunitários em relação à frequência de infecção por *H. pylori*, tendo em conta a virulência das estirpes infectantes. Os casos foram internados nos Serviços de Cirurgia do Hospital de S. João e do Instituto Português de Oncologia Francisco Gentil, ambos no Porto. Os controlos foram parte de uma amostra representativa da população adulta do Porto. A análise caso-caso, foi realizada para

comparação dos cancros do cardia e não-cardia no que respeita às características histológicas da mucosa neoplásica e não neoplásica e foi estratificada por estado de infecção nos casos, para fornecer evidência sobre a frequência de casos de cancro do cardia de tipos etiologicamente distintos. A infecção por *H. pylori* e o estado de infecção CagA foram avaliados por ELISA e Western Blot, respectivamente. Foram calculados OR e respectivos IC 95% por regressão logística multinomial para quantificar a associação entre infecção por *H. pylori* e o cancro do cardia e não-cardia e a regressão logística não condicional para comparar casos de cancro do cardia e não-cardia.

Foi observada uma associação negativa para a infecção por *H. pylori*, mas a infecção com estirpes cagA-positivas associou-se a um risco aumentado de cancro não-cardia (OR=1,97, IC95%: 1,35-2,87). Trinta e um (75,6%) dos casos de cancro do cardia, predominantemente do tipo intestinal (67,7%), tinham atrofia da mucosa não-neoplásica, bem como os 173 (75,6%) casos de cancro não-cardia (54,3% do tipo intestinal). Entre os casos que ocorrem em doentes sem atrofia da mucosa, a proporção dos cancros do tipo intestinal de Laurén's foi de 70,0% para o cardia e 39,3% para o cancro não-cardia.

Conclusões

A avaliação exaustiva da associação entre a infecção por *H. pylori* e o cancro gástrico do cardia fornece evidência quantitativa de uma relação positiva nas populações com elevado risco de cancro gástrico. Os resultados obtidos no estudo conduzido em Portugal demonstraram que os cancro do cárdia e não-cárdia eram semelhantes relativamente à relação com a infecção, tipo histológico e características da mucosa gástrica não neoplásica, de acordo com a hipótese do predomínio de cancros do cárdia determinados pela infecção nesta população Ocidental com elevado risco de cancro gástrico.

Os nossos resultados apoiam a existência de dois tipos etiologicamente distintos de cancro gástrico do cardia, um dos quais positivamente associado com a infecção por *H. pylori* e mais frequente em populações com elevada incidência de cancro gástrico.