HHS Public Access

Author manuscript

Gut. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

Gut. 2018 July ; 67(7): 1239–1246. doi:10.1136/gutjnl-2016-311685.

Dynamics of *Helicobacter pylori* infection as a determinant of progression of gastric precancerous lesions: 16-year follow-up of an eradication trial

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Abstract

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Competing interests None declared.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval Committees on Ethics of Universidad del Valle and Hospital Departamental de Nariño in Colombia; Institutional Review Boards of Louisiana State University Health Sciences Center and of Vanderbilt University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Coded data from all subjects in our study are available for sharing as required by other investigators.

Contributors RMM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: PC, RMM and LEB. Acquisition of data: PC, LEB, MBP, JCB, MCY and JLR. Analysis and interpretation of data: PC, RMM, MCC, MBP and KTW. Statistical analysis: RMM. Drafting of the manuscript: RMM, MCC, MBP, KTW and BGS. Critical revision of the manuscript for intellectual content: all authors. Obtained funding: KTW, PC, LEB, MCY and RMP. Administrative, technical or material support: KTW, PC, LEB, DRM, AGD, MCY, JRG and JLR. Study supervision: PC, LEB and MCY.

Objective—To evaluate the long-term effect of cumulative time exposed to *Helicobacter pylori* infection on the progression of gastric lesions.

Design—795 adults with precancerous gastric lesions were randomised to receive anti-*H. pylori* treatment at baseline. Gastric biopsies were obtained at baseline and at 3, 6, 12 and 16 years. A total of 456 individuals attended the 16-year visit. Cumulative time of *H. pylori* exposure was calculated as the number of years infected during follow-up. Multivariable logistic regression models were used to estimate the risk of progression to a more advanced diagnosis (versus no change/regression) as well as gastric cancer risk by intestinal metaplasia (IM) subtype. For a more detailed analysis of progression, we also used a histopathology score assessing both severity and extension of the gastric lesions (range 1–6). The score difference between baseline and 16 years was modelled by generalised linear models.

Results—Individuals who were continuously infected with *H. pylori* for 16 years had a higher probability of progression to a more advanced diagnosis than those who cleared the infection and remained negative after baseline (p=0.001). Incomplete-type IM was associated with higher risk of progression to cancer than complete-type (OR, 11.3; 95% CI 1.4 to 91.4). The average histopathology score increased by 0.20 units/year (95% CI 0.12 to 0.28) among individuals continuously infected with *H. pylori*. The effect of cumulative time of infection on progression in the histopathology score was significantly higher for individuals with atrophy (without IM) than for individuals with IM (p<0.001).

Conclusions—Long-term exposure to *H. pylori* infection was associated with progression of precancerous lesions. Individuals infected with *H. pylori* with these lesions may benefit from eradication, particularly those with atrophic gastritis without IM. Incomplete-type IM may be a useful marker for the identification of individuals at higher risk for cancer.

Introduction

Helicobacter pylori (*H. pylori*) infection is categorised as a class I carcinogen for the development of gastric cancer (GC).[1] In the pathogenesis of intestinal-type non-cardia adenocarcinoma, *H. pylori*-induced chronic gastric inflammation slowly progresses through the following series of lesions known as Correa cascade: non-atrophic gastritis (NAG), multifocal atrophic gastritis without intestinal metaplasia (MAG), intestinal metaplasia (IM) and dysplasia.[2,3] The latter three are considered premalignant stages, and studies in different populations have shown that the risk of GC increases with the severity of precancerous lesions.[4–6]

