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Association between Helicobacter pylori status and self-rated health – a population based cross sectional study.

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Association mellan *Helicobacter pylori*-status och självskattad hälsa – en populationsbaserad tvärsnittsstudie.

Bakgrund: Halva jordens befolkning är beräknad att vara infekterad av *Helicobacter pylori*, men i 80% av fallen är infektionen asymptomatisk. Det är inte känt huruvida infektionen med dess potentiellt kroniska låggradiga inflammation har en effekt på självskattad hälsa. ***Syfte*:** Att undersöka huruvida *H. pylori* -infektion är associerad med dåligt självskattad hälsa. ***Material och Metoder:*** Gastroskopi utfördes på 360 individer från Östhammars kommun, Uppland. Biopsier från magsäckens slemhinna togs för histologisk analys, samt blodprover för serologisk analys, för att upptäcka förekomst av *H. pylori*. Individerna skattade sin hälsa på en skala från 1-5. ***Resultat*:** Fjorton individer var positiva i serologi, medan 10 individer var positiva i histologi för *H. pylori*. Analys med logistisk regressionsmodell visade ingen significant statistisk association mellan *H. pylori* och självskattad hälsa. ***Slutsats*:** Vi fann inte någon korrelation mellan förekomst av *H. pylori*-infektion och lägre eller högre självskattad hälsa.

Association with *Helicobacter pylori* status and self-rated health – a population based cross sectional study.

Introduction: Half of the world's population is estimated to be infected with *Helicobacter pylori*, although in 80% of the cases the infection is asymptomatic. It is not known whether the infection with its potential chronic low-grade inflammation has an effect on self-rated health. ***Aims:*** To investigate if *H. pylori* infection is associated with poor self-rated health. ***Materials and Methods:*** Esophagogastroduodenoscopy was conducted at 360 individuals from Östhammar municipality, Uppland, Sweden. Biopsies for histology from the ventricular mucosa as well as serology from blood samples were taken in order to detect *H. pylori*. The individuals rated their health on a scale of 1-5. ***Results:*** Fourteen individuals were positive on serology, while 10 individuals were positive on histology for *H. pylori*. Analyzing with logistic regression model, we did not find a significant statistical association between *H. pylori* and self-rated health. ***Conclusions:*** We have not found any correlation between *H. pylori* infection and lower or higher self-rated health.

Introduction

Helicobacter pylori

It happens rarely in modern medicine that a completely new etiology for a disease is found – especially a microbial one. Ever since the revolutionizing discovery of *Helicobacter pylori* (*H. pylori*) and its correlation to gastritis and peptic ulcers in 1982 by Barry Marshall and Robin Warren (1), *H. pylori* has also been shown to be a major cause of duodenal and gastric ulcer disease, and to be associated with gastric lymphoma and gastric cancer in humans (2). The infection most commonly occurs during the first years of life and persists lifelong, if untreated. The prevalence varies with socioeconomic status during childhood, and thus also varies between countries and generations. Around half of the total population in the world is estimated to be infected by *H. pylori*, making it one of the most common human infections in the world (3). The prevalence in Sweden is estimated to be around 30%, ranging from 15% among the youngest and >50% among the elderly – a difference that is thought to depend on living standards during childhood. In lower income countries, around >90% can be infected (4). Since its discovery, over 50 extra-gastric manifestations have also been reported and discussed in relation to *H. pylori* in medical literature in a wide range of different medical areas, with more or less compelling evidence (3).

Symptoms in the epigastrium that are supposed to originate from the upper gastrointestinal tract are called dyspepsia. Dyspepsia can be either idiopathic, which is most common, or be caused by an organic disease, where symptomatic *H. pylori* infection is one potential etiological agent. When no cause is found, the term *functional dyspepsia* or *non-ulcer dyspepsia* is used, according to the internationally used Rome criteria (5). Functional dyspepsia is defined as having one or more of the symptoms bothersome postprandial fullness, early satiation, epigastric pain and epigastric burning. For a more severe *H. pylori* infection, causing ulcers or even cancer, other symptoms or signs such as anemia, dysphagia and bloody stool can occur (4).

The aim in international clinical practice has consequently been to eradicate *H. pylori* when found in dyspeptic patients, after an upper GI endoscopy or EGD (esophagogastroduodenoscopy) has shown an ulcer. Eradication therapy (antibiotics and proton pump inhibitors) is sometimes given even without testing for *H. pylori* when duodenal ulcers are found. The reverse method is also accepted for practice, with the newer "test and

treat" strategy patients with dyspeptic symptoms showing positive for *H. pylori* using urea breath test (UBT) or Fecal Helicobacter pylori-Antigen ELISA (F-Hp) are given eradication therapy without a preceding EGD (4).

However, with around half of the global population infected with *H. pylori*, the bacterium leaves its host asymptomatic in around 80% of all cases. It is thus one of few infections that cause a chronic inflammatory response without giving disease or symptoms in the majority of the infected patients (4).

Ecosystem hypothesis

In recent years, new hypotheses concerning a potential protective role of *H. pylori* infections have been explored. The theory of evolution claims that if there is a survival benefit for the host, the organisms within its body will continue to thrive. Maixner *et al* have demonstrated *H. pylori* genome findings from the glacier mummy "Ötzi", estimated to have lived 5300 years ago (6), while Linz *et al* suggested a human-bacterial coexistence as far as 58 000 years back, based on their findings that *H. pylori* genetic diversity follow similar patterns as human migration from east Africa (7). It is thus rather clear that humans have been infected for a long period of time. Furthermore, *H. pylori* only has one niche for living: the human stomach (1). The question is hence posed: is this relationship mutualistic, commensal or parasitic? The explanatory model for the human body as an ecosystem has gained a lot of interest, especially in research of autoimmune diseases, where the immune system is proposed to be so "unstimulated" that it instead attacks the own tissues (8). Popularly, this has been called the hygiene hypothesis (9).

