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THE EPIDEMIOLOGY OF HELICOBACTER PYLORI INFECTION IN EUROPE AND THE IMPACT OF LIFESTYLE ON ITS NATURAL EVOLUTION TOWARDS STOMACH CANCER: A SYSTEMATIC REVIEW

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PREFACE

This dissertation is an important part of my Master study in Medicine at the Ghent University. During the past two years, I have researched the epidemiology of Helicobacter pylori infection in Europe. Furthermore, I have studied the impact of lifestyle factors in association with this bacterium because this could be an important cost-effective manner to prevent stomach cancer.

It was an interesting learning experience to write this systematic review. It required a lot of energy, but I gained a better critical attitude towards scientific literature.

First of all, I would like to thank my promotor Professor Koen Van Herck and my co-promotor Professor Inge Huybrechts for their accurate guidance and their helpful feedback whenever I needed it. For being the co-reviewer, I would like to thank Doctor Lieve Vandendaele. The quality of this review improved noteworthy through this cooperation.

I want to thank my loving parents, my little brother and the rest of my fantastic family. They have given me the opportunity to study Medicine, they always believed in me and they supported me at any time. Finally, I would like to thank my loving partner Aaron Vanheede for all his patience and support. He was always there for me for whatever, whenever.

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Kimberly Venneman

TABLE OF CONTENTS

DUTCH ABSTRACT

DOELSTELLING Helicobacter pylori (H. pylori) is door het *International Agency for Research on Cancer* (IARC) erkend als een kankerverwekkende bacterie. Eens men met deze bacterie geïnfecteerd is, kan men maagkanker ontwikkelen. Dit gebeurt via verschillende tussenfases zoals de ontwikkeling van een chronische atrofische gastritis. Dit proces richting maagkanker is multifactorieel. Verschillende factoren zoals het immuunsysteem van de gastheer, de kiem van de bacterie en de omgeving spelen een rol. Het is onvoldoende gekend hoeveel geïnfecteerde Europeanen er zijn. Nochtans is het overlevingscijfer na de diagnose van maagkanker in deze regio uitermate slecht. Het kan hierom belangrijk zijn om epidemiologische gegevens over deze infectie te verzamelen zodat een toekomstig risico op maagkanker kan ingeschat worden. Door het gebrek aan deze kennis, werd de prevalentie van de H. pylori infectie geanalyseerd bij Europese asymptomatische gezonde volwassenen. Op basis van deze informatie kunnen preventieve maatregelen uitgewerkt worden, wat voornamelijk van belang zal zijn in de gebieden waar het meest aantal individuen geïnfecteerd zijn. Het benadrukken van een gezonde levensstijl zou een kosteneffectieve maatregel kunnen zijn om H. pylori geassocieerde maagkanker te vermijden. Het mogelijke voordeel van leefstijlfactoren na een infectie met H. pylori werd hierom ook systematisch onderzocht in deze masterproef.

METHODOLOGIE Een systematisch literatuuronderzoek werd uitgevoerd. Epidemiologische studies werden geïncludeerd indien onderzoek gedaan werd naar de infectie prevalentie van een gezonde volwassen populatie binnen de Europese Unie. Globale studies over de invloed van leefstijlfactoren in de ontwikkeling van maagkanker na infectie met H. pylori werden eveneens geïncludeerd. De databases *PubMed* en *Web of Science* werden gebruikt. De screening op basis van abstract werd uitgevoerd door twee onafhankelijke onderzoekers. Nadat een overeenkomst bereikt werd, werden de overige artikels verder gescreend. De artikels werden alsook gecontroleerd op methodologische kwaliteit.

RESULTATEN Onafgezien van de tijdsperiode werden de laagste prevalenties van H. pylori infectie gevonden in Noord – Europa. De meest recente epidemiologische gegevens omtrent H. pylori infectie toonden aan dat er een opmerkelijk hoge graad van infectie is in bepaalde regio's van Europa. Deze prevalenties lopen op tot meer dan 84% in Polen. Indien de bacterie het CagA-domein heeft, dan is dit meer kankerverwekkend. Uit de studies die de graad van infectie met dit

subtype bijkomend bestudeerden, bleek dat minstens een derde van de H. pylori – geïnfecteerde mensen met deze specifieke stam geïnfecteerd was. Vrijwel alle studies toonden aan dat de infectie met H. pylori stijgt met ouderdom. Bijna even veel mannen als vrouwen waren geïnfecteerd en immigratie zou een belangrijke invloed kunnen hebben.
Nadat men geïnfecteerd is, kunnen verschillende leefstijlfactoren nog steeds een belangrijke impact hebben wat betreft de ontwikkeling van H. pylori geassocieerde maagkanker. Studies waarbij de impact van roken, de inname van zout en het eten van vlees onderzocht werd, konden een verhoogd risico aantonen. Andere studies toonden dan weer een verminderd risico aan wanneer een gemiddeld hoeveelheid aan fruit en groenten of een grote hoeveelheid aan vitamines gegeten werd. Hoewel significante risico's voor maagkanker aangetoond werden, waren de studies niet eensgezind over een significante associatie tussen deze leefstijlfactoren en H. pylori. Wat betreft alcohol en fysieke activiteit, werd geen enkele invloed gevonden.

CONCLUSIES Hoewel er sprake is van een dalende tendens wat de prevalentie van H. pylori infectie betreft, tonen recente studies aan dat de graad van infectie nog hoog is in bepaalde regio's van Europa. Dit kan een waarschuwing zijn voor nog vele toekomstige vermijdbare sterfgevallen door H. pylori geassocieerde maagkanker. Verdere studies zijn absoluut noodzakelijk om van elke regio meer recente informatie te hebben over hun graad van infectie. Hierbij hoort men ook de specifieke kiemen per regio te kennen. Zo kan in Europa de problematiek van maagkanker in de toekomst beter ingeschat worden. Bovendien is het ook nodig om de hoog-risico groepen te identificeren, aangezien dit de groepen zijn die het meeste baat hebben bij gerichte interventies. De bevindingen suggereren dat leefstijlfactoren een impact kunnen hebben in het proces naar maagkanker bij reeds geïnfecteerde personen. Leefstijlfactoren kunnen gecorrigeerd worden, wat de progressie naar de kanker kan vertragen. Meer onderzoek hiernaar is daarom absoluut nodig omdat dit een alternatieve kosteneffectieve manier kan zijn om H. pylori geassocieerde maagkanker tegen te gaan in Europa. Hiervoor is meer onderzoek binnen een Europese populatie nodig vooraleer kosteneffectieve preventieve programma's of strategieën uitgewerkt kunnen worden.

ABSTRACT

OBJECTIVE The bacterium Helicobacter pylori (H. pylori) is a recognized carcinogen as classified by the International Agency for Research on Cancer (IARC). Once an individual is infected, this bacterium may lead to the development of gastric cancer via the intermediate stage of chronic atrophic gastritis. It is a multifactorial process depending on the host immune system, the specific strain of the bacterium and the multiple environmental factors. Little is known on the H. pylori infection rate in Europe where the survival rate once diagnosed with gastric cancer is still very bad. Due to the lack of such important information in order to estimate the risk for developing stomach cancer in a defined population, our objective was to analyze the prevalence of H. pylori infection in the asymptomatic healthy adult population in Europe. Such information may demonstrate the interest to organize more preventive efforts in high-risk regions. Focusing on particular lifestyle factors could be a cost – effective manner to prevent H. pylori - associated stomach cancer. Therefore, the potential benefit of these lifestyle factors after H. pylori infection was also systematically investigated in this dissertation.

METHODS A systematical literature search was conducted using the databases PubMed and Web of Science. Epidemiological studies were included if research was conducted into the prevalence of infection among healthy adult populations in the European Union. Worldwide studies on the impact of lifestyle factors towards the development of gastric cancer after infection with H. pylori were also included. The screening based on abstract was independently conducted by two investigators. After a consensus was achieved, the remaining articles were screened based on full text. A quality control on the methodology was carried out thereafter.

RESULTS Irrespective of time, the lowest H. pylori infection rate was found in Northern Europe. Most recent data on H. pylori infection showed a significantly high infection rate in several regions in Europe, up to 84% in Poland. Studies providing additional information about the more carcinogenic CagA – strain revealed that at least a third of the infected individuals was infected herewith. Practically all studies demonstrated that the risk for H. pylori infection increased with age. Almost as many men as women were infected. Moreover, an important impact of immigration is suggested. After being infected with H. pylori, certain lifestyle factors might have a significant impact towards the development of the bacterium - associated gastric cancer. Studies on smoking, salt consumption and the consumption of meat could demonstrate increased risks

among the H. pylori infected individuals. Others studies could demonstrate decreased risks among infected individuals consuming median intakes of fruit and vegetables or consuming high intakes of (vitamin-)antioxidants. However, across all studies, the trends for interaction were conflicting to prove association - hypotheses. Furthermore, no association could be found for the remaining two lifestyle factors studied, namely alcohol consumption and physical activity.

CONCLUSIONS Despite a declining trend, recent data show that the H. pylori infection rate is still high in several regions in Europe. This could be a predictor for many preventable future deaths due to the H. pylori - related stomach cancer. Further studies are absolutely needed in order to have more recent data on the infection rates and the specific strains present by region for predicting the future burden of stomach cancer. Moreover, it is necessary to identify the high-risk subpopulations who could possibly benefit most from interventions. These findings suggest that even among infected individuals, some correctable lifestyle factors may inhibit the progression towards stomach cancer. The focus on correctable lifestyle factors after being infected could be an important alternative cost-effective manner to prevent H. pylori – associated stomach cancer in Europe. Therefore, more research concerning an European population is needed to explore the possibilities for preventive strategies.