Multiple studies have assessed the effect of anti-*H. pylori* therapy on gastric premalignant and malignant lesions, but they have provided conflicting results.[7] A study by Leung *et al* in Chinese individuals reported that, after 5 years of follow-up, *H. pylori* eradication significantly slowed the progression of precancerous lesions.[8] Another trial in China, the Shandong Intervention Trial, demonstrated that anti-*H. pylori* therapy significantly reduced the prevalence and severity of precancerous lesions[9] as well as reduced the incidence, but not the mortality of GC, after 15 years of follow-up.[10] Further analyses stratified by subgroups found that antibiotic treatment was associated with a significant decrease in GC incidence and mortality at ages 55 years and older and a statistically significant decrease in

incidence among those with IM or dysplasia.[11] The latter finding contrasts with the main conclusion of another Chinese study by Wong *et al* that reported significantly decreased GC incidence after a follow-up of 7.5 years after eradication of *H. pylori* only in the subgroup of patients without precancerous lesions at enrolment.[12] More than 40% of the GC cases worldwide occur in China; therefore, it is not surprising that most randomised trials have been conducted in this country. A recent meta-analysis of 24 studies (observational and randomised controlled trials) reported significant benefit in eradicating *H. pylori* in populations where baseline GC incidence was higher than 150 per 100 000 person-years.[13]

Our research group conducted a randomised, placebo-controlled, three-way factorial trial in the Colombian Andes Mountains evaluating the effects of anti-*H. pylori* therapy with or without vitamin supplementation (ascorbic acid and/or beta-carotene) on individuals with gastric precancerous lesions.[14] We found that, after 6 years of follow-up, eradication of *H. pylori* and both vitamin supplements were significantly associated with regression of precancerous lesions.[14] At 12 years of follow-up, the effects of the vitamin interventions were lost, but *H. pylori* eradication remained significantly associated with regression of precancerous lesions.[15] To expand on our previous findings, the aim of this analysis was to evaluate the effect of cumulative time of *H. pylori* infection on progression of gastric lesions over a period of 16 years.

Methods

Study subjects, treatment and follow-up

Details of subject characteristics and results of the 6-year and the 12-year follow-up of our trial were reported previously.[1415] Briefly, 1219 volunteers aged 29–69 years from two Colombian towns (Pasto and Túquerres) in a high-risk area for GC were screened in 1991. Among these, 795 individuals with histological diagnosis of gastric precancerous lesions were randomly assigned to receive 14 days of anti-*H. pylori* therapy (amoxicillin, metronidazole and bismuth subsalicylate, referred to as ‘antibiotics’) or placebo, with or without supplementation for 6 years with beta-carotene and/or ascorbic acid or their corresponding placebos, resulting in eight possible treatment combinations: (1) antibiotics + beta-carotene; (2) antibiotics + ascorbic acid; (3) antibiotics + beta-carotene + ascorbic acid; (4) antibiotics + placebo; (5) placebo + beta-carotene; (6) placebo + ascorbic acid; (7) placebo + beta-carotene + ascorbic acid; and (8) placebos only. Individuals assigned to anti-*H. pylori* therapy who tested positive for *H. pylori* at 3 years of follow-up were treated again for 14 days with amoxicillin, clarithromycin and either omeprazole or lansoprazole. After 6 years of follow-up, the trial was unblinded, and anti-*H. pylori* therapy was offered to those not treated. Individuals were then followed for an additional 10 years (figure 1). Informed written consent was obtained from all participants. The institutional review boards of Louisiana State University Health Sciences Centre and of Vanderbilt University and the Committees on Ethics of Universidad del Valle and Hospital Departamental de Nariño in Colombia approved the protocol of this study.

Histopathology and *H. pylori* infection assessment

Upper GI tract endoscopies were performed by the same two experienced gastroenterologists at baseline and at 3, 6, 12 and 16 years of follow-up. At the time of each endoscopy, four gastric mucosa biopsy samples were obtained for histology: antrum (lesser and greater curvature, both within 5 cm from the pylorus), *incisura angularis* and one from corpus (anterior wall). The specimens were fixed in formalin and embedded in paraffin, and 4-micron-thick sections were stained with H&E for regular histology and with the modified Steiner technique[16] to detect *H. pylori*. Special mucin stains (Alcian blue pH 2.5/periodic acid Schiff and high-iron diamine/Alcian blue) were performed for the detection or classification of IM into complete and incomplete types[1718] when needed, but most cases were classified on the basis of morphology on H&E-stained sections.[19] Histological sections from all gastric tissues collected at 16 years were examined independently by two experienced pathologists (MBP and JCB) unaware of both treatment assignment and the results of prior histopathological evaluations. All biopsy sets of individuals with discordance in assignment of the histological diagnosis between the two pathologists, as well as with discordance in the grading of dysplastic lesions, were reviewed by a third experienced senior pathologist (PC) to reach a consensus.