In an article, Smyk *et al*, presented that over one hundred autoimmune diseases have been studied concerning their correlation to *H. pylori*, with very mixed results (10). In a review on the controversial topic, Daugule *et al* show that could be some evidence from observational studies supporting a weak inverse relation with *H. pylori* and allergy, but on the other hand points out that this is difficult to adjust for confounders such as geography and socioeconomic factors, known to influence *H. pylori* prevalence. Furthermore, the authors proposed that *H. pylori* is a major stimulus for the generation of T-regulatory immune cells, that is important in the development of a healthy immune system (11). The possible correlation between autoimmunity and *H. pylori* is however debatable, and was recently dismissed by well-respected *H. pylori* researcher David Graham, who claimed that the evidence was weak and that *H. pylori* could merely be a marker for poor hygiene. An environment generally richer of

different organisms is what the hygiene hypothesis really comprises, he argues – not one single bacterium (12).

H. pylori and the stomach

The main pathology associated with *H. pylori* is gastritis and consequently peptic ulcer disease (PUD) (1). The correlation between the bacteria and mucosal inflammation was also the findings Robin and Marshall made in their first article about the "unidentified curved bacilli" (13).

Since the discovery of *H. pylori*, the prevalence has decreased steadily in more developed countries, most often explained by increased living standards and improved sanitation (14). Oral-to-oral and fecal-to-oral is believed to account for most transmission of the infection, although there is not yet complete understanding in the medical society of the transmission routes (1). During the same time period, effective medical treatment for PUD has dramatically improved, with proton pump inhibitors (PPI:s) and antibiotic treatment for ulcers in clinical practice (15). As a consequence, elective surgery for ulcers has virtually disappeared, which Paimela *et al* concluded in a Finnish nationwide database study (15). Between the years 1987 and 1999 elective PUD surgery decreased from 15,7 to 1,7 operations per 100 000 inhabitants ($p < 0,05$) in Finland. On the contrary, in the same study emergency surgery increased from 5,2 to 7 operations per 100 000 inhabitants ($p < 0,05$). Manuel *et al* demonstrates a similar paradoxical trend: in their study including five large US teaching hospitals from 1996 to 2005, PUD as a primary or secondary discharge diagnosis did not significantly decrease, although *H. pylori* prevalence had steadily decreased in the country (16). The authors discussed whether their study might combine two opposing trends, as use of non-steroid anti-inflammatory drugs (NSAIDs), the second most common etiology for PUD, had increased during the same time period. A systematic review conducted by Sung *et al* including 18 studies with at least 1000 subjects in sample size, demonstrated that both incidence and prevalence of PUD have decreased over the last decades (17). However, the authors also recognize that hospitalizations and complications of PUD were not decreasing in some of these studies, and also attributed this to use of low-dose ASA, NSAIDs and incomplete application of gastroprotective medication.

While ulcers are the clear main pathology associated with *H. pylori*, in some of the infected individuals the inflammation could lead to atrophy and intestinal metaplasia of the ventricular mucosa – and eventually develop into gastric cancer. If these cell changes already have

occurred, the increased risk of gastric cancer persists even though *H. pylori* is successfully eradicated – "the point of no return" (4). But how important is the carcinogenic potential of the bacteria? In a systematic review by Cochrane from 2015, the researchers investigated whether treating those infected with *H. pylori*, using antibiotics, could decrease the number of new cases of gastric cancer. Six randomly controlled trials were included, where patients were given either antibiotics or placebo to *H. pylori* positive asymptomatic adults. Fifty-one (1.6%) of 3294 participants given treatment developed gastric cancer, compared with 76 (2.4%) of 3203 given placebo (RR: 0.66, 95% CI 0.46 to 0.95). The subjects were followed during at least two years. The authors concluded that the evidence for that a search and eradicate strategy in preventing gastric cancer was limited, although this evidence was of moderate-quality (18). However, the Japanese health insurance system started in 2013 to cover eradication for all patients infected with *H. pylori* in order to prevent gastric cancer, which might entirely eradicate the infection in the country (19).

H. pylori and the esophagus

Epidemiological studies have repeatedly demonstrated a negative correlation between *H. pylori* infection and erosive esophagitis, Barrett's esophagus and esophageal adenocarcinoma (20). The strategy of the bacteria to survive the acidic conditions in the stomach is to have a large enzyme that produces urease, creating an alkaline environment around it (2). Moreover, the immune response to the infection involves several cytokines, such as IL-1β, which exerts a 100-fold and 6000-fold more potent inhibition of acid secretion than the common drugs for decreasing symptomatic stomach acidity (PPIs and histamine-2 receptor antagonists respectively) (19). In addition, the mucosal atrophy that the chronic inflammation leads to decreases the acid secretion even more. In total, the reduced acidity in the stomach is thought to lead to decreased reflux damage on the esophagus, and hence explain the negative correlation between *H. pylori* and esophageal conditions (20).

On the other hand, whether eradication therapy really does induce gastroesophageal reflux disease (GERD), which is considered to be a pre-state to the other esophageal diseases mentioned, is debatable. Large review studies have come to discordant conclusions: Tan *et al* examined 16 cohort studies and found that *H. pylori* eradication therapy had no significant effect on the occurrence at GERD (OR: 0,87, CI: 95%), (21) while Xie *et al* examined 43 case-control, cohort and randomized-controlled studies and found an increased risk for GERD after *H. pylori* eradication (RR from 1,70 to 2,50, CI: 95%) (22). However, considering the epidemiologically shown negative association with the other diseases in the esophagus

discussed previously, it would seem like *H. pylori* infection might be protective for the esophagus (20).

H. pylori and anemia

Persisting inflammation in the gastric mucosa could not only induce metaplasia and/or gastric cancer, it could also result in atrophic gastritis, leading to a significant reduction in the production of the important intrinsic factor (IF). Vitamin B12, essential for DNA synthesis, is dependent on IF for its uptake more distally in the gastrointestinal tract, and the first manifestation of vitamin B12 deficiency is a reduced production of blood cells. Pernicious anemia, caused by vitamin B12 deficiency, was the first extragastric manifestation of *H. pylori* recognized, and eradication of the bacteria has been included in international management guidelines when this deficiency is found (3).