ABBREVIATIONS

H. pylori	Helicobacter pylori
IARC	International Agency for Research on Cancer
EU	European Union
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
CagA	Cytotoxin - associated gene A
VacA	Vacuolating cytotoxin A
pH	Power of hydrogen
OR	Odds ratio
CI	Confidence Interval

INTRODUCTION

In 2008, 2 million cases of the 12.7 million new cancer cases were attributable to infections, especially caused by hepatitis B viruses, hepatitis C viruses, human papillomaviruses and Helicobacter pylori (H. pylori). [1] In the latest updates, the International Agency for Research on Cancer (IARC) estimated that 6.2% of all cancers were attributable to the gram-negative bacterium H. pylori. [2]

Chronic infection with H. pylori is carcinogenic to humans, classified by the IARC. The bacterium is a recognized cause of gastric carcinoma. [3] Stomach cancer itself is the fifth most common malignancy in the world. It is the third leading cause of cancer death in both sexes worldwide, but twice as many men than women are being diagnosed. There is a significant international variation in its occurrence with more than 70% of cases occurring in developing countries. [4] A decline in the world's incidences and mortality rates of stomach cancer has been observed but it is compensated by growth and aging of the population. Consequently, the burden of this multifactorial disease is still highly present. [5]

The routes of transmission of H. pylori still remain unclear. An interhuman transmission seems to be the main route. [6-8] H. pylori colonization in the stomach which usually occurs in childhood, leads to chronic atrophic gastritis and to intestinal metaplasia. These are the important precursor lesions towards the development of gastric cancer. The carcinogenic effect of H. pylori infection is dependent on several factors such as the production of toxic cytokines by specific strains, the immune response of the host and some environmental factors including life style factors. [9]

Survival for stomach cancer is not good, even not in Asiatic countries such as Japan where active screening is applied. In Western Europe, the survival rate would be about only 27%. [10] Despite the important morbidity and mortality of gastric cancer, only a few countries have established control efforts. Screening and eradication programs are mainly implemented in Asia. As a result, the detection rate of early stomach cancer is much higher in Japan which is important because of the much better 5-year survival rate over there. Mass-eradication of H. pylori, which is a population screen-and-treat strategy based on antibiotics and proton pump inhibitors, could reduce the gastric cancer incidence and could be cost-effective. However, a precise estimation of overall benefits and adverse consequences such as antibiotic resistance remains difficult, especially in lower risk regions. Furthermore, uncertainties remain about the effectiveness and

about the generalizability of the results. [11] However, recommendations emphasize the need for considering H. pylori eradication in high-risk areas around the world, including Europe. [12]

Two sets of guidelines addressing stomach cancer prevention strategies in Europe emphasize the need for gastric cancer risk stratification. [12, 13] International experts working at the IARC emphasize the acute need to manage more public health resources in order to control gastric cancer. Collecting information on H. pylori infection prevalences will be useful for predicting the future burden of stomach cancer and other related H. pylori - associated diseases. It might help to identify the subpopulations that could benefit most from interventions. [11] [14]

There is an enormous amount of studies since the discovery of the bacterium by Robin Warren and Barry Marshall. This discovery resulted in the awarding of the 2005 Nobel Prize in Physiology or Medicine. Nevertheless, there is a lack of thorough understanding about the epidemiology in healthy symptomless European populations which will be systematically investigated in this medical master thesis. The overall prevalence of H. pylori infection in Europe is estimated to be 20 – 40% depending on region and increasing with age. [15] Infection rates are linked to socio-economic factors such as hygienic standards and educational levels which have improved in Europe since the 1950's. This could have influenced the current infection prevalence. [16]

This epidemiological information could emphasize the interest to organize more preventive efforts in high-risk regions. Based on such information, European countries could further develop strategies against stomach cancer in a cost-effective manner. [17] One of these strategies could be based on lifestyle. In Brasil for example, there is already attention for a prevention strategy based on improving lifestyle factors (diet, tobacco use, alcohol consumption and physical activity) for all cancers. [11] Correctable lifestyle factors could have an impact on reducing the risk of getting H. pylori - associated gastric cancer. [18] Studies investigate usually the independent impact of lifestyle factors towards gastric cancer, but less is known about the impact of lifestyle in association with this bacterium. To date, no overview on this topic exists. Therefore, the impact of lifestyle factors after H. pylori infection is systematically investigated in this dissertation to provide an overview of the current knowledge. This information could be useful in the elaboration of preventive strategies. This could be an alternative cost-effective way to reduce the morbidity and mortality of stomach cancer in Europe.

In February 2014, a systematic review by Peleteiro et al. was published about the prevalence of H. pylori infection worldwide. They included 37 studies addressing the prevalence of H. pylori in twenty-two countries, including ten European countries. [19] This dissertation which is designed as a systematic review, only focuses on the epidemiology in Europe defined by the European Union (EU) using another search strategy in two databases and including regional studies. Additionally, the impact of lifestyle after infection is being investigated.

PATHOPHYSIOLOGY

The initial H. pylori colonization in the stomach usually occurs in childhood. The routes of transmission of H. pylori still remain unclear. An interhuman transmission seems to be the main route through oral-oral or oral-fecal ways. [6-8] A route via contaminated water also remains possible. [20, 21]

The H. pylori colonization can persist in the human stomach for decades, causing continual damage despite the immune response of the host. After the colonization in the stomach, it is able to survive in this acid environment by its production of enzymes such as urease to maintain a neutral pH. Before the attachment to the epithelium surface, the bacterium has to cross the thick mucus layer first. By the presence of flagella, H. pylori can stick to the mucosal surface. The bacterium moves quickly due to these flagella to the epithelium surface where the pH is higher, permitting the growth of the bacterium. All infected individuals develop inflammatory responses to H. pylori, but only a minority develop clinical signs. [22, 23]
The associated diseases such as gastric ulcer, dyspepsia, acute or chronic gastritis, gastric adenocarcinoma or primary lymphomas occur predominantly in adults. The risk of developing these disorders in the presence of H. pylori infection depends on a variety of environmental, host and bacterial factors (see Figure 1). [9]

The inflammatory response depends also on its gastric localization. Patients with H. pylori infection localized in the distal portion of the stomach can develop gastritis, which can lead to ulceration. These patients almost never develop gastric cancer in contrast to patients with corpus-predominant atrophic gastritis, which is accompanied by hypochlorhydria and hypergastrinemia. [22, 23] A schematic representation is shown in Figure 1.
The hypochlorhydria is caused by the inactivated function of the parietal cells, leading to a reduced acid secretion. This condition of reduced stomach acid becomes permanent when corpus atrophy occurs due to the loss of gastric epithelial cells. This insufficient replacement by regenerative epithelium or the replacement by an intestinal-type epithelium (intestinal metaplasia) is important in the pathogenesis. [24]

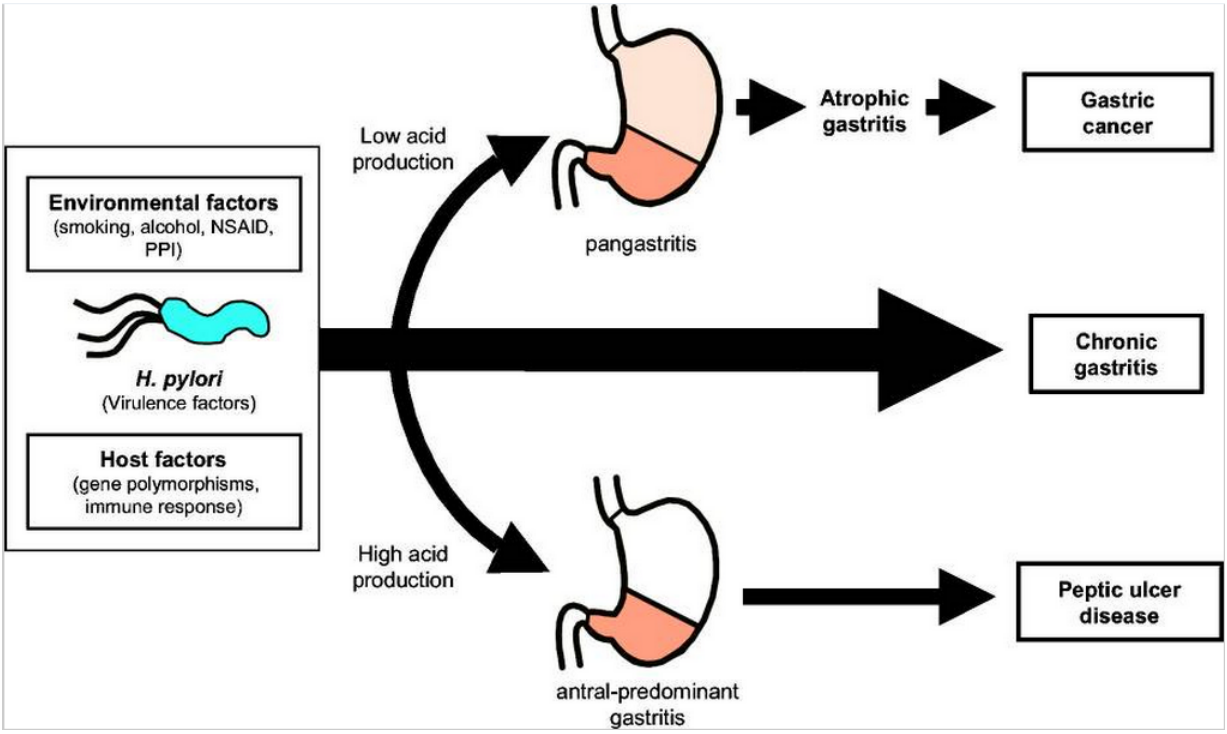

Figure 1. Schematic representation of the factors contributing to gastric pathology and disease outcome in H. pylori infection. *Reproduced with permission from Clin Microbiol Rev, 2006. 19(3): p. 449-90. Copyright © 2006, American Society for Microbiology. All rights reserved.*

Gastric carcinogenesis is a multifactorial process. As mentioned before and as shown in Figure 1, the interaction of three major factors is important: the host immune response, the virulence of the H. pylori and the environmental factors including lifestyle.

Stomach cancer can occur when gastritis is accompanied by an over-expression of COX-2 protein, by a subsequent release of prostaglandins, by an up-regulation of the oxidative stress system and by an over-expression of cytokines such as IL-1β and TNF-α. [22]

Secondly, some specific strains of the H. pylori are more virulent than others. Cytotoxin-associated antigen (CagA)-positive strains are more virulent and are associated with higher grades of inflammation compared to CagA-negative strains. [25] The CagA-positive strain and the secreted vacuolating cytotoxin (VacA)-strain interact directly with immune cells, causing more damage to the mucosa. [22] Cover et al. provided an overview of H. pylori strains correlating with gastric cancer risk (see Table 1). [26]

At last, environmental factors including lifestyle factors such as dietary high salt intakes could have a significant impact in the gastric carcinogenesis. [27]

Table 1:An overview of H. pylori strains correlating with stomach cancer risk

Gene/Region		Encoded proteins
cag PAI	cytotoxin-associated gene pathogenicity island	T4SS
cagA	cytotoxin-associated gene A	Effector protein
vacA	vacuolating cytotoxin A	Secreted toxin
babA	blood group antigen–binding adhesion	OMP
sabA	sialic acid–binding adhesin	OMP
homB	helicobacter pylori outer-membrane protein B gene	OMP
oipA (hopH)	outer inflammatory protein A	OMP
hopQ	helicobacter pylori outer membrane protein Q	OMP
dupA	duodenal ulcer promoting A	VirB4 homolog

T4SS: Type IV secretion systems; OMP: outer membrane proteins; hopH: helicobacter pylori outer membrane protein H; Vir: virulence region. *Table adapted from Cover et al., Helicobacter pylori Diversity and Gastric Cancer Risk. MBio, 2016; 7(1). Table 1, Strain-specific H. pylori features that correlate with gastric cancer risk; p.2.*

Figure 2 shows a schematic overview of the gastric carcinogenesis in the presence of H. pylori infection. The persistence of the bacterium, the associated degree of inflammation and the influence of the other abovementioned factors during longstanding infection determines the accumulation of mutations in the genome of gastric epithelial cells, leading to malignant transformations and thus leading to stomach cancer.