Histological diagnosis categories—Gastric lesions were classified according to internationally accepted guidelines for the diagnosis of NAG, MAG, IM, dysplasia and GC.[20–22] We assigned numerical codes to each diagnostic category following the Correa cascade: 1=normal, 2=NAG, 3= MAG, 4=IM, 5=dysplasia and 6=GC. The most advanced lesion observed in the set of biopsies of a given subject at each endoscopic procedure was considered as the histological diagnosis.

Interobserver agreement—To determine interobserver agreement in histology assessment over the 16-year study period, 100 individuals were randomly selected from the study set of 456 who attended the 16-year follow-up visit. One of the pathologists (MBP) blindly re-evaluated the original histological sections from all sets of biopsies collected at baseline and at 6, 12 and 16 years. Among the 100 selected individuals, 86 attended the 6-year visit and 96 attended the 12-year visit. A total of 382 sets of biopsies were re-evaluated for histological diagnosis and the results were compared with the previous consensus diagnoses. The re-evaluation of the sections of the 16-year follow-up was performed at least 1 year after the initial review. Overall agreement had a kappa value of 0.64 (95% CI 0.59 to 0.72), with year-specific values of 0.64, 0.69, 0.72 and 0.53 for baseline, 6, 12 and 16 years, respectively. The highest kappa value was observed at year 12 and the lowest was observed at year 16, with no significant temporal trend and overlapping CIs.

Correa histopathology scoring system—For a more detailed analysis of progression, we used our previously developed histopathology score,[1523] which we have named herein the Correa Histopathology Scoring System, and referred to from now on as Correa score (table 1 and online supplementary table 1). Based on the above-described histological diagnosis categories (coded from 1 to 6), the Correa score assigns numerical values to the various levels of extension, type and grade of the precancerous lesions MAG, IM and

dysplasia. All biopsies (antrum, *incisura angularis* and corpus) in each set are equally considered for the score without grouping them by anatomic location.

For individuals with MAG or IM, the extension of both atrophy and IM was scored in each biopsy sample (0=absent; 1=mild; 2=moderate; 3=marked) according to the visual analogue scales of the updated Sydney System.[21] To better reflect the heterogeneity of the precancerous lesions, the Correa scoring system adds the following values to the diagnosis of MAG (coded as 3): indefinite for atrophy (0.25), mild (0.50), moderate (0.75) and marked (1.0), resulting in scores from 3.25 to 4.0. Similarly, the Correa scoring system adds values to the diagnosis of IM (coded as 4) according to type and extension. IM type includes four categories: complete (0.1), mixed predominantly complete (0.2), mixed predominantly incomplete (0.3) and incomplete (0.4). IM extension is graded 0–3 (0=absent; 1=mild; 2=moderate; 3=marked) in each biopsy and the average of each set of biopsies is given a value of 0.2, 0.4 or 0.6, for mild, moderate or marked, respectively. Values for type and extension are added to the histological diagnosis of IM, resulting in scores ranging from 4.3 to 5.0. Dysplastic lesions are classified according to the Padova International Classification for Dysplasia.[22] The Correa scores for dysplasia (coded as 5) are indefinite for dysplasia (5.25), low grade (5.50) and high grade (5.75) (table 1 and online supplementary table 1). For MAG, IM type and dysplasia, the most advanced grade seen (maximum value) among all biopsies was used.

Operative link for gastritis assessment (OLGA) and operative link on gastric intestinal metaplasia assessment (OLGIM) staging systems—To evaluate the OLGA and OLGIM systems[2425] as predictors of progression to GC, all sets of baseline biopsy specimens were reclassified by a single experienced pathologist (MBP). Review of the histological sections was done unaware of the treatment assignment and the results of subsequent histopathological evaluations. The extent of atrophic-metaplastic lesions (for OLGA) and that of IM (for OLGIM) were scored as percentages in all antrum, *incisura angularis* and corpus biopsies. Combined scores of antrum and *incisura angularis* biopsies represented the antral compartment. Stages were obtained combining antrum and corpus scores.