Iron, the other big part in deficient anemia has also been connected to *H. pylori* infection (2). Here, the pathophysiology is not yet fully understood, apart from the evident bleeding from *H. pylori* ulcers. The recently discovered hormone hepcidin has been proposed to be of importance in anemia without a known cause. It is released by the liver and regulates iron metabolism in erythrocytes, and rises as an acute phase reactant in response to the mucosal inflammation following *H. pylori* infection. Studies have shown that serum hepcidin is increased in *H. pylori* infected individuals, and decreases after eradication. Anemia of inflammation or chronic disease is seen in many other conditions – *H. pylori* infection is both inflammatory and chronic (3). The Maastricht guidelines include unexplained iron deficiency anemia in the cases where *H. pylori* should be sought and eradicated (2).

H. pylori and coronary heart disease

Cardiologists have also turned their attention to *H. pylori* infection. The theory of systemic low grade inflammation being a trigger for atherosclerosis has been a research field of interest, with correlation between IL-6, C-reactive protein and coronary heart disease (CHD) in particular (24, 25). Different microbial associations to CHD such as *Chlamydia pneumonia, Hepatitis C virus, cytomegalovirus* and *H. pylori* have all been proposed and to different extent significantly supported (26-28). *H. pylori* has been found in the coronary arterial wall in patients undergoing coronary bypass grafting (29), but the importance of the bacteria's general risk increase for CHD seemed in that study to be of less importance compared to other risk factors for CHD (30, 31).

Self-rated health

The question is therefore raised: is *H. pylori* a friend or a foe? Giving the well-known direct causal relation to PUD and gastric cancer, the answer should be that the bacteria is a mere parasite, but the reality might be more complex. Today there is no consensus concerning the coexistence between humans and the bacteria: who benefits and who becomes ill – and if there is a price to extinguishing *H. pylori* from the human flora. Risks and comorbidity aside, for this study we have chosen to look at how carriers of *H. pylori* rated their health. Given that the infection is asymptomatic in 80%, the question arises whether the potentially low grade inflammation could impact the perceived general health status.

Poor self-rated health has been proven an independent predictor of both mortality and morbidity, including diseases such as diabetes, cancer and cardiovascular disease (32). A single general question of the type "How would you rate your general health status?" (different wordings used), with a 5-point response scale, from "very good" to "very poor" has a predictive capacity of objective health status independent of age, gender, socioeconomic status, country of origin and cognitive function (33).

There have been many theories regarding the mechanisms of this correlation (33), where the most interesting for our study concerns inflammation and cytokines. Many studies have previously shown independent correlations between inflammatory factors and self-rated health (33). In one study, published by Lekander *et al* (34), the correlation between self-rated health, cytokine levels and physician rated health was examined. The physicians rated the subjects' health using a scale of 1-5 (Healthy/1, Healthy with slight problems/2, Fair/3, Rather poor/4, Poor/5) strictly based on medical criteria in a questionnaire filled out by the doctor after consultation – a method that has proven good interrater reliability (35). The authors found that poor self-rated health was correlated to high cytokine levels independently of physicians' rating (34). It is plausible that asymptomatic *H. pylori* infection could elicit a low-grade inflammatory response, detectable in the measure of self-rated health.

Modern medicine is a discipline focused on objective findings; laboratory results, radiological findings, histological diagnostics and visual, hearable and palpable abnormalities. Although very well validated, self-rated health is currently not used in clinical practice, even though there often is a large discrepancy between patient-rated and physician-rated symptom burden and functional limitations (36). This study will not answer the underlying question whether *H. pylori* is on average beneficial or harmful to humans concerning mortality or morbidity.

However, it will examine whether people infected with *H. pylori* might have a worse self-rated health than those non-infected, which to our knowledge has not been studied before.

Aims

To investigate if *H.pylori* infection is associated with poor self-rated health.

Our hypothesis is that that individuals positive to *H. pylori* biopsy (i.e. ongoing infection) have a lower self-rated health, due to an increased risk of dyspepsia, but also the potential negative impact of low-grade chronic inflammation on self-rated health.

Do individuals with *H. pylori* positivity on histology and/or serology rate their health as worse compared to individuals without *H. pylori* positivity, independent of age, gender and education?

Materials and methods

The study has used a cross-sectional design within a longitudinal population based study (LongGERD) (37), as we were interested in the general health experience of being a *H. pylori* carrier, not the risk of future complications.
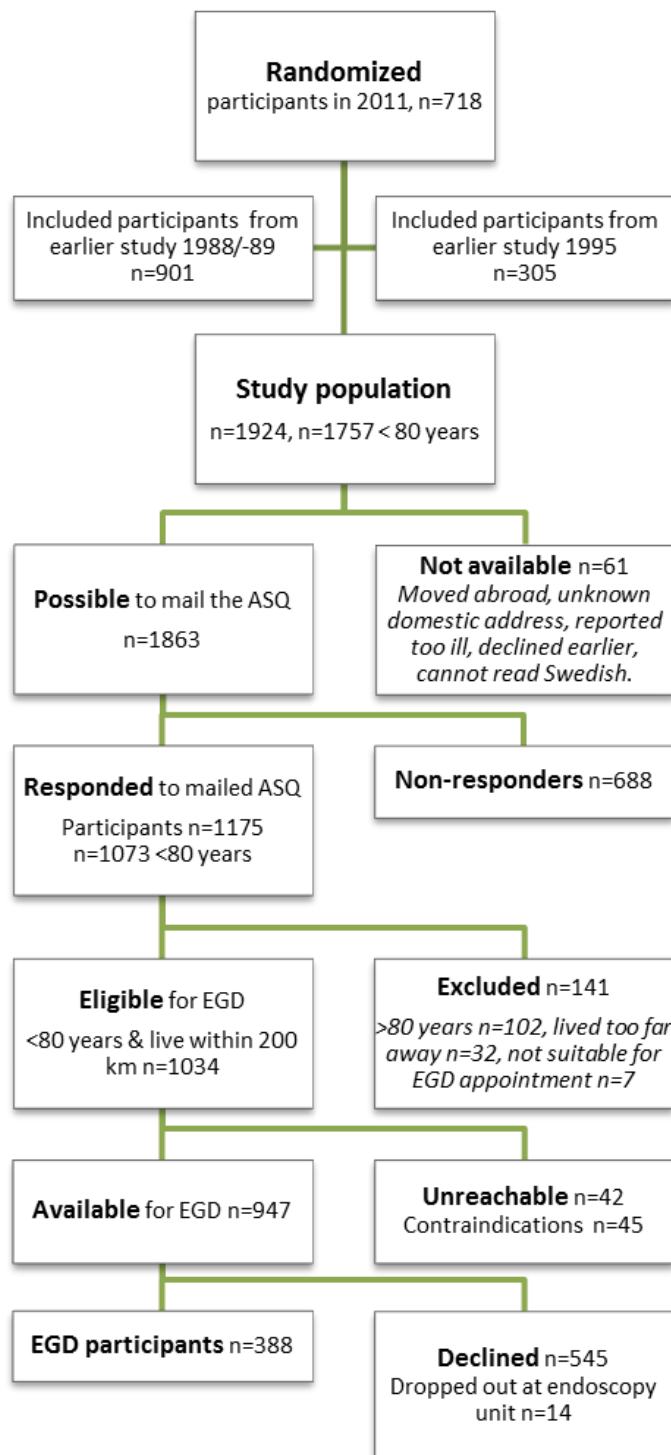
Population