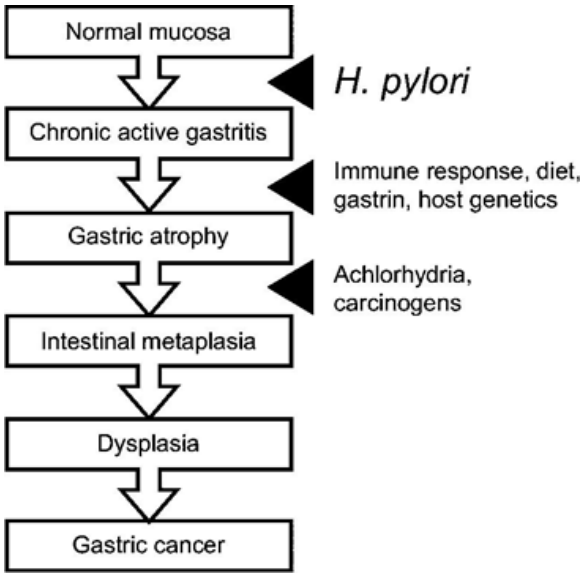

Figure 2. A schematic overview of the multifactorial process in H. pylori induced gastric carcinogenesis. *Reproduced with permission from Clin Microbiol Rev, 2006. 19(3): p. 449-90. Copyright © 2006, American Society for Microbiology. All rights reserved.*

METHODOLOGY

Search strategy

At first, a search for relevant reviews on the topic was carried out. Then, we modified our specific subject in order to have no overlap with existing literature. The search strategy used in the search engines PubMed and Web of Science, was discussed and agreed upon within the review panel in order to find all relevant literature. The appropriate keywords were found by exploring the MesH database and by exploring interesting studies or (systematic) reviews including their references. The search strategy was continuously improved by repetitive small screenings in order to find all relevant articles. In the end, the following keywords were used with the Boolean technique: helicobacter infections, helicobacter pylori, campylobacter pylori, stomach neoplasms, gastritis, tobacco use, diet, life style, risk factors, transmission, prevalence, incidence, epidemiology, seroepidemiologic studies, socioeconomic factors and Europe. Subheadings have been used to ensure that only the most relevant studies would be found and to have an achievable number of articles for evaluation. The specific implementation can be found in the attached flowchart (see Figure 3).

Selection of the studies

A systematic literature search was conducted in PubMed and Web of Science until October 2015. There was no time restriction because of the important lack of knowledge on this subject. The only preselected limit used in PubMed was "humans".

Articles on the epidemiology of H. pylori infection in healthy adults in European countries defined according to the European Union (EU); and articles on the possible impact of lifestyle factors after H. pylori infection towards the associated stomach cancer were eligible for this review. Adulthood was defined on the age of fifteen years and older. Studies were excluded according to predefined criteria which were extensively described further (see Table 2).

The design of this dissertation is a systematic review. A checklist by the Preferred Reported Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines was used to achieve a properly substantiated review. [28] The references were imported into the software program Endnote for a first screening based on title by one investigator. In case of doubt, the articles were retained for further assessment based on abstract. The screening based on abstract was conducted

independently by two investigators. This resulted in a 59.7% agreement after the first revision. The major discrepancy was observed in the selection relating to epidemiology. This issue was discussed, leading to a better clarification of the exclusion criteria, especially for the definition of symptomatic subpopulations. Hereby, the agreement improved to 70.6%. The remaining differences in the selection by abstract were searched by one researcher and these were all discussed in the review panel afterwards. After clarifying the criteria and achieving a consensus, one investigator further screened the selection based on full text. An update on new articles based on the same search strategy was investigated by one researcher from June 2015 up to March 2016 in both search engines. The entire selection process of this updated search based on title, abstract and full text was investigated only by this one reviewer.

In case a full text could not be retrieved, a request was sent twice via ResearchGate, which is an online social platform for scientists and researchers to share information. In this way, several articles could still be obtained for further screening. Relevant articles were also searched through the reference lists of interesting studies.

Because of the importance of the methodological quality of studies to prove some evidence, the tool by Fowkes et al. was used. [29] This checklist is attached in annex (see appendix 1). An appropriate study design was one of the important criteria. Concretely, this meant a cross-sectional design to measure prevalence for example. Studies should be representative, so a careful description of the study sample was also important. Furthermore, the studies were evaluated in terms of quality, completeness and corrections for distorting influences. In case of case – control studies, the control group was also evaluated by using this tool. In case a study had clearly some lacks at multiple criteria or guidelines, it was excluded. Notable issues regarding the methodology of the selected studies were described in the attached tables in order to estimate existing bias (see also in appendix 3 - 4).

In the end, fifty-two publications were included in this dissertation. The selection process is shown in further detail in the attached flowchart (see Figure 3).

Data extraction

The following data were extracted from the full texts of relevant epidemiological articles to implement in a table: study country, reference, publication year, period of data collection, description of the adult study population including number and age range, prevalence of H. pylori infection in the adult population, the method of H. pylori detection and when investigated, also the CagA – positivity.

Figure 3: Flowchart of the methodology (last update 05/03/2016)

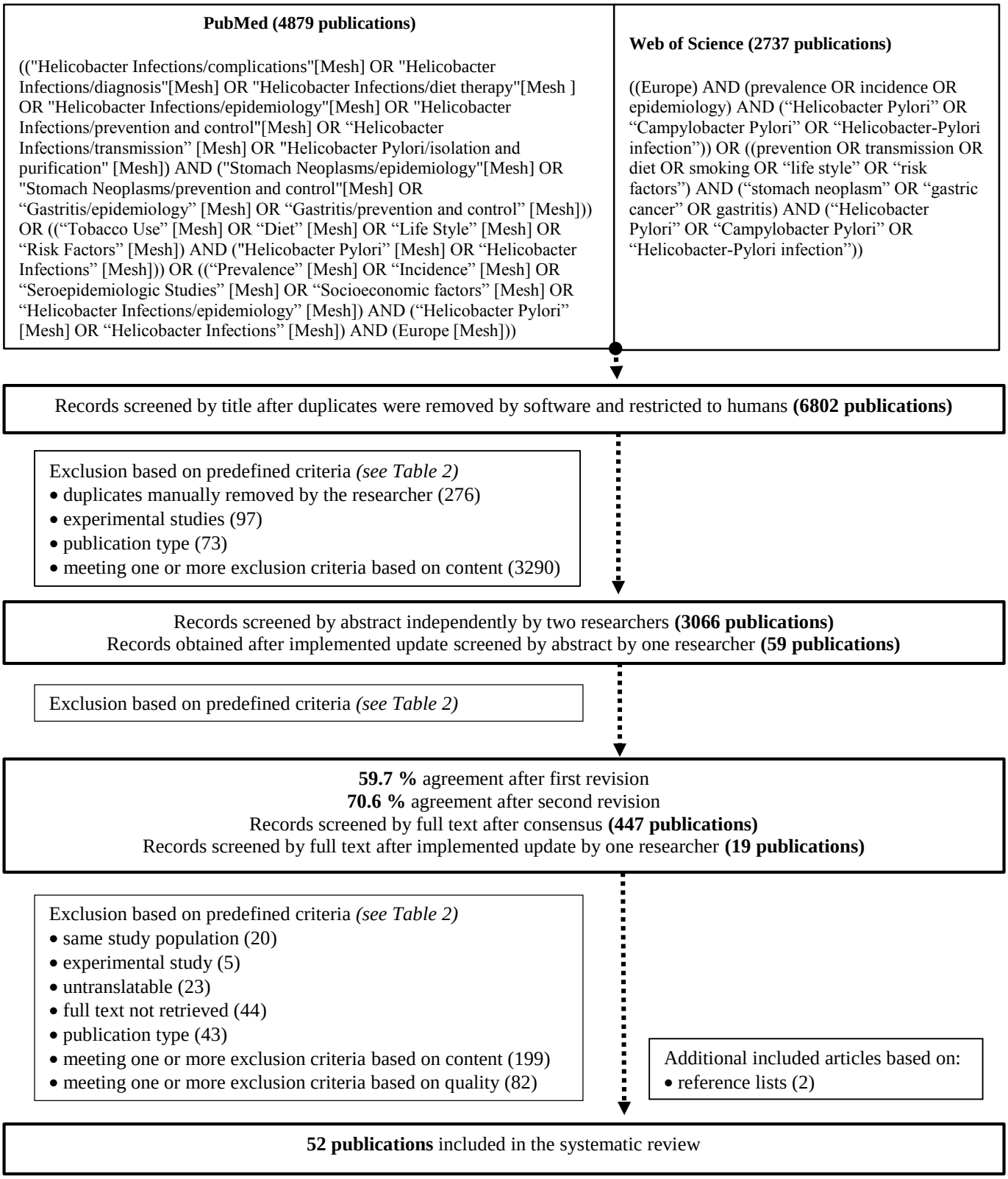

Table 2: Inclusion and exclusion criteria

Inclusion criteria

- Data relating to Helicobacter pylori infection in physically and mentally healthy adult populations (i.e. 15 years or more) in Europe defined by the European Union or articles worldwide focusing on the impact of lifestyle factors in the natural progression towards H. pylori - related stomach cancer after the infection.

  Considered as lifestyle factor: smoking, diet, alcohol consumption and physical activity

Exclusion criteria

General exclusion

- Animal or in vitro experimental studies
- Full text not retrieved
- Unable to translate (i.e. Spanish, Portuguese, Italian, Rumanian, Czech, Croatian, Polish, Finnish, Danish, Hungarian, Japanese, Korean, Chinese and Russian)
- Duplicates or epidemiological publications from the same study population
- Publication types: reviews, editorials, comments, case reports, meta-analyses, guidelines

Exclusion by content

- Data relating to epidemiological data other than population-based prevalences of Helicobacter pylori (e.g. the prevalence of gastric cancer) or relating to children (i.e. under 15 years old)
- Studies relating to the epidemiology of Helicobacter pylori in a sick or symptomatic subpopulation (e.g. ischemic heart diseases, cirrhosis, kidney failures, diabetes, dyspepsia, gastro-intestinal diseases,…) and in the population of health care workers including medical students because of the potential overestimation in a general population
- Studies only focusing on the prevalence of specific Helicobacter pylori strains among an infected population (e.g. prevalence of vacA genes in Helicobacter pylori isolated from adults in Poland)
- Studies relating to (lifestyle) factors before Helicobacter pylori infection and/or relating to person-to-person transmission including familial transmission or transmission in medical setting (e.g. by endoscopy)
- Data relating to other lifestyle factors than considered or articles relating to the considered lifestyle factors but not associated with H. pylori infection in the associated evolution from infection – gastritis – metaplasia – dysplasia – to gastric cancer (i.e. not towards peptic ulcer, not towards non-ulcer dyspepsia)
- Studies focusing on the eradication therapy of Helicobacter pylori by pharmaceutical medications (e.g. antibiotics, vaccinations); or studies relating the lifestyle topic but during or after eradication therapy because of the focus on the natural evolution (e.g. the effect of smoking on failure for eradication therapy)
- Studies explaining the mechanisms behind the impact of considered lifestyle factors in the Helicobacter pylori - associated process to stomach cancer (e.g. regulatory B cell function is suppressed by smoking and obesity in infected subjects)
- Studies only relating to histological features (e.g. the effect of smoking on gastric histology in infected subjects)

Exclusion by quality

- Data not representative for an entire adult population (e.g. only men, only the elderly, only shepherds)
- Insufficient quality based on a tool by Fowkes et al.