Statistical analysis

For each subject, the cumulative time exposed to *H. pylori* was calculated by adding the number of years infected during all intervals between endoscopic visits. For each interval, the time infected with *H. pylori* was estimated as: (1) the number of years between two positive endoscopies or (2) the midpoint in years between a negative and a positive (0–3 years was 1.5; 3–6 years was 1.5; 6–12 years was 3; and 12–16 years was 2). All times were added, even though there may have been a lapse or interim period of no exposure.

Poisson regression models were used to compute incidence rates of reinfection/recrudescence and clearance of *H. pylori*. Multivariable logistic regression models were used to estimate the risk of progression to a more advanced diagnosis (versus no change/regression), as well as the risks of GC by OLGA and OLGIM systems (stages III and IV

versus others) and by IM type (incomplete versus complete). For these analyses, individuals with both types were classified as having incomplete-type IM.

Generalised linear regression models with a Gaussian link were used to estimate the effect of *H. pylori* cumulative exposure on the difference in the histopathology score from baseline to 16 years, adjusting for interventions and potential confounders. The following independent variables were included in the initial model: randomised treatment (ie, antibiotics plus beta-carotene and ascorbic acid, antibiotics plus beta-carotene, antibiotics plus ascorbic acid, antibiotic plus placebo), cumulative time of *H. pylori* exposure and *H. pylori* status at 16 years. Covariates were age, gender, town and baseline diagnosis. The initial model was fitted with randomised treatment without any post-randomisation variables. *H. pylori* status was modelled as follows: (1) cumulative time exposed, (2) last status and (3) status at each endoscopic visit (3, 6, 12 and 16 years). Variables were removed from the final model if they were not statistically significant at the 0.05 level. All statistical analyses were performed using STATA V.14 (STATACorp).

Results

Of the 795 randomised individuals at baseline, 456 returned for the 16-year follow-up visit. Characteristics of the study subjects at each endoscopic visit are presented in table 2.

Person-time rates of reinfection/recrudescence and clearance among individuals that did not receive anti-*H. pylori* treatment at baseline or immediately after the 6-year follow-up were computed for three periods: from baseline to 6 years; from 6 to 12 years; and from 12 to 16 years (table 3). Reinfection/recrudescence rates did not change significantly over time, but the clearance rate doubled between the first 6 -year period and the following 6 years. After the 12-year endoscopic visit, individuals positive for *H. pylori* were referred to their primary care physicians, since a new round of treatment was not part of the study protocol and therefore not recorded. The higher clearance rate observed between 12 and 16 years compared with previous periods may be in part explained by the inclusion of individuals that may have received anti-*H. pylori* therapy during this period.

Prevalence of histological lesions by anatomic subsite

To assess the distribution of the lesions by anatomic subsite in individuals attending both baseline and 16 years endoscopy visits, the most advanced histological lesion was considered separately for antrum (one biopsy from *incisura angularis* and two from antrum) and corpus (one biopsy). As presented in figure 2, at baseline, 100% of the individuals had precancerous lesions (MAG, IM or dysplasia) in the antrum and 58% had precancerous lesions in the corpus. At 16 years, 82% and 54% of individuals had precancerous lesions in the antrum and in the corpus, respectively. The antral prevalence of NAG, MAG, IM and dysplasia (including indefinite for dysplasia) at baseline was 0%, 27%, 64% and 9%, respectively. Comparatively, the corresponding antral prevalence at 16 years was 18%, 10%, 56% and 16%, indicating mainly a reduction in MAG diagnosis accompanied by an increase in NAG. The corpus prevalence of NAG, MAG, IM and dysplasia at baseline was 42%, 38%, 20% and 0%, respectively. As a comparison, the corresponding corpus prevalence at 16

years was 46%, 30%, 24% and 1%, showing also a decrease in individuals with MAG diagnosis.