In December 2010, the municipality of Östhammar, Uppsala county, had 21 373 inhabitants. It is partly a coastal region with its shore on the Baltic Sea, situated approximately 100 km north of Stockholm. Historically, the municipality was chosen for the study partly due to that the distribution by age, gender, family size, income, occupation, and other socioeconomic variables is largely similar to the national average. Ninety-two percent of the residents were Swedish citizens (37).

The study participants were found through mailing of a symptom questionnaire (The Abdominal Symptom Questionnaire, ASQ) to all adults above 20 years old in Östhammar municipality born on day 3, 12 or 24 in each month. The invitation procedure was equivalent to random sampling, as well as it allowed for follow up studies. Two individuals had protected identity, nine individuals had moved out of the country and four individuals had denied participation in the prior studies in LongGERD, and these were all excluded. The research team chose to include all participants from the three prior studies who had moved out of the municipality. These participants had been included in the prior studies using the same methodology. This resulted in a final study population of 1924 individuals. Sixty-one individuals of these were unavailable, so 1863 individuals were mailed the questionnaire in late 2011. A total of 1175 (63,1%) responded. See fig. 1.

Serology and biopsies

In the group of the 1175 responders, 1034 were under the age of 80 years and lived within 200 km from the study center, and were eligible for esophagogastroduodenoscopy (EGD). Participants >80 years old were excluded due to it not being considering ethical given their age to conduct such an invasive examination as EGD. Forty-five individuals were excluded due to the preset contraindications for EGD, see fig 2. The remaining individuals were invited by telephone by research assistants, who gave verbal information on informed consent. A total of 402 (42,5%) accepted to participate and signed a written consent form. Out of these, 14 individuals discontinued the process, leaving 388 (40,8%) completing the EGD. See fig. 1.

Randomized
participants in 2011, n=718

Included participants from earlier study 1988/-89 n=901

Included participants from earlier study 1995 n=305

Study population
n=1924, n=1757 < 80 years

Possible to mail the ASQ
n=1863

Not available n=61
Moved abroad, unknown domestic address, reported too ill, declined earlier, cannot read Swedish.

Responded to mailed ASQ
Participants n=1175
n=1073 <80 years

Non-responders n=688

Eligible for EGD
<80 years & live within 200 km n=1034

Excluded n=141
>80 years n=102, lived too far away n=32, not suitable for EGD appointment n=7

Available for EGD n=947

Unreachable n=42
Contraindications n=45

EGD participants n=388

Declined n=545
Dropped out at endoscopy unit n=14

- unstable angina or myocardial infarction
- heart disease or failure
- recent cerebral infarct or bleeding
- mental retardation or dementia,
- psychosis and other severe mental disorders
- anticoagulation therapy or known bleeding tendency
- known esophageal varicose veins alcoholism
- previous upper gastrointestinal surgery (excluding cholecystectomy)
- lung disease with low respiratory capacity
- current malignant disease
- pregnancy

Fig. 1. Inclusion of subjects in the *Helicobacter pylori* Östhammar study. Flow chart of inclusion of study subjects for endoscopy (EGD) comprising drop-out and reasons for drop-out. The subjects were identified based on day of birth in every month (3rd, 12th, 24th), which gives a random selection. Abbreviations: ASQ = Abdominal Symptom Questionnaire, EGD = esophagogastro-duodenoscopy.

Fig. 2. Exclusion criteria in the *Helicobacter pylori* Östhammar study - contraindications for endoscopy. List of previously defined contraindications that excluded potential participants from undergoing endoscopy.

At the day EGD was performed, blood samples for standard blood tests and serology were drawn. Biopsies for histology were taken from multiple sites by five experienced endoscopists, who were unaware of the patient's medical history and *H. pylori* status of the participants. A positive result of *H. pylori* serology reflected whether an individual had encountered the bacteria and had a competent immune response, while biopsies detected an ongoing infection. One of the patients in the study reported having gone through eradication treatment.

Serum stored in -70 degrees C was used for a test panel on levels of IgG class antibodies to H. pylori (GastroPanel, Biohit Plc., Helsinki, Finland). Five blood tubes were drawn, and CRP, Hb, cholesterol, creatinine and ALAT was also analyzed for future studies. Two biopsies were taken from both the antrum and the corpus for H&E staining and W.S. staining, and assessed by the Sydney System by two experiences pathologists. The pathologists were blinded, but afterwards the histology for the participants that were *H. pylori* positive on serology and negative on W.S. staining on histology was reviewed.