EPIDEMIOLOGY OF HELICOBACTER PYLORI INFECTION IN EUROPE

Detection

The detection of H. pylori can be done by using various invasive or noninvasive tests. Each test has its (dis)advantages in different settings. The noninvasive urea breath test and the invasive histology or culture biopsy tests, each having a sensitivity and specificity > 95%, are the preferred methods to be used in routine screening because of the adequate ability to detect a current infection or inflammation. However, these tests require more expertise and more money. Therefore, the use of serology having a sensitivity and specificity of 80-90%, is the detection method mainly used for large epidemiological studies. [9]

Prevalence

Data concerning the prevalence of H. pylori infection in European adults is shown in Table 3 for the following countries: Belgium, Denmark (including Greenland), Germany, Greece, Italy, Poland, Portugal, Slovenia, United Kingdom, Ireland, Croatia, Spain, Czech Republic, Estonia, Sweden, The Netherlands, Finland, Norway and Latvia.

The prevalence of H. pylori infection varies considerably according to region. In a regional randomized cross-sectional study by Thjodleifsson et al., the infection prevalence of the bacterium was much higher in Estonia (69.2%) in comparison with its prevalence in Sweden (11.2%) using the same manner of data collection in the same time period. [30]

Overall, the highest prevalence rates of H. pylori – infection could be found in Eastern Europe and Southern Europe. By contrast, the lowest prevalence rates were found in Northern Europe (see Table 3).

Table 3: The prevalence of H. pylori infection in Europe

Country	Ref. (publication year)	Period of data collection	Study population	Age range (years)	Number	Prevalence (%)	Bias / Remarks
NORTHERN EUROPE							
Denmark	Wildner - Christensen et al. (2003) [31]	⊦	Randomized selection from the population of Odense and surrounding towns (rural and urban)	40 – 64	5749	18♦	Randomization applied Infection confirmed by ^{13}C – urea breath test
	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	140	23[A]	Randomization applied
	Milman et al. (1998) [33]	1982 – 1984	Randomized selection from the civil registration system	30 – 60	2794	26	Randomization applied Exclusion of Danes of foreign origin 95% was healthy based on self-reports
Greenland (Denmark)	Koch et al. (2005) [34]	1996 – 1998	Selection based on a population-based survey to measure the seroprevalence of several infectious agents	15 – 87	392	58	Unclear whether randomization was applied
Norway	**Asfeldt et al. (2008)** [35]	**2004**	All adult inhabitants of Sørreisa were invited	20 – 69	398	**24**▪	No randomization applied Prevalence among those without dyspepsia Exclusion of reported peptic ulcer
	Breckan et al. (2009) [36]	**2004 – 2005**	A representative sample in Bodø drawn by Statistics Norway was invited and all residents of Sørreisa were invited	18 – 85	1414	**33**▪	No randomization applied Calculations made for sample size
Sweden	Sörberg et al. (2003) [37]	1995 – 1999	Voluntary blood donors across Sweden and a random sample based on a population register of Stockholm	17 – 80+	3502 1030	18 25	Randomization applied but not for the voluntary blood donor group across the country
	Thjodleifsson et al. (2007) [30]	1990 – 1994	Randomized selection from the population of Uppsala	20 – 44	359	11	Randomization applied

Table 3 continued: The prevalence of H. pylori infection in Europe

Country	Ref. (publication year)	Period of data collection	Study population	Age range (years)	Number	Prevalence (%)	Bias / Remarks
Finland	Kosunen et al. (1997) [38]	1994	Randomized selection from the National Population Register by computer	15 – 74	504	31	Randomization applied
Ireland	Buckley et al. (1998) [39]	⊦	Voluntary blood donors	18 – 60	1000	43	No randomization applied Exclusion of volunteers who previously had received H. pylori - eradication therapy or who had a history of gastric surgery
	Murray et al. (1997) [40]	1986 – 1987	Randomized selection from three population surveys using the same protocol	20 – 64	3874	57	Randomization applied
United Kingdom	Lane et al. (2002) [41]	1996 – 1999	Selection from registries of seven primary care centers in Bristol and surrounding areas (England)	20 – 59	10 537	16♦	Unclear whether randomization was applied Exclusion if allergy to the study medication or severe renal impairment
	Woodward et al. (2000) [42]	1995	Randomized selection from lists of randomly sampled general practitioners (Schotland)	25 – 64	1631	66	Randomization applied Self – assessed expected number of infected individuals in North Glasgow
	Webb et al. (1999) [32]	⊦	Randomized selection from the lists of general practitioners	25 – 64	294	28[A]	Randomization applied
CENTRAL EUROPE							
Belgium	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	188	36 [A]	Randomization applied
Germany	Seher et al. (2000) [43]	1997 – 1999	Representative sample selection from population registries in 120 communities	18 – 79	6748	40	Unclear whether randomization was applied Exclusion if insufficient knowledge of the German language
	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	522	49[A]	Randomization applied

Table 3 continued: The prevalence of H. pylori infection in Europe

Country	Ref. (publication year)	Period of data collection	Study population	Age range (years)	Number	Prevalence (%)	Bias / Remarks
The Netherlands	**van Blankenstein et al. (2013) [44]**	**2001 – 2010**	Voluntary blood donors from two regional blood banks	18 – 70	1551	**32**	Randomization applied after donation Non-European immigrants not included
	De Vries et al. (2008) [45]	**2004 – 2005**	Selection from the civil registration system in Rotterdam	18 – 65	288	**72**	Randomization only mentioned in abstract Remarkable low participation rate (16%)
Czech Republic	**Bureš et al. (2012) [46]**	**2011**	Randomized selection from fifteen centers of general practitioners	15 – 98	1406	**29**♦	Randomization applied
SOUTHERN EUROPE							
Greece	Webb et al. (1999) [32]	⊦	Randomized selection from health-screening programs	25 – 64	209	64[A]	Randomization applied
	Pateraki et al. (1990) [47]	⊦	Recruits from all provinces and blood donors from blood banks in Athens	20 – 50	610	69	Unclear whether randomization was applied
Portugal	**Bastos et al. (2013) [48]**	**1999 – 2003**	Randomized selection from the population of Porto	18 – 92	2067	**84**	Randomization applied
	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	119	63[A]	Randomization applied
Italy	**Luzza et al. (2014) [49]**	**2002 – 2003**	Selection from an informed population in Sorbo San Basile after invitation	Adults	595	**72**♦	No randomization applied No definition adulthood Exclusion of individuals who received H. pylori eradication therapy
	Russo et al. (1999) [50]	1995 – 1997	Voluntary blood donors	30 – 60	2586	45	No randomization applied
	Mayr et al. (2003) [51]	1990	Randomized selection from all inhabitants of Bruneck	40 – 79	684	80	Randomization applied
	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	189	30[A]	Randomization applied

Table 3 continued: The prevalence of H. pylori infection in Europe

Country	Ref. (publication year)	Period of data collection	Study population	Age range (years)	Number	Prevalence (%)	Bias / Remarks
Spain	**García et al. (2006)** [52]	**1999 – 2002**	Randomized selection by using random numbers applied to a list of persons	20 – 93	383	**69♦**	Randomization applied Calculated sample size Exclusion if psychiatric incapacity to understand, physical inability to attend, (sub)total gastrectomy and inability to stop the use of acid secretion inhibitors for four weeks
	Senra-Varela et al. (1998) [53]	1997	Randomized selected residents of Ubrique, Grazalema and Barbate	18 – 60+	332	43	Randomization applied Selected individuals did not receive prolonged antibiotic or antiacid treatment Exclusion of chronic diseases and addiction to intravenous drugs
EASTERN EUROPE							
Poland	**Laszewicz et al. (2014)** [54]	**2002 – 2003**	Randomized selection drawn from ten regions representing the country	19 – 89	3307	**84**	Randomization applied
	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	158	80[A]	Randomization applied
Latvia	**Leja et al. (2012)** [55]	**2008 – 2009**	Randomized selection from the national population registry	24 – 74	3564	**79**	Randomization applied
Estonia	Thjodleifsson et al. (2007) [30]	1990 – 1994	Randomized selection from the population of Tartu	20 – 44	240	69	Randomization applied
Slovenia	Webb et al. (1999) [32]	⊦	Randomized selection from population-based registries	25 – 64	182	56[A]	Randomization applied
Croatia	Babus et al. (1998) [56]	⊦	The first people of corresponding age and sex signing up at two local hematology laboratories	25 – 64	456	51	No randomization applied Exclusion of digestive disorders and the blood sample had to be provided for a certain reason

Only the most recent prevalence data within the studies are shown. Data collected in the 21th century are highlighted in bold font type.
Ref.: reference; ⊦: information not obtained; HIV: Human Immunodeficiency Virus; HBV: Hepatitis B Virus; [A] Average of the age groups and the Eurogast centers. Used as detection method: ♦ ^{13}C – urea breath test; ■ Stool sample; remaining studies used blood samples

As shown in Table 3, studies from Northern Europe with the exception of Ireland showed relatively low prevalences of H. pylori infection. Additionaly, two regional studies showed a remarkable difference in the United Kingdom within approximately the same time period. The prevalence rate in England was four times higher than it could be observed in Scotland, demonstrating regional differences within one country.

The most remarkable observation in Central Europe was the major difference in the observed infection rates between studies in the Netherlands, both conducted in the first decade of the 21st century. Non-European immigrants were not included in the study by Van Blankenstein et al. in contrast to the study by De Vries et al., which could explain the remarkable difference in infection rates (31.7% versus 72%).

In Eastern Europe and Southern Europe the highest H. pylori infection rates were found, especially in Portugal, Poland and Latvia. Almost all these studies from these two European regions showed that the majority of the individuals (in other words more than 50% of their population) was infected.