Progression of precancerous lesions using the histological diagnosis

Using the histological diagnosis categories (normal, NAG, MAG, IM, dysplasia and GC), we performed analyses of the progression of precancerous lesions (versus no change/regression) using logistic regression models. Progression was defined as the change to a more advanced histological diagnosis between baseline and 16 years. The first model included the amount of time that individuals were exposed to *H. pylori*. Among individuals who were always positive for *H. pylori*, the probability of progression over a 16-year period was 0.59 (95% CI 0.48 to 0.71) for those with MAG at baseline and 0.69 (95% CI 0.60 to 0.77) for those with IM at baseline. In contrast, the probability of progression among individuals who cleared the infection at baseline and were always negative thereafter was 0.34 (95% CI 0.22 to 0.46) and 0.44 (95% CI 0.34 to 0.54) for those with MAG and with IM at baseline, respectively. These differences in progression due to the presence of *H. pylori* were statistically significant (p=0.001). In a second model, we assessed the progression of precancerous lesions by baseline anti-*H. pylori* treatment assignment (ie, intention-to-treat analysis), without adjusting for *H. pylori* status, and we found no significant effect at 16 years (p=0.287).

Progression of precancerous lesions using the Correa score

Correa score and randomised treatment—A multivariable generalised linear model of the difference in the Correa scores between baseline and 16 years found that individuals in two of the four groups who received anti-*H. pylori* therapy at baseline had a significantly lower score when compared with placebo. The average difference in the score for those randomised to antibiotics plus beta-carotene and ascorbic acid was −0.48 (95% CI −0.11 to −0.58; p=0.011), while the average score difference for those randomised to antibiotics plus beta-carotene was −0.42 (95% CI −0.25 to −0.48; p=0.032).

Correa score and baseline histological diagnosis—Baseline diagnosis also had a statistically significant effect on the difference of score between baseline and 16 years (p<0.001). Independent of covariates and randomised treatment, individuals who started with MAG changed the most during 16 years of follow-up (−0.20 net regression of Correa score; 95% CI −0.11 to −0.39) with less change for IM (−0.07 regression; 95% CI −0.19 to 0.03).

Correa score and *H. pylori* infection status—Introducing the *H. pylori* status at 6, 12 and 16 years of follow-up, separately or together, into the above-mentioned multivariable model made the association of intervention with the score non-significant, indicating that the effect of intervention was mainly explained by *H. pylori* clearance. The association of baseline diagnosis with the difference in the Correa score between baseline and 16 years remained statistically significant, and the *H. pylori* status at 16 years made the other two (*H. pylori* status at 12 and 6 years) not significant when evaluated together in the same model. The effect of *H. pylori* status at 16 years on the difference in Correa score was statistically significant, with individuals infected at 16 years having a net progression of 0.22 (95% CI 0.08 to 0.35), while those negative for infection showed a net regression of −0.25 (95% CI

−0.12 to −0.36); p<0.0001 for the comparison between infected and non-infected individuals. Average differences in scores between baseline and 16 years by *H. pylori* status at 12 and 16 years are shown in figure 3. Individuals who were *H. pylori*-negative at 12 and 16 years had a net regression of −0.34 units (on the average score), while the individuals that were *H. pylori*-positive at 12 and 16 years had a net progression of 0.26 units; p<0.01 for the comparison between these two groups of individuals.

Adding cumulative time of *H. pylori* exposure to a model with age, gender, town, randomised treatment, 16-year *H. pylori* status and the interaction between cumulative time of *H. pylori* exposure and baseline diagnosis resulted in a significant effect for cumulative time of *H. pylori* exposure, with an average estimated change of 0.20 score units/year (95% CI 0.12 to 0.28; p<0.0001). Therefore, our model estimated that continuous exposure to *H. pylori* during 16 years might result in a change of 3.2 units (95% CI 1.8 to 4.5) for those who started with less severe lesions.