Self-rated health

Self-rated health was included in the mailed questionnaire. The question was formulated "How would you rate your general health status?" rated on a five point scale. The response alternatives were *very good, rather good, neither good nor poor, quite poor* or *very poor* (coded 1-5). The first round of questionnaires was sent out in October 2011. The first endoscopies started in beginning January 2012. The individuals did thus respond to their health status some weeks or months earlier than they were examined with EGD and blood samples, depending on how fast they answered and when they were scheduled for EGD. Hypothetical maximum time between questionnaire and EGD were six months. If considering that *H. pylori* could be a chronic infection, this time gap was not considered to be of any significant importance for the analysis. The possibility of new infections occurring during the time gap was very low, since infection most often occurs the first years of life (38). As the self-rated health variable was available from all individuals that responded to the questionnaire (63,1%) it was used to investigate any bias in self-rated health between those who had the EGD and those who did not.

Statistical method

Logistic regression was used when analyzing whether subjects with a lower self-rated health were more prone to go through the EGD investigation, using self-rated health as the independent variable and EGD participation as the dependent variable. Logistic regression analysis was used to test if persons with *H. pylori* positivity reported worse self-rated health by using *H. pylori* status as the dependent variable and self-rated health as the independent variable. This was done for histology and serology positivity respectively. Logistic regression was used as *H. pylori* status and EGD participation respectively were dichotomous variables while self-rated health was an ordinal variable. In these analyses, self-rated health was analyzed as a categorical variable using the response alternative "very good" as reference. All analyses were adjusted for age, gender and educational level. Self-rated health was also tested as a continuous variable in a logistic regression to investigate whether self-rated health, age or education affected the likelihood to go through the EGD.

Ethical considerations

All participants have given oral and written consent. Five blood tubes were drawn and 2 serum and 2 plasma tubes were stored in Uppsala Biobank for future studies. Conventional blood status analysis was made (CRP, hemoglobin, cholesterol, creatinine and ALAT), as well as serology analysis of *H. pylori*. Furthermore was brush test of the esophagus, stomach and duodenum performed at the same sites as the biopsies, for future studies.

Participants were given information on the risks of EGD. The patients underwent a very unpleasant examination, associated with some risks. Sedatives (sublingual diazepam) were offered if patients requested in order to reduce suffering. However, they were treated if pathologies were found, which could be seen as a benefit outweighing the risks for the participants. It could also be an incentive for the subjects to choose to participate which could lead to a selection bias of the population. This possibility was taken into consideration in the data analysis. Approval for the study was obtained from the Ethics Committee of Uppsala University on January 26th, 2011 (Dnr. 2010/443).

Results

Data for self-rated health was missing for 30 out of the 947 who responded to the questionnaire and were eligible for EGD. Educational level data was missing for 16 of these participants, leaving 901 available for analysis. See fig. 3. Similarly self-rated health data, correct biopsy and educational data were missing for 28 of the EGD examined individuals, leaving 360 out of the original 388 available for analysis. See fig. 4. For all of these 360 individuals serology data was available.
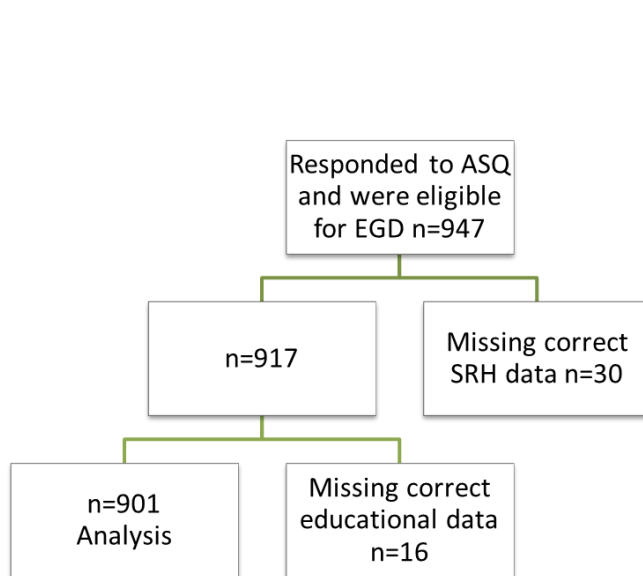
Figure 3. Data fall-out of the total subject group in the *Helicobacter pylori* Östhammar study. Flow chart depicting how many subjects dropped out from statistical analyses due to incomplete responses. Abbreviations: ASQ = Abdominal Symptom Questionnaire, EGD = esophagogastro-duodenoscopy.
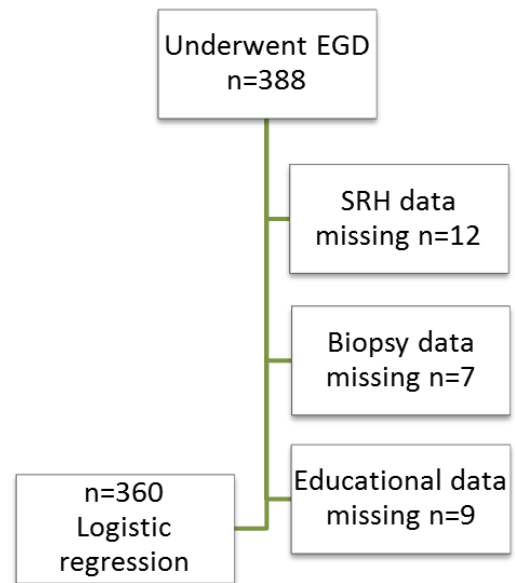
Figure 4. Data fall-out of the group that underwent endoscopy in the *Helicobacter pylori* Östhammar study. Flow chart depicting how many subjects dropped out from statistical analyses due to incomplete responses or missing biopsy data. Abbreviations: EGD = esophagogastroduodenoscopy.

The population characteristics are presented in table 1. Higher education was defined as completed college or university studies. The population who conducted EGD was very similar to the population who declined. The average age for those who are positive for *H. pylori* is slightly higher than in the other groups, which is expected, since infection often occurs in childhood and is correlated to living standards (4).

Table 1. Subject population demographics in the *Helicobacter pylori* Östhammar study.
Population characteristics for all of the endoscopy eligible participants, the population who conducted the endoscopy and the populations who were positive and negative for *H. pylori* on histology and serology.