The prevalence of H. pylori infection changes throughout the years. In the Czech Republic, the infection rate was significantly lower in 2011 in comparison with the infection rate ten years before. The overall prevalence was 23.5% compared with an overall prevalence of 41.7% in 2001. The studies used the same detection method, applied in the same region. [46] Same trend could be found in Finland whereby the prevalence of H. pylori infection significantly decreased over a 21-year period in all age groups in both genders. [38] Among non-dyspeptic infected individuals in a representative community in Norway, the H. pylori infection rate also decreased within a time period of 17 years. [38] Therefore, the overall trend in current infection rate appears to be a declining trend in Europe. However, recent data show that the absolute infection rates remain very high in Europe. Recent European data, collected in the 21th century, showed the lowest prevalence of H. pylori infection in the Czech Republic, where almost a third of the individuals was infected. Other studies from this century showed even higher infection rates, up to 84% in Portugal and Poland (see Table 3).

Multiple studies gave additional information concerning the strain of the bacterium. As shown in Table 4, the positivity for the high-virulent CagA – strain among the H. pylori infected was relatively high in Europe. Overall, about half of the H. pylori positives were infected with this

CagA – strain. However, there were distinct differences between studies and regions. In Rotterdam, a multicultural city in the Netherlands, 58.2% (120/206) tested CagA – positive among the infected subjects. Once infected, there was no significant difference in CagA – seropositivity between the Duth natives on the one hand and the immigrants on the other hand. [45] Though, several factors could have some effect in the CagA – prevalence rate. For example, Bastos et al. observed more CagA – infected individuals among the less educated seropositives. [48]

Table 4: CagA – positivity among the healthy H. pylori infected adults in Europe

Reference	**Data collection**	**Country**	**CagA (%) among infected**
Thjodleifsson et al. [30]	1990 - 1994	Estonia	62.5
		Sweden	69.3
Van Blankenstein et al. [44]	NA	The Netherlands	28
De Vries et al. [45]	2004 - 2005	The Netherlands	58.2
Bastos et al. [48]	1999 - 2003	Portugal	61.7
Webb et al. [32]	NA	Portugal	40.8
		Denmark	48.4
		Germany	52.1[A]
		Greece	36.8
		Poland	56.7
		Belgium	66.2
		Slovenia	59.4
		United Kingdom	70.3[A]
		Italy	46.6
Luzza et al. [49]	2002 - 2003	Italy	60
Mayr et al. [51]	1990	Italy	38.5

CagA: cytotoxin - associated gene A; [A] Average of the age groups and Eurogast centers

The majority of these studies could not show a significant difference in prevalence of H. pylori infection between men and women. If there was a gender difference, the infection rate was higher in men. Nearly all these European epidemiological studies did find a strong, mostly significant, increase in prevalence of H. pylori infection in the older age groups. However, two studies showed conflicting results. The Spanish study by García et al. showed a declining trend in the oldest age groups (older than 75 years) but these groups consisted of less participating

individuals. [52] In the Swedish study by Sörberg et al., the positive seroprevalence increased with age in the population sample, but among the blood donors the prevalence of H. pylori infection decreased again after the age of 50. [37]

There is less clarity about all other potential risk factors for H. pylori infection. Generally, several studies show the importance of the socio – economic class (including educational background). However, there are several contradictions. This also applies to the other potential risk factors such as smoking. However, this was not the focus of this dissertation.

IMPACT OF LIFESTYLE FACTORS AFTER INFECTION

Cost – effective strategies against H. pylori – related gastric cancer in Europe could be based on lifestyle; namely diet, alcohol, tobacco intake and physical activity. Correctable and preventable lifestyle factors could potentially reduce the risk of getting H. pylori – associated gastric cancer. This is why this aspect was further systematically investigated. It could be an important cost – effective way to reduce the morbidity and mortality of stomach cancer in Europe.

1. SMOKING

Considering that smoking is a known negative predictor for various diseases outcomes, the importance of this lifestyle parameter is investigated in the occurrence of H. pylori – associated stomach cancer.

1.1 ATROPHIC GASTRITIS

The influence of smoking towards the intermediate stage in the process to H. pylori – related gastric cancer was investigated. An Asian matched case – control study could not find any significant difference for developing gastric atrophy among the infected individuals. The H. pylori infected ever-smokers (including current smokers) had a slightly decreased risk for developing gastric atrophy (OR 0.81; 95%CI 0.59-1.12), but they had an increased risk for developing the severe form of gastric atrophy (OR 1.30; 95%CI 0.84-2.02). Nevertheless, both outcomes adjusted for age and sex were not significant. [57] An univariate analysis in an European population infected with the bacterium could not demonstrate any significant association either towards this stage. However, the proportion of smokers was quite small. [58]

1.2 STOMACH CANCER

As shown in Table 5, multiple studies demonstrate a significant increased risk among smokers for developing H. pylori – associated stomach cancer. A Japanese matched case – control study found an increased risk for non-cardia stomach cancer in the H. pylori-positive smoker group. After the applying of adjustments, this group had 3 times the risk of developing non-cardia cancer compared to the infected individuals without smoking history, but the interaction term was not significant (p – value for interaction 0.52). [59] Another matched case – control study conducted in Hawaii showed a similar outcome after adjustments for sex, age and ethnicity. H. pylori-positive smokers, but only those who smoked more than 38 pack per years, had also

approximately 3 times the risk of developing gastric cancer compared to non-smoking seropositives. The interaction term was significant (p – value for trend 0.0004). [60]

A third case – control study could also confirm this association among the infected individuals. Within the H. pylori infected group without the cytotoxin-associated gene A (CagA), there was a significant increased risk for developing non-cardia gastric cancer among the smokers compared to never-smokers. When a smoker was infected with the CagA - positive strain, the risk increased spectacularly (OR 19.5; 95%CI 10.3-42.2). They found a significant interaction between smoking and the positivity for CagA, suggesting a synergistic association (p – value 0.021). [61]

At last, the same conclusion could be found in an European country, namely Sweden. Smokers had also a significantly increased risk for getting stomach cancer compared to the non-smoking infected group. However, there was no significant interaction term and the number of cases was limited. [62]

Table 5: Overview of the studies on the impact of smoking after H. pylori infection

Study reference	Type of cancer	OR	95% CI	Adjusted for
Simán et al. [62]	Stomach	2.3	1.1 – 4.7	occupation (as an indicator of socio-economic status)
Machida-Montani et al. [59]	Non – cardia	3.0	1.4 – 6.6	family history of gastric cancer, one specific diet, tot vegetable intake, total fruit intake and salt intake
Epplein et al. [60]	Non - cardia	3.2	1.7 – 6.2	sex, age and ethnicity
Wang et al. [61]	Non – cardia	2.7	1.3 – 4.9	family history of gastric cancer, education and alcohol consumption

Studies from European regions are highlighted in bold font type.
All risks compared to infected non-smoking individuals. OR: odds ratio; CI: confidence interval

2. ALCOHOL CONSUMPTION

2.1 ATROPHIC GASTRITIS

An association between alcohol use and H. pylori – related atrophic chronic gastritis could not be shown. In an European infected population, the risk was not associated with drinking alcohol after an unadjusted analysis. [58]

2.2 STOMACH CANCER

Alcohol consumption at least once a week was not associated with an increased risk for developing H. pylori – associated stomach cancer after adjustments for sex, age and ethnicity in the study by Epplein et al. conducted in Hawaii. Compared with abstaining alcohol among H. pylori infected individuals, the risk was slightly increased but not significant (OR 1.3; 95%CI 0.8-2.1). [60] Similarly, in the Korean cohort study by Ma et al. no significant association could be demonstrated between drinking alcohol and the development of gastric cancer among the infected residents. Not the number of years of alcohol drinking, nor the drinking frequency, nor the average dose was associated compared to infected alcohol abstainers after adjustments for age, sex, body mass index, educational level and smoking status. [63]

3. DIET

Multiple recent studies investigated the possible impact of dietary factors on the development of gastric cancer after H. pylori infection. On the one hand, there are studies relating to traditional food patterns; on the other hand, there are studies relating to worldwide diet patterns such as fruit and vegetable intakes.

3.1 ATROPHIC GASTRITIS

Towards the intermediate stage, namely the stage of H. pylori-related atrophic gastritis, some dietary factors may have an important influence. A high rice intake increased the risk significantly in female seropositive participants in Japan after adjustment for age (OR 1.6; 95%CI 1.1-2.3; p – value 0.02). Other dietary factors such as salted or dried fish, sodium, vitamin C, carotene and several types of vegetables or fruits were not associated with atrophic gastritis; neither an inverse protective effect was observed. [64] These last-mentioned dietary factors plus the consumption of meat and potatoes were also not associated with atrophic gastritis among an European dyspeptic population. Only the consumption of coffee was after a multivariate analysis significantly associated with atrophic gastritis compared with the H. pylori infected individuals having non-atrophic gastritis (OR 2.35; 95%CI 1.07-5.16; p – value for interaction 0.033). [58]

In a randomized placebo controlled study with fifty H. pylori-positive volunteers having gastritis, the intervention group was instructed to consume sulforaphane-rich broccoli sprouts. The gastritis

in this group was reduced afterwards possibly by an antibacterial effect on H. pylori. [65] However, the cross – sectional study by Montani et al. could not show an inverse trend towards atrophic gastritis in the category of high intake of broccoli or cabbage which are sources of sulforaphane. [64]

3.2 STOMACH CANCER

Towards the final stage in the gastric carcinogenesis, namely the associated stomach cancer, the pathway may also be influenced by dietary factors. The consumption of specific dietary components could amplify or inverse the risk towards it.

Bastos et al. investigated the association between several dietary patterns and the development of stomach cancer in Portugal. In association with H. pylori, there was one dietary pattern whereby the risk for developing gastric cancer was significantly increased after adjusting for age, gender, education and total energy intake (OR 1.78; 95% CI 1.31-2.41). This pattern consisted of a less frequent consumption or smaller portions in the consumption of dairy products, fish, fruits, salads, vegetables and meat. However, the trend for interaction was not significant (p – value 0.166). [66]

Wang et al. investigated the association towards non-cardia cancer between soya products, pickled food and H. pylori in Japan. Compared to non-infected individuals, the adjusted risks among the infected persons were significantly higher when high intakes for pickled food (OR 18.3; 95% CI 8.7-49.7); when low intakes for soya products (OR 13.7; 95% CI 4.9-50.2) among the infected. Although the increased risks, the interaction terms were not significant (p – values respectively 0.727 and 0.855). [67]

Other studies investigated the association between other specific nutritional components and H. pylori – associated stomach cancer. These are further categorized below.