In the same multivariate model, *H. pylori* status at 16 years was significantly associated with progression of Correa scores, even after adjusting for cumulative time of *H. pylori* exposure (0.34 units; 95% CI 0.08 to 0.60; p=0.011). Moreover, there was a significant interaction between cumulative exposure to *H. pylori* and baseline diagnosis, with the effect of *H. pylori* infection being greater at the less severe baseline diagnoses and increasing with the passage of time. The effect of cumulative *H. pylori* exposure was greater for MAG and less marked for IM and dysplasia. The net difference on the score was 0.78 units (p<0.001) for IM and 0.29 units (p=0.395) for dysplasia.

Figure 4 represents the average difference in Correa scores by baseline diagnosis and *H. pylori* status at 16 years. As a specific theoretical example, a 65-year-old subject at baseline who was randomised to placebo, never cleared the *H. pylori* infection, was offered treatment at the end of the trial (6 years) but did not take it, was still positive at the 16-year biopsy and started as a mild MAG (Correa score 3.5) then progressed 1.1 units (95% CI 0.74 to 1.47) to IM (Correa score 4.6). In contrast, a theoretical 40-year-old subject, who was randomised to antibiotic therapy, cleared the *H. pylori* infection, was negative at 3, 6, 12 and 16 years and started as a mild MAG (Correa score 3.5) then regressed −0.83 units (95% CI −1.2 to −0.48) to NAG. Regarding other baseline covariates, there were no significant differences by gender and town.

GC incidence

Our trial is not powered to detect a significant difference in GC incidence among the intervention groups. However, during the 16 years of follow-up, 10 individuals (seven males, three females) developed GC; of these, five had been randomised to anti-*H. pylori* therapy and five had been randomised to placebo. Individuals who developed GC were significantly older at baseline than the set of randomised individuals who did not develop GC (56 vs 51 years, p=0.04). All individuals who progressed to GC had IM (n=7) or dysplasia (n=3) as the most advanced lesion at baseline, with an average baseline Correa score significantly higher than that of the individuals who did not develop GC (5.0 vs 4.2, p=0.004). Six of the seven individuals with IM at baseline had mixed (complete and incomplete) types, and one had only complete type, although extensive. Among the three individuals with dysplasia at

baseline, two had lesions classified as indefinite for dysplasia and one had low-grade dysplasia. All dysplastic lesions were observed in a background of incomplete or mixed IM. The *H. pylori* status at each endoscopic visit of the individuals who developed GC is included in online supplementary table 2.

IM subtype at baseline and progression to GC

Rate of progression to GC during the 16 years among individuals with complete IM at baseline was 0.6% (95% CI 0% to 1.7%), while the rate among those with incomplete IM at baseline was 6.1% (95% CI 1.9% to 10.1%). The OR of GC was 11.3 (95% CI 1.4 to 91.4) among those with incomplete IM when compared with those with complete IM. We also found that the proportion of individuals with incomplete-type IM increased significantly ($p<0.001$) with OLGIM stages: 27%, 73%, 96% and 83% for consecutive stages I to IV.

Progression to GC according to OLGA and OLGIM stages at baseline

A total of 396 sets of baseline biopsies were classified according to the OLGA system and a total of 402 sets of baseline biopsies were classified using the OLGIM system, after excluding 10 individuals with low-grade dysplasia at baseline. For all analyses, stages 0, I and II were considered low risk, and stages III and IV were considered high risk in both systems. There were no individuals with OLGA stage 0 at baseline, as all participants had at least a histological diagnosis of MAG.

Individuals with high-risk OLGA stages at baseline were 19.9 times (95% CI 2.46 to 160.9; $p=0.005$) more likely to progress to GC compared with those with low-risk stages. An alternative analysis for linear trend from OLGA stages I to IV was also significant ($p\leq0.0001$), with an OR of 5.4 (95% CI 1.96 to 14.9) for a change of one stage to another. In addition, the GC progression rate for low-risk OLGA stages was 0.4% (95% CI 0% to 1.1%) and that for high-risk OLGA stages was 6.7% (95% CI 2.2% to 11.2%).

Using the OLGIM system, individuals with high-risk stages were 38.2 times (95% CI 7.62 to 191.3; $p<0.0001$) more likely to progress to GC as compared with those with low-risk stages. An alternative analysis for linear trend from OLGIM stages 0 to IV was also significant (OR, 4.0; 95% CI 2.2 to 7.4; $p<0.0001$). Progression rates to GC were 0.55% (95% CI 0% to 1.3%) for low-risk stages and 17.5% (95% CI 5.7% to 29.2%) for high-risk stages.