Population information	All eligible	Done EGD	Positive Histology	Negative Histology	Positive Serology	Negative Serology
Higher education (%)	62,15	62,50	42,86	65,09	48,21	65,13
Women (%)	52,16	51,11	38,09	52,83	48,21	51,64
Mean age (years) and SD (years)	53,42 (SD 14,42)	54,10 (SD 13,21)	59,30 (SD 12,68)	53,41 (SD 13,14)	60,71 (SD 12,34)	52,88 (SD 13,02)
Minimum age (years)	20	20	28	20	28	20
Maximum age (years)	79	79	78	79	78	79
Mean and SD of SRH (1-5)	1,93 (SD 0,79)	1,98 (SD 0,74)	1,83 (SD 0,54)	2,00 (SD 0,76)	1,96 (SD 0,85)	1,98 (SD 0,72)
Total number (n)	901	360	42	318	56	304

Abbreviations: SRH = self-rated health, EGD = esophagogastroduodenoscopy. SD = standard deviation.

Potential selection bias

EGD is an uncomfortable examination to undergo and there is a risk for selection bias: The individuals choosing to participate could have more gastric symptoms and lower self-rated health which might make them more prone to undergo invasive investigations. In the present study self-rated health did not influence participation rate. The data for self-rated health the two different groups is presented in table 2. There is no compelling difference in the population that answered the questionnaire (n=901) and the EGD population (n=360) considering self-rated health when analyzing with logistic regression.

Individuals rating their self-rated health as level 2 and 4 were more prone to agree to EGD than individuals with very good health (self-rated health 2/*rather good* odds ratio (OR)=1.77, p=0.00; self-rated health 4/*quite*

Table 2. Self-rated health of endoscopy willing and non-willing subjects in the *Helicobacter pylori* Östhammar study. Self-rated health status (1-5) for those who conducted the esophagogastroduodenoscopy (EGD) and those who did not.

SRH	No EGD	Did EGD	Total
1	175	86	261
2	260	224	484
3	77	45	122
4	11	14	25
5	7	2	9
Total	530	371	901

poor OR=2.69, p=0.02, adjusted for age, gender and educational level). On the other hand, there was no significant difference for those who rated their health as level 3/*neither good nor poor* or 5/*very poor*. There is thus a slight tendency that those who went through EGD had a worse self-rated health, but it is not convincing. Furthermore, when analyzing self-rated health as a continuous variable there were no increased tendency to go through EGD concerning SHR, age or educational level (data not shown).
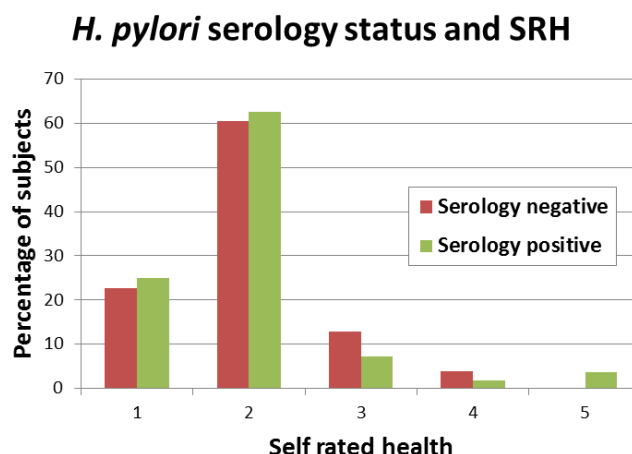
Helicobacter pylori and self-rated health

For the analysis of *H. pylori* status and its association to self-rated health logistic regression was used. The binary dependent variable was *H. pylori* positivity and self-rated health was the independent variable. Gender, age and educational level were included as covariates. Data for self-rated health, serology and histology status are presented in table 3 and 4, and fig. 5 and 6. The group of previously but not currently infected (serology: n=56 minus histology: n=42 gives n=14) was too small to be analyzed. The analysis showed that there was no significant difference in self-rated health between participants that were *H. pylori* positive and participants that were negative, neither for histology nor serology. The odd's ratio, p-value and confidence interval for the different self-rated health levels are shown in table 5. For the analysis of histology, self-rated health 3-5 (neither good nor poor, rather poor and very poor) has been summarized due to too few positive subjects.

Table 3. *Helicobacter pylori* **serology status of partipants in the Östhammar study.** Number of individuals positive and negative on *H. pylori* serology test, presented in relation to their answer of self-rated health (1-5).

SRH	Negative Serology	Positive Serology	Total
1	69	14	83
2	184	35	219
3	39	4	43
4	12	1	13
5	0	2	2
Total	304	56	360

Abbreviation: SRH = self-rated health.
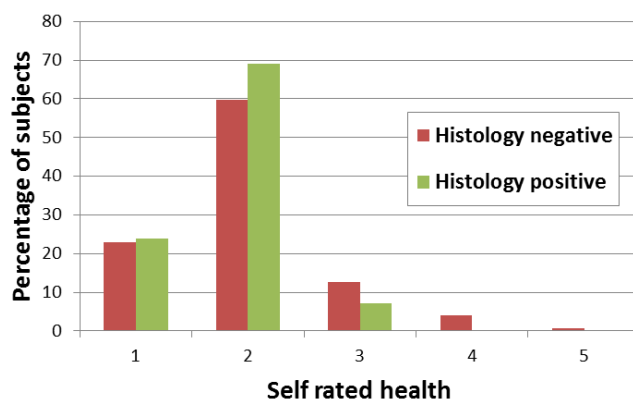

H. pylori serology status and SRH

Figure 5. *Helicobacter pylori* **serology status of partipants in the Östhammar study.** Percentage of total group positive and total group negative on *H. pylori* serology test, presented in each self-rated health (SRH) level. The data is presented in percentage due to the large variation in number size of the group of positive and negative individuals.

Table 4. *Helicobacter pylori* **histology status of partipants in the Östhammar study.** Number of individuals positive and negative on *H. pylori* histology, presented in relation to their answer of self-rated health (1-5).