3.2.1 SALT

In a Japanese prospective study, salt intake more than 10g/day significantly increased the risk for developing stomach cancer among the H. pylori – infected individuals (age- and sex- adjusted HR 2.43; 95%CI 1.29-4.60; p – value < 0.01). Especially the infected individuals who also had atrophic gastritis were at increased risk (HR 2.87; 95%CI 1.14-7.24; p – value < 0.05). [68] The higher the salt intake, the higher the risk was for developing non-cardia cancer in another

Japanese case – control study in both the uninfected and the infected group. These outcomes were adjusted for a family history of gastric cancer, for a specific membership and for total vegetable/fruit intake. The highest risk was observed among the H. pylori positives with high salt intakes compared to H. pylori negatives with low intakes (OR 14.2; 95%CI 3.9-52.3). However, the trend for interaction was not significant (p – value 0.56). [59]

An European study demonstrated a significant interaction between salt consumption and the risk for developing stomach cancer among the infected individuals after adjustments. However, this significant interaction could only be found by using food frequency questionnaires (p – value for interaction 0.045). When other methods such as a visual analogical scale or the questioning for the use of table salt were used in order to verify a total dietary salt intake, then the risk was increased but the interaction terms were not significant (p – values > 0.05). Furthermore, in association with sodium intake, there was no significant risk difference for developing gastric cancer when the infected group was stratified by virulence of the bacterium (whether or not being infected with the CagA – strain). [69]

3.2.2 ANTIOXIDANTS

Antioxidants are substances capable to inhibit the oxidation by free radicals. An imbalance between protective antioxidants and harmful radicals results in oxidative stress. This implies damage to cells and tissues, possibly leading to cancer in the end. Antioxidants (for example vitamin C) prevent this damage to tissues by a neutralizing process. The human body itself produces antioxidants, but it also retrieves these components from diet.

As shown in Table 6, three European studies investigated the association between the total intake of fruits, vegetables and stomach cancer in interaction with H. pylori infection. The European nested case – control study by Gonzalez et al. could not demonstrate any statistically significant association. [70] A Portuguese case – control study demonstrated a significant decreased risk for developing stomach cancer among the H. pylori negatives in the group with the highest intake of fruit and vegetables (OR 0.21; 95% CI 0.06-0.81). Among the infected individuals, not the group with the highest intakes, but the group with median intakes of fruit and vegetables had a significantly reduced risk for developing stomach cancer. Nevertheless, the interaction term was not significant (p – value for interaction 0.25). [71] A similar conclusion was found by Serafini et al. in Sweden. They specifically investigated the total antioxidant potential of fruit and vegetables

through calculations based on food databases. The H. pylori infected individuals with median intakes of total calculated antioxidant potential, had a lower risk compared to the infected ones with high intakes. The p – value for trend was significant (p – value < 0.05). [72]

In Hawaii, Epplein et al. demonstrated a significant decreased risk when consuming higher quantities of vegetables. The trend for interaction was significant (p – value 0.02). [60] Among the infected Japanese subjects in the case – control study by Machida-Montani et al., the adjusted risk for developing non-cardia cancer was lower within the subgroups with high intakes of fruits and vegetables. However, the confidence intervals were wide and the interaction terms were not significant (p – value 0.60 and 0.32 for respectively total vegetable intake and fruit intake). [59] This is in contrast with the result of another Asiatic study. A significant increased risk was observed among the infected individuals consuming low quantities compared to non-infected persons consuming high quantities of both vegetables and fruits. Significant interactions could be found between vegetables, fruit and H. pylori infection (p – values for interaction less than 0.05). [67]

Table 6: Overview of the studies on the impact of total fruit and vegetable intake after H. pylori infection

Study reference	Diet factor	Cancer	Intake	HR/ OR	95% CI	Adjusted for
Gonzalez et al. [70]	Vegetables	Stomach Cardia Non-cardia	High	1.11 1.42 1.25	0.71-1.74 0.58-3.45 0.71-2.20	sex, age, center, date of blood extraction, height, weight, education level, tobacco smoking, cigarette smoking, work and leisure physical activity, alcohol intake, energy intake, red meat intake, processed meat intake
	Fruit	Stomach Cardia Non-cardia	High	0.98 0.76 1.10	0.81-1.20 0.48-1.22 0.87-1.39	
Lunet et al. [71]	Fruit and vegetables	Stomach	High Median	0.54 0.42	0.26-1.10 0.24-0.74	age, sex, education, number of siblings, vitamin and mineral supplement use, total caloric intake, vitamin C, vitamin E and carotenoids intake
Serafini et al. [72]	Total antioxidant potential of fruit and vegetables	Stomach	High Median	0.56 0.41	0.30-1.06 0.22-0.78	age, sex, body mass index, salt intake, total caloric intake and number of meals per day
Epplein et al. [60]	Vegetables	Non-cardia	High	0.4	0.2-0.8	age, sex, ethnicity, cigarette smoking, education, NSAID use, family history of cancer and total calories
Machida-Montani et al. [59]	Vegetables	Non-cardia	High Low	7.6^{HR} 8.5^{HR}	2.3-25.2■ 2.4-29.9■	JA membership, family history of gastric cancer, total vegetable or fruit intake, salt intake and total energy intake
	Fruit		High Low	5.8^{HR} 10.6^{HR}	2.0-16.9■ 3.3-33.9■	
Wang et al. [67]	Vegetables	Non-cardia	High Low	0.8 2.9	0.5-3.5■ 1.5-9.7■	education, smoking, alcohol consumption, family history, total fruit intake or total vegetable intake, pickled food and soya products
	Fruit		High Low	0.9 2.0	0.3-3.1■ 1.2-6.7■	

Studies from European regions are highlighted in bold font type.
OR: odds ratio; HR: hazard ratio; CI: confidence interval; JA: Japan Agriculture cooperatives; NSAID: non-steroidal anti-inflammatory drugs; ■ compared to H. pylori negative individuals (reference)

As shown in Table 7, Ekstrom et al. showed that higher dietary intakes of specific antioxidants such as ascorbic acid (vitamin C) and β – carotene significantly decreased the risk for developing non-cardia cancer among European H. pylori seropositive subjects. [73] The same trend was observed in the case – control study by Epplein et al. whereby higher intakes of β – carotene, vitamin C and vitamin E significantly decreased the risk for non-cardia gastric cancer among those infected with the bacterium. In contrast to the previous study, this study population was not European, additional adjustments were implemented and the trend for interaction was significant (p – value 0.03). [60] Another European study concerning the association with vitamin C, vitamin

E and vitamin A equivalents could not demonstrate any difference in the risk for developing H. pylori - related stomach cancer. [71]

The Korean case - control study showed significant interactions for vitamin C and vitamin E (p – values respectively 0.015 and 0.028). In other words, high intakes of these vitamins showed a significant protective effect towards the associated stomach cancer among H. pylori infected individuals. No association was found for other vitamins, nor for folate (OR 0.56; 95% CI 0.12-2.68). [74]

The case – control study of Kim et al. conducted a stratification based on vitamin C intake. Compared to H. pylori uninfected subjects, the risk among H. pylori positives was significantly increased only in the subgroup of low vitamin C intake. In contrast, high intakes slightly decreased the risk among the infected ones suggesting a protective inhibition of H. pylori activity (see Table 7). [75] In association with the more virulent type of the bacterium, namely the CagA-positive strain, a matched case-control study showed no significant interaction with vitamin C towards stomach cancer (p – value for interaction 0.874), nor could a significant interaction be demonstrated with nitrite intake (p – value for interaction 0.919). [76]

Among all the infected in the study by Miyazaki et al., the subjects with a high dietary vitamin A intake were more at risk for developing stomach cancer compared to those with low intakes of this vitamin. However, there was no significant interaction between vitamin A intake and H. pylori for the development of gastric cancer (p – value for trend 0.27). [77] The interaction term was also not significant in the study by Epplein et al. (p – value for interaction 0.56), but they observed among the infected an inversed association at higher intakes of vitamin A. An exclusion of cardia gastric cancer cases was applied in this study. [60]

Flavonoids are specific color giving components which can act as antioxidants in fruits and vegetables. Woo et al. investigated this in association with H. pylori infection. Among H. pylori infected individuals, the risk of getting gastric cancer was reduced within the group with the highest intakes of total flavonoid. However, after additional adjustments, the trends were not significant for most flavonoids, except for flavones within the group of women (p – values < 0.05). The outcomes were adjusted for total energy intake, age, education, smoking status, alcohol consumption, BMI, physical activity, consumption of pickled vegetables, red or processed meat and additionally for fruits and vegetable consumption. [78]

Table 7: Overview of the studies on the impact of vitamin intake after H. pylori infection

Vitamin	Study reference	Type of cancer	Intake	HR/ OR	95% CI	Adjusted for
Vitamin C	**Ekstrom et al. [73]**	Cardia Non-cardia	High High	0.8 0.5	0.3-2.0 0.3-0.9	age, sex, socio-economic status and total caloric intake
	Lunet et al. [71]	Stomach	High	0.80	0.40-1.59	age, sex, education, number of siblings, vitamin and mineral supplement use, total caloric intake, vitamin C, vitamin E and carotenoids intake
	Epplein et al. [60]	Non-cardia	High	0.5	0.2-0.9	age, sex, ethnicity, cigarette smoking, education, NSAID use, family history of cancer and total calories
	Kim et al. [75]	Stomach	High Low	0.72 4.68	0.32-1.65▪ 1.97-11.1▪	age, sex, history of gastritis or gastric ulcer and educational level
	Kim et al. [74]	Stomach	High	0.10	0.02-0.63	age, sex, socioeconomic status, family history, refrigerator use, supplement use and specific foods•
β – carotene	**Ekstrom et al. [73]**	Cardia Non-cardia	High High	0.6 0.5	0.2-1.5 0.3-0.8	age, sex, socio-economic status and total caloric intake
	Epplein et al. [60]	Non-cardia	High	0.3	0.2-0.7	age, sex, ethnicity, cigarette smoking, education, NSAID use, family history of cancer and total calories
	Kim et al. [74]	Stomach	High	0.54	0.12-2.52	age, sex, socioeconomic status, family history, refrigerator use, supplement use and specific foods•
Vitamin A	**Lunet et al. [71]**	Stomach	High	1.48	0.82-2.69	age, sex, education, number of siblings, vitamin and mineral supplement use, total caloric intake, vitamin C, vitamin E and carotenoids intake
	Epplein et al. [60]	Non-cardia	High	0.3	0.2 – 0.6	age, sex, ethnicity, cigarette smoking, education, NSAID use, family history of cancer and total calories
	Miyazaki et al. [77]	Stomach	High	2.00 HR	1.08-3.70	age, sex, body mass index, diabetes, serum total cholesterol, smoking habit, alcohol intake, regular exercise and other dietary factors
	Kim et al. [74]	Stomach	High	0.32	0.07-1.52	age, sex, socioeconomic status, family history, refrigerator use, supplement use and specific foods•
Vitamin E	**Lunet et al. [71]**	Stomach	High	1.15	0.64-2.07	age, sex, education, number of siblings, vitamin and mineral supplement use, total caloric intake, vitamin C, vitamin E and carotenoids intake
	Ekstrom et al. [73]	Cardia Non-cardia	High High	0.3 0.7	0.1-1.0 0.4-1.1	age, sex, socio-economic status and total caloric intake
	Epplein et al. [60]	Non-cardia	High	0.4	0.2-0.9	age, sex, ethnicity, cigarette smoking, education, NSAID use, family history of cancer and total calories
	Kim et al. [74]	Stomach	High	0.16	0.03-0.83	age, sex, socioeconomic status, family history, refrigerator use, supplement use and specific foods•

Studies from European regions are highlighted in bold font type.
HR: hazard ratio; OR: odds ratio; CI: confidence interval; NSAID: non-steroidal anti-inflammatory drugs; • specific foods included charcoal grilled beef, spinach, garlic, mushroom and kimchi types; ▪ compared to H. pylori negative persons (reference); remaining studies compared to infected individuals with low intakes of the specific component

3.2.3 MEAT

The association between meat intake and the risk of developing gastric cancer in interaction with H. pylori infection was investigated by Gonzalez et al. in a nested case – control study from an European prospective study. They demonstrated a significant association for total meat intake and processed meat, especially for the development towards gastric non-cardia adenocarcinoma. A daily intake increase of 100 grams total meat increased the risk for developing stomach cancer (adjusted OR 2.57; 95% CI 1.25-5.25). More specifically, it led to an increased risk for developing non-cardia gastric cancer among the infected subjects (adjusted OR 5.32; 95%CI 2.10-13.4). No association could be found for red meat or poultry, in contrast to processed meat. A daily intake increase of 50 grams processed meat resulted in a risk twice as high for developing stomach cancer (adjusted OR 2.00; 95%CI 1.06-3.79). More specifically, it led to an increased risk towards non-cardia gastric adenocarcinoma (adjusted OR 2.67; 95%CI 1.20-5.93). [79]

In Hawaii, Epplein et al. found a similar association for processed meat among the H. pylori infected, but not for bacon. A daily food intake of 8.7-25.4 grams of processed meats increased the risk for developing gastric cancer compared to those consuming less than 8.7 grams (OR 2.7; 95%CI 1.4-5.2). Consuming this type of meat more than 25.4 grams a day also increased the risk, but not in proportion with the higher intakes (OR 2.0; 95% CI 1.0-4.0). [60] High intakes of meat compared to non-infected Japanese people with low intakes of meat was associated with an increased risk too in the study by Wang et al. (OR 3.0; 95% CI 1.1-8.8). [67]

However, the interaction terms were not significant in all abovementioned studies (p – values > 0.5). The non-European studies excluded cardia gastric cancer thus this type of stomach cancer was not investigated in these studies.

4. PHYSICAL ACTIVITY

Only one qualitative study relating to the association between physical activity and H. pylori related stomach cancer could be retrieved. The sample consisted of participants of the *European Prospective Investigation into Cancer and nutrition* (EPIC), an European prospective study which investigated lifestyle, metabolic and genetic determinants of cancer and other chronic diseases. Different types and intensities of physical activity during working hours as well as during leisure time were studied but no association could be found. [80]

DISCUSSION

It is remarkable that the infection rates of H. pylori are still high in Europe. Although most studies date from the past century, we found multiple recent studies demonstrating very high prevalences of H. pylori infected Europeans. The lowest percentage in the 21th century was found in the Czech Republic. However, almost a third of the screened population was infected which is not negligible. In other countries providing recent data, the infection rates were even higher up to 84% in Poland and Portugal.

The number of infected individuals varies considerably according to region. In Europe, the infection rates were clearly lower in Northern Europe (irrespective from time). Even in one single country such as the United Kingdom, remarkable differences in approximately the same period of data collection could be found as observed in Wales and England (respectively by Woodward et al. and by Lane et al.). These regional differences in infection rate could be explained by the multiple risk factors leading to infection. They could be more common in one region than another. Repeatedly documented risk factors include age, sex, large family size, poor sanitation, source of water supply, unhygienic conditions, lower socioeconomic class (including educational grade), smoking, living in rural areas and overcrowding. [81] [42] Nevertheless, there might be some protective factors such as breast-feeding, which could be protective but mainly in less developed areas. [82]

Throughout time, the environment changes and consequently the prevalence of H. pylori infection can change as found in the Czech Republic, Finland and Norway. This is one of the reasons why not all these studies can be compared with each other. A constantly changing environment includes also some immigration trends from time to time, which could have a significant impact on the recent infection prevalence in populations as demonstrated in the studies from the Netherlands. Migrants originating from developing countries might already be infected with this persistent bacterium in childhood due to less hygienic and sanitation conditions. [83]

When comparing the infection rates, one should also take into account the various detection methods used, the different sample sizes studied and the different inclusion or exclusion criteria used. These aspects for each study are summarized in the attached tables as remarks to be aware of this potential bias.

Serology is the most commonly used detection method for H. pylori infection in epidemiological studies. Being not able to prove ongoing infection due to the immunological memory is an important disadvantage of this method. Depending on regional variations, its sensitivity and specificity ranges between 80-90%, which is less than the golden standard methods namely the invasive histology test and the noninvasive urea breath test both having a sensitivity and specificity of more than 95% but requiring more expertise and more money. [9]

There were wide variations regarding the sample sizes between the studies. Only two studies could justify the size of its study sample. However, this is important information since this determines the representativeness of a study for a general population.
At last, not all studies were just as clear about the inclusion or exclusion criteria. There were also some disparities in the criteria used which may affect the conclusion. As discussed before, immigrant trends could be an important influence, but most studies did not specify whether or not this specific subpopulation was enrolled.

Moreover, dyspepsia and other gastrointestinal diseases are very common in communities so it is difficult to include a truly asymptomatic population causing potentially an overestimation of the H. pylori infection rate in some of the reviewed epidemiological studies. [39] Furthermore, health workers, especially those having close contacts with patients, seem to be more infected than others. [84-86] In this dissertation, studies relating the epidemiology of H. pylori infection among sick individuals or among health workers only were excluded to avoid such overestimates. Otherwise, this would have given incorrect conclusions about particular regions and would have made it more difficult to compare the study results.

Studies about the impact of lifestyle factors after H. pylori infection towards stomach cancer are showing similar as well as conflicting results. Especially the association – hypotheses (interaction terms) derived from the observed risks were conflicting. The trends for interaction were significant if the p – value for trend was less than 0.05 ($p < 0.05$).

Towards the intermediate stage of atrophic gastritis, no significant association could be found except for coffee consumption in one study.
Towards H. pylori – associated stomach cancer, a significant increased risk was generally found among smokers, high salt consumers and those eating lots of meat (mainly processed meat). However, the results were conflicting regarding the trends for interaction. Concerning other

dietary components, the studies were inconclusive. A median intake of fruit and vegetables decreased the risk among European H. pylori infected individuals in two studies, but the interaction terms were conflicting. Studies investigating specifically the association towards non-cardia cancer between (vitamin-)antioxidants and H. pylori infection demonstrated significant decreased risks among the infected individuals. However, the trends for interaction were again conflicting. Furthermore, no association was found for the remaining two lifestyle factors studied, namely alcohol consumption and physical activity.

A part of the studies was from non-European regions which complicates the generalizability of these results towards an European population. The actual impact of lifestyle factors after being infected with H. pylori could be very specific per host genetics and per particular present strain of the bacterium. [87-89] Furthermore, the majority of the studies did not calculate their necessary power (sample size) in order to prove evidence which possibly affect the value of the results. In addition, some studies applied different or more extended adjustments and criteria. These were retrieved and shown in the attached tables or in appendix (see appendix 3-4).

During the selection process, a part of the articles could not be retrieved despite consulting the online platform ResearchGate twice making it possible that some relevant studies have been missed. More than half of that group of articles had no abstract and exclusion based on title alone of these articles could not be applied because it was too unclear. During the selection steps whereby only one reviewer was involved, the probability was also higher to miss certain relevant studies. Finally, a part of the studies was untranslatable. Consequently, relevant information could also have been missed by this last-mentioned aspect. The untranslatable languages are listed in the criteria section.

A strength of this dissertation is the fact that it implemented a methodological quality control for each selected study. The tool used was an aid to assess articles by several methodological aspects. The exclusion of studies based on their methodological quality was carried out by one reviewer based on his weighting of all subcomponents. The main reasons for exclusion were a non-representative sample and an inappropriate control group. The strengths and weaknesses of the remaining selected studies are shown in the attached tables as remarks in order to be aware of a potential bias (see also in the attached tables in appendix 3 – 4). Nevertheless, it remains a selection step done by only one reviewer which possibly influenced the results.

CONCLUSIONS

The prevalence of H. pylori infection in a community is depending on the exposure to infection. Secondly, it is depending on its capacity to survive in the gastric environment of the host with its specific immune system. Lastly, the loss or the eradication of the infection determines also the current infection prevalence. [39]

This dissertation clearly demonstrates that the H. pylori infection rate is still high in several regions in Europe. Moreover, studies also investigating the prevalence of the CagA – strain among the H. pylori infected Europeans showed that at least a third was infected with this more carcinogenic strain. This is definitely important because only information about the infection rate itself is not enough. The specific strain in interaction with the specific host immune response will determine who will or will not develop stomach cancer. [9] Therefore, further studies are absolutely needed to have more recent data on the infection rates and to know the specific strains present by region for predicting the future burden of gastric cancer. Also recommended by the IARC, it is necessary to identify the high – risk subpopulations who could possibly benefit the most from interventions. [14]

This dissertation showed that residents in particular European regions were more vulnerable to be infected than other Europeans. This could be explained by the different present risk factors for infection by region. Documented risk factors for infection are a low socioeconomic status, overcrowding living circumstances and poor hygienic conditions.

There is an epidemiological difference between the developing and the developed countries. In industrialized regions, the prevalence of H. pylori infection is low early in childhood and slowly rises with increasing age. [9] Increasing infection rates with age could also be demonstrated in this dissertation within the group of adults from European industrialized countries. This finding could be explained by a cohort effect. Older individuals could be infected with H. pylori in childhood when the infection was more prevalent than currently. [90] However, this was not the focus of this dissertation. Only the impact of lifestyle factors after H. pylori infection were systematically investigated in order to find the infected groups at most risk for getting stomach cancer.

After being infected with H. pylori, certain lifestyle factors could have a significant impact towards the development of the bacterium - associated gastric cancer. Studies on smoking, salt consumption and the consumption of meat could demonstrate increased risks among the infected individuals. A median intake of fruit and vegetables decreased the risk among European H. pylori infected individuals in two studies. Studies investigating specifically the association towards non-cardia cancer between (vitamin-)antioxidants and H. pylori infection demonstrated significant decreased risks among the infected individuals. However, across all studies, the trends for interaction were conflicting to prove association - hypotheses. Furthermore, no association could be found for the remaining two lifestyle factors studied, namely alcohol consumption and physical activity.

However, there are not enough qualitative studies concerning a European population to draw any conclusions. Studies investigate usually the independent impact of lifestyle factors towards gastric cancer, but more research is needed about the impact of lifestyle in association with this bacterium. Knowing how this specific interaction affects the development to stomach cancer is important to take the most appropriate action. More research on the possibilities and mechanisms is therefore definitely useful. This focus on correctable lifestyle factors could be an important cost effective manner to prevent stomach cancer in Europe.