SRH	Negative Histology	Positive Histology	Total
1	73	10	83
2	190	29	219
3	40	3	43
4	13	0	13
5	2	0	2
Total	318	42	360

Abbreviation: SRH = self-rated health.

H. pylori histology status and SRH

Figure 6. *Helicobacter pylori* **histology status of partipants in the Östhammar study.** Percentage of total group positive and total group negative on *H. pylori* histology, presented in each self-rated health (SRH) level. The data is presented in percentage due to the large variation in number size of the group of positive and negative individuals.

Table 5. Logistic regression analyses of individuals positive for *Helicobacter pylori* on histology and serology in the Östhammar study. Logistic regression analysis was used to test if persons with *H. pylori* positivity reported worse self-rated health by using *H. pylori* status as the dependent variable and self-rated health as the independent variable. Self-rated health was analyzed as a categorical variable using the response alternative "very good" as reference. All analyses were adjusted for age, gender and educational level.

SRH	Odd's ratio	P-value	Confidence interval 95%
Serology			
2	1,05	0,89	0,52-2,11
3	0,52	0,28	0,155-1,73
4	1,68	0,48	0,39-7,20
Histology			
2	1,17	0,7	0,53-2,57
3	0,42	0,21	0,11-1,63

Discussion

It is not known how and if asymptomatic *H. pylori* infection affects the subjective health, as well as to which extent it is a protective or a risk factor for other diseases. This study was aimed at investigating the subjective health of people infected with *H. pylori* compared to those not. The prevalence of *H. pylori* was tested using blood serology and biopsies from the ventricular mucosa, while self-rated health was measured using a questionnaire. The hypothesis was postulated that individuals positive to *H. pylori* biopsy (i.e. ongoing infection), have a lower self-rated health, due to increased risk of mainly dyspepsia and anemia, but also the potential negative impact of low-grade chronic inflammation on self-rated health. In the study, we could however not find any such association that was statistically significant.

The lack of association between self-rated health and *H. pylori* infection in the present study is in line with previous research on the relationship between self-rated health and other "silent" pathogens. In a study from 2009, Steptoe *et al* did not find a correlation between self-rated health and *Chlamydia pneumonia*, cytomegalovirus, *HSV-1*, and seropositivity for Borrelia Ig (39). To the best of our knowledge, this is the first study on self-rated health and *H. pylori*.

Furthermore, asymptomatic pathogens such as *H. pylori* have been investigated concerning mortality. In a previous epidemiological study, Simanek *et al* investigated whether total pathogen burden as well as single and combined burden of *CMV*, *HSV-1*, *HSV-2* and *H. pylori* were associated with all-cause mortality. The study included 6522 individuals and no statistically significant relationship between infectious burden and all-cause mortality was found (40). Even though our study has not investigated the mortality, *H. pylori* infection as a risk factor for gastric cancer and maybe a potentially protective factor for esophageal cancer, and hence mortality, is at the center of clinical decision-making on eradication. Our findings may add to this decision-making, as we cannot find an indication for eradication therapy based on self-rated health. Low ratings of self-rated health are in itself an independent predictor of mortality (33), something that *H. pylori* positive subjects in this study did not show.

On the other hand, our results may seem to be in contrast with previous findings that *H. pylori* was associated with higher Interlekukin-6 (IL-6) (41) – the most widely studied cytokine that has in its turn been associated with worse self -rated health independently of background

factors (33). In the specific study, Nakagawa *et al* found that *H. pylori*-positive individuals with a higher anti-*H. pylori* IgG titer had a higher concentration of IL-6, adjusted for age, sex and atrophic gastritis (β=0,18, p=0,012). According to the authors of the specific study, this suggests that *H. pylori* infection could cause a systemic inflammation, which in its turn could be associated with a worse self-rated health (33). However, the subjects included in the study had come to a clinic because they were worried about *H. pylori*, and could therefore potentially be more symptomatic than the average asymptomatic carrier which we have been investigated in the present study.

We did not have the opportunity to investigate inflammatory factors in our study, but Nakagawa and colleagues' findings are interesting when considering research on cytokines and self-rated health. Undén *et al* have examined the correlation between self-rated health, symptoms (defined as "disease behavior") and cytokines in 174 female consecutive primary health care patients (42). As thought, women with more disease behavior (based on ratings of energy, sleep, appetite, fitness, memory and pain), showed lower self-rated health and higher cytokine levels. Importantly, self-rated health and cytokine levels were associated even in the asymptomatic women, mainly for TNF-α (18-64 years, β=0,29 p<0,001, >65 years β=0,34 p<0,001). Although the cross sectional design of the study precludes any conclusions about causality, it may be plausible that low-grade inflammation without symptoms still can affect the perceived health, which would contrast to our findings.

Strengths and limitations

In the present study we did not find a significant association between *H. pylori* status and self-rated health. The included population was generally very healthy and few individuals were positive for *H. pylori* serology and histology which reduced the power of the statistical analyses. Since EGD is an invasive investigation, 45 subjects were excluded due to contraindications, e.g. for recent myocardial infarction and no serology for *H. pylori* was taken. If these subjects were too sick to be included, their self-rated health would potentially be lower, and this combined with their *H. pylori* status could have been a way to increase the variance in health status among the participants. Furthermore, *H. pylori* and low-grade inflammation as a risk factor for cardiovascular disease is much debated in medical literature as previously discussed in this paper, why individuals suffering from more severe heart disease would have been interesting to include in the study.

Self-rated health is a very well validated measurement, although broad. The different diseases associated with *H. pylori* in both negative and positive manner come with a range of symptoms, which would be interesting to investigate in a more extensive study. Possibly, the *H. pylori* positive subjects are generally more tired, or have more problems concerning their stomach, without it affecting the rough measurement of self-rated health. In the questionnaire used in the inclusion, symptoms like this were asked for using a widely utilized questionnaire called *Short form health survey* (SF-36). Analysis of these answers might have made this study more nuanced in the understanding of the eventual impact of *H. pylori* on the individuals' health. SF-36 is a measure of health related life quality, and not a measure of self-rated health – although the two measurements combined could create a fuller understanding of seemingly asymptomatic *H. pylori* infection.