Current strategies to prevent H. pylori – associated stomach cancer include screen-and-treat programs. A current eradication treatment consists of a triple therapy of a proton pump inhibitor with clarithromycin and amoxicillin or metronidazole. However, a decrease in efficacy of this triple therapy has been observed due to the increase of H. pylori resistance to clarithromycin. The most recent guidelines advice to use higher doses of proton pump inhibitors and to extend the duration of the therapy from 7 up to 14 days to improve the eradication success. [12] However, uncertainties remain about the cost-effectiveness for routine screen-and-treat programs in European countries. [14]

Furthermore, uncertainties persist about the adverse consequences such as the increasing antibiotic resistance which is already a major health problem. Therefore, alternative options such as the development of a vaccine have been investigated but still without success. [91] Moreover, H. pylori may have a protective role against allergic diseases (atopy) which is also a major health problem in the industrialized countries. [92] It is also possible that the bacterium disappears

naturally which means that it does not have to cause a problem thus causing medicalization. However, studies are not convincing. [93]

All of this makes it difficult to simply implement these quite expensive preventive programs against H. pylori infection. If there is a significant impact by lifestyle factors among H. pylori infected individuals, than this could be an important alternative cost – effective prevention strategy against stomach cancer which again emphasizes the importance of further research.

Despite limitations as discussed above, this dissertation provided important systematically researched data on the epidemiology of H. pylori infection among healthy adult Europeans. The infection rate is still high in several regions which could be a predictor for many preventable future deaths due to H. pylori - related stomach cancer. Moreover, this dissertation provided an overview of the recent insights on the impact of lifestyle factors after being infected. These findings suggest that even among infected individuals, some correctable lifestyle factors could have the capacity to inhibit the progression towards stomach cancer.

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APPENDIX

Appendix 1: Critical appraisal to a medical article, guidelines and checklist from Fowkes et al. [29]

Guidelines and checklist for appraising a medical article

Guideline	*Checklist*		
(I) Study design appropriate to objectives?	Objective:	Common design:	
	Prevalence	Cross sectional	—
	Prognosis	Cohort	—
	Treatment	Controlled trial	—
	Cause	Cohort, case-control, cross sectional	—
(2) Study sample representative?	Source of sample		—
	Sampling method		—
	Sample size		—
	Entry criteria/exclusions		—
	Non-respondents		—
(3) Control group acceptable?	Definition of controls		—
	Source of controls		—
	Matching/randomisation		—
	Comparable characteristics		—
(4) Quality of measurements and outcomes?	Validity		—
	Reproducibility		—
	Blindness		—
	Quality control		—
(5) Completeness?	Compliance		—
	Drop outs		—
	Deaths		—
	Missing data		—
(6) Distorting influences?	Extraneous treatments		—
	Contamination		—
	Changes over time		—
	Confounding factors		—
	Distortion reduced by analysis		—

Appendix 2: Permission for the reproduction of the figures

Appendix 3: Overview of the studies on the impact of smoking and alcohol consumption after H. pylori infection

Study reference	Design	Sample	Risk factors	Remarks
Ma et al. [63]	cohort	Voluntary participants from four urban and rural areas in Korea	Alcohol	No randomization applied Exclusion: individuals younger than 20 years, *
Megraud et al. [58]	cross – sectional	Infected individuals consulting for dyspepsia from 14 European countries	Smoking Alcohol	No randomization applied More cases of non-atrophic chronic gastritis (76%) Exclusion: *
Epplein et al. [60]	case – control	Gastric cancer patients as cases; controls matched by sex, ethnicity and age from residents lists	Smoking Alcohol	Exclusion: cardia gastric cancer, *
Machida et al. [59]	case – control	Gastric cancer patients as cases; controls matched by sex, age and residence area randomly selected from a health check-up program	Smoking	Hospital based case – control study Controls were confirmed to be free of cancer and to have no history of cancer Low number of uninfected cases Exclusion: cardia gastric cancer, extreme caloric intake
Hishida et al. [57]	case – control	Gastric cancer patients as cases; controls matched by age and sex	Smoking	75% of the controls was infected About one third of the controls had gastric atrophy Exclusion: *
Wang et al. [61]	case – control	Gastric cancer patients as cases; randomly selected controls from the same community matched by sex and age	Smoking	Exclusion cases: cardia gastric cancer Exclusion controls: cancer in medical history, diabetes or gastrointestinal disorders
Simán et al. [62]	case – control	Gastric cancer patients as cases; four controls per case matched by age, sex and date of enrolment	Smoking	Low number of cases Exclusion: cancer diagnosis before enrolment, wrong cancer diagnosis, no histological diagnosis, *

* Exclusion in case of inadequate or incomplete information

Appendix 4: Overview of the studies on the impact of diet after H. pylori infection

Study reference	Design	Number	Sample	Remarks
Megraud et al. [58]	cross – sectional	267	Infected individuals consulting for dyspepsia from following European countries: Belgium, Finland, France, Germany, Greece, Ireland, Italy, Portugal, Spain, Bulgaria, Romania, Czech Republic, Estonia, Hungary and Poland	No randomization applied Questionnaire filled out by clinician or a research nurse No details of OR nor 95%CI More cases of non-atrophic chronic gastritis (76%) Exclusion: *
Ekstrom et al. [73]	case – control	267 cases 238 controls	Gastric cancer patients as cases; two controls per case randomly selected from population register based on age and gender in Sweden	Questionnaire by professional interviewers Dietary habits 20 years prior to interview Exclusion: history of gastrectomy, *
Epplein et al. [60]	case – control	212 cases 336 controls	Gastric cancer patients as cases; controls matched by sex, ethnicity and age from residents lists in Hawaii	Questionnaire by trained interviewers Exclusion: cardia gastric cancer, *
Gonzalez et al. [79] and [70]	nested case – control from European cohort study	241 cases 1141 controls	Gastric cancer patients as cases; randomized selected controls from cohort study matched by age, center and date of blood sample collection from following European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom	Most centres via self – administered questionnaire Second dietary measurement applied Exclusion: prevalent cancer cases, lost to follow-up, Norway cohort, prevalent cancer cases, nonadenocarcinomas of the esophagus, gastric lymphomas, gastric stump cancers, other nonadenocarcinoma gastric cancers, unspecified malignant neoplasms of the stomach, extreme high or low caloric intake, *
Kim et al. [75]	case – control	295 cases 295 controls	Gastric cancer patients as cases; controls matched by sex and age selected from patients without chronic diseases in Korea	Hospital based case – control study Questionnaire by an interviewer Calculated number of cases and controls Exclusion controls: chronic diseases
Machida et al. [59]	case – control	122 cases 235 controls	Gastric cancer patients as cases; two controls per case matched by age, sex and residence area selected at random from a health program in Japan	Hospital based case – control study Controls were confirmed to be free of cancer and to have no history of cancer Questionnaire by interview Low number of uninfected cases Exclusion: cardia cancer, extreme caloric intake, *
Miyazaki et al. [77] and Shikata et al. [68]	cohort	2467	Residents of Hisayama (Japan) selected undergoing health screenings every 1-2 years up to 14 years	No randomization applied Self – administered questionnaire checked by dietitians and nutritionists Exclusion: history of gastrectomy/gastric cancer, *
Serafini et al. [72]	case – control	505 cases 1116 controls	Gastric cancer patients as cases; two controls per case selected randomly from population registers based on age and gender in Sweden	Questionnaire by professional interviewers Low number of never infected cases Exclusion: history of partial distal gastrectomy and energy intake <700kcal/day, *

Appendix 4 continued: Overview of the studies on the impact of diet after H. pylori infection

Study reference	Design	Number	Sample	Remarks
Woo et al. [78]	case – control	334 cases 334 controls	Gastric cancer patients as cases; controls matched by sex, education and age from health-screening examinations in Korea	Self – administered questionnaire Exclusion cases: diabetes, other present cancer, advanced gastric cancer, severe systemic of mental disease, pregnant women or giving breastfeeding Exclusion controls: cancer, diabetes, gastric or duodenal ulcer and previous H. pylori treatment, *
Yanaka et al. [65]	randomized double blinded control study	50	Fifty H. pylori positive volunteers randomly divided into two groups	Treatment: sulforaphane Two dropouts in placebo group Compliance checked by diary Exclusion: antibiotic use, proton pump inhibitors, or antiulcer drug use
Montani et al. [64]	cross – sectional	1071	Seropositive participants from a health check-up program in Japan and a subgroup from a prospective study on cancer and cardiovascular diseases	No randomization applied Self – administered questionnaire Exclusion: history of gastrectomy or gastric cancer and extreme low or high caloric intake, *
Bastos et al. [66]	case – control	449 cases 1306 controls	Gastric cancer patients as cases; controls randomly recruited and matched by age and gender randomly selected in Portugal	Trained interviewers and food frequency questionnaire Exclusion: no previous cancer diagnosis (except skin non-melanoma), no subtotal gastrectomy for benign conditions, no mental disorders based on Mini Mental Examination (score less than 18), changed food intake more than one year before interview because of gastrointestinal symptoms
Peleteiro et al. [69]	case – control	422 cases 649 controls	Gastric cancer patients as cases; controls randomly recruited in Portugal	
Lunet et al. [71]	case – control	233 cases 311 controls	Gastric cancer patients as cases; controls randomly recruited and matched by age and gender in Portugal	
Lopez-Carrilo et al. [76]	case – control	211 cases 454 controls	Gastric cancer patients as cases; minimum two controls per case matched by age, gender and residence recruited in Mexico	No thorough randomization applied Questionnaire by trained interviewers Exclusion: no previous cancer diagnosis, less than 20 years old, lived in the study area less than six months, a diet related disease, immunosuppressive condition
Maria Huerta et al. [80]	cohort (EPIC)	420 449	Participants from 9 European countries: Denmark, France, Germany, Greece, Italy, Spain, Sweden, the Netherlands and the United Kingdom	Exclusion: prevalent cancer cases, Norway cohort, lost to follow-up, *
Kim et al. [74]	Case – control	136 cases 136 controls	Gastric cancer patients as cases; controls recruited from the departments orthopedic surgery, ophthalmology, dermatology, plastic surgery or family medicine and matched by sex and age	No randomization applied Interviews by trained dietitians Exclusion controls: confirmed to have no severe stomach problems

Appendix 4 continued: Overview of the studies on the impact of diet after H. pylori infection

Study reference	Design	Number	Sample	Remarks
Wang et al. [67]	case – control	257 cases; 514 controls	Gastric cancer patients as cases; two controls per case matched by age and gender, randomly selected from the same residential community	Randomization applied Face-to-face interviews Exclusion: cardia cancer, other chronic diseases such as diabetes or gastrointestinal disorders, other forms of cancer and abnormal caloric consumption

* Exclusion in case of inadequate or incomplete information