Furthermore, due to technical problems with the self-rated health questionnaire collection on the day of EGD, self-rated health data from the inclusion questionnaire was used for the analysis instead. This means that the subjects responded to their health status some weeks or months before the EGD and blood samples, with the hypothetical maximum time between the questionnaire and EGD of six months. *H. pylori* is a chronic infection, and it occurs mostly during the first years of life (38), thus this flaw is not thought to influence the results significantly. However, it is methodically not intended and not correct in a cross-sectional study to have such a large time gap between two variables that are hypothesized to be associated.

Another limitation in the study is that we did not measure the subjects' cytokine levels. Since these could be important in the discussion of self-rated health and potential low-grade inflammation, it would have made this study more robust and relevant in its conclusions.

The strength of this study is that the cohort is large. We have used two of the most reliable measuring methods available for detecting *H. pylori*, serology and histology. The specificity and sensitivity of the serology test for discovering *H. pylori* antibodies were 97% and 96% respectively (38), and the pathologists that studied the biopsies were blinded. Another strength of our study is that the population that was investigated with EGD was a generalizable sample compared to the Swedish population.

Clinical significance

The newest Maastricht Guidelines from 2012 state that "*H. pylori*-positive patients with fear of gastric cancer should receive eradication treatment." (2). By stating this, the authors make a strong case for global eradication, i.e. antibiotic treatment of half of the world population – since no one wishes to have cancer. The effect of this is not known. *Klebsiella*, *Candida* and *Streptococcus viridans* are organisms viewed as part of the normal human microbial flora, with the potential of becoming opportunistic pathogens, especially in the elderly or immunocompromised population (43), but there is no attempt to eradicate these from the bodies of worried patients. The comparison is however haltering since these potential pathogens do not cause cancer, but only infections. Nevertheless, the effect on the fecal microbiota of widespread eradication antibiotic use is not known, nor is the risk of *Clostridium difficile* associated diarrhea or subsequent antibiotic resistance. The Maastricht approach or the "test and treat" strategy (4) does not take this into account. The complex biological interplay between humans and the commensal organisms harboring in our bodies is only in the beginning of being understood. Pflughoeft *et al* states: "Human biology can no longer concern itself only with human cells: Microbiomes at different body sites and functional metagenomics must be considered part of systems biology." (44)

Our study has not found any significant association between *H. pylori* positivity and self-rated health. If these patients should be treated with eradication therapy, it would be in order to calm their worries, or to reduce risks of future diseases – not to increase their general health status. However, the potential reduction in risk for esophageal cancer with *H. pylori* infection has to be evaluated compared to the increased risk for gastric cancer. Likewise, although more speculative and with less compelling evidence, should the potentially increased risk for coronary heart disease with *H. pylori* infection be evaluated compared to a potentially protective effect on allergy. Lastly, of course, the risk for peptic ulcer disease with following complications needs to be added into this cost-benefit equation. Which disease of these is affects the most and is the most harmful? A thorough evaluation of these questions is needed.

Future studies

It was originally our intention to compare those individuals positive on serology and not histology, i.e. seroconverted, with those positive on histology, i.e. currently infected. *H. pylori* is generally a life-long infection, (38) but the vast use of antibiotics in modern health care is thought to contribute to "accidental" eradication (37). Unfortunately, the seroconverted group

was too small (n=14) for analysis. The two groups are interesting from different hypotheses. If the hygiene hypothesis for *H. pylori* is true and significant (i.e. *H. pylori* infection in early age helps to educate and train the immune system and prevents autoimmune diseases, see the earlier discussion in this paper) the previously infected individuals could reap the benefits of the infection but after that be protected from the risks of e.g. ulcers, gastric cancers and potentially coronary heart disease. Future studies if potential benefits of *H. pylori* infection are dependent on age when infected and age for eradication would be interesting to see. A study of a larger sample group, and/or a sample group where *H. pylori* is more prevalent than in Östhammar would be needed in order to investigate this question.

The diseases previously studied in relation to *H. pylori* (ulcers, gastric cancer, GERD, esophageal cancer, asthma and allergy, autoimmune diseases, anemia and coronary heart disease) are different regarding *risk for disease* or on *ongoing impact* of the perceived health. The overall question whether *H. pylori* overall is harmful or beneficial for humans cannot be answered in a cross-sectional study investigating solely self-rated health. Eventual impact of dyspepsia, anemia, general feelings of sickness, asthma and allergy could here have been detected, but for diseases like cancer and coronary heart disease a prospective longitudinal study concentrating at risk would be better. Since the study is a part of LongGERD that has run since 1988 (37) this could be a future follow-up study.

In the present study, blood test for hemoglobin (Hb) was also taken together with serology and other standard analyses. Considering *H. pylori's* known correlation to anemia, it would have been interesting to study whether anemia or low Hb was more prevalent in positive individuals. Furthermore, the symptoms of anemia (mainly tiredness and physical fatigue) is associated with lower self-rated health, why the three parameters' relationship would have been interesting to see in further studies.

In our study we have not detected any association between self-rated health and *H. pylori*, which could come from poor variance in the material and thus low power. For future studies it would be interesting to examine whether *H. pylori* infection is correlated to specific extragastric symptoms, which previously have been associated to increased inflammatory factors such as tiredness, pain and depression for example. Moreover, studies investigating risk, and studies concerning perceived health and symptoms using more specific and sensitive methods when living as a carrier with *H. pylori*, would combined come closer to a more complete understanding about *H. pylori* and human coexistence.

For other "silent" pathogens, no correlation has been shown to SRH in previous studies (39). If the hypothesis is that silent pathogens elicit a systemic inflammatory response and that higher levels of circulating inflammatory factors influences the experienced well-being – it does not seem that self-rated health is a measurement sensitive enough to prove it. Furthermore, a study design that measures all three components hypothesized to interplay would be the most accurate for our aim; cytokines, infections burden, symptoms and self-rated health.

Conclusions

We have not found any correlation between *H. pylori* infection and lower or higher self-rated health.

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