Discussion

The long-term follow-up of our Colombian trial has allowed us to evaluate the effect of the dynamics of *H. pylori* infection on progression of gastric lesions. Individuals who were continuously infected with *H. pylori* for 16 years had a significantly greater probability of progression to a more advanced diagnostic category along the precancerous cascade than those who cleared the infection and remained negative after baseline. Consistent with our analysis at 12 years of follow-up,[15] and using the Correa score, our current study shows that continuous exposure to *H. pylori* infection for 16 years led to a significant progression of precancerous lesions. We found a significant interaction between cumulative time of exposure to *H. pylori* and baseline diagnosis, with the effect of total time of exposure on the

progression of the Correa score being greater for the group of individuals with MAG, less marked for those with IM and not significant for those with dysplasia (including the category indefinite for dysplasia). Aside from total time with infection, *H. pylori* status at the 16-year time point had an independent effect. The Correa score in individuals who were *H. pylori*-negative at 16 years was significantly reduced, while the score in those who were *H. pylori*-positive at 16 years was increased.

Exposure to *H. pylori* infection has not been stable in our cohort, as the reinfection/recrudescence rate has hovered around five new infections per 100 person-years of exposure. The rate of *H. pylori* clearance in individuals who did not receive anti-*H. pylori* therapy increased from 2 per 100 person-years during the 6 years after the initiation of the trial to 4 per 100 person-years during years 6–12 of follow-up. The overall *H. pylori* clearance rate during years 12–16 was almost double that of the rate in the preceding 6-year period, but this rate includes all individuals, whether treated or not after the 12-year visit. Although 25% of the cohort was lost to follow-up during years 12–16, those returning for follow-up were a representative subset of the initial randomised group, as there were no significant differences in demographic variables or in the distribution of baseline diagnoses.

H. pylori is the single most important risk factor for non-cardia GC, and current international clinical guidelines support *H. pylori* eradication in individuals at high risk for GC.[26–28] However, there is controversy about the efficacy of anti-*H. pylori* therapy for the prevention of progression along the carcinogenesis cascade in the presence of advanced precancerous lesions. Data from animal models suggest that antibiotics are highly effective at preventing progression to GC when administered during early stages of the *H. pylori* infection, but still they remain effective in presence of dysplastic lesions.[2930] Results from the 15-year follow-up of the Shandong Trial[11] showing the benefit of *H. pylori* treatment in reduction of GC incidence in individuals with advanced precancerous lesions suggest that (1) even a short-term interruption of the *H. pylori* infection can slow the progression to cancer and that (2) anti-*H. pylori* treatment may modify progression of precancerous lesions through mechanisms unrelated to *H. pylori*, perhaps by eliminating other members of the stomach microbiome that may be critical during later stages of progression to cancer.[1131] These observations that *H. pylori* eradication may be beneficial in advanced precancerous lesions contrast with our current findings and those by Lee *et al*[32] that suggest improvement occurs in individuals with early precancerous lesions.

Regarding the distribution of histological lesions by anatomic subsite, a greater prevalence of precancerous lesions was observed in the antral compartment (antrum and *incisura angularis* biopsies) than in the corpus. This finding is consistent with previous studies by our group and others.[3334] During the 16-year period, the overall prevalence of MAG in the antrum showed a decrease accompanied by an increase in NAG. However, the prevalence of the more advanced lesions (IM and dysplasia) did not decrease, considering that all dysplastic lesions, from any gastric location, were observed in a background of IM. Lesions in the corpus displayed similar trends.

It should be emphasised that our trial is not powered to detect differences in GC risk among intervention groups. During the 16-year follow-up, ten individuals developed GC. All of

them had IM or dysplasia as the most advanced lesion at baseline, with an average baseline Correa score significantly higher than that of the individuals who did not develop GC. Staging systems (OLGA and OLGIM) have been proposed for the stratification of the gastritis-associated GC risk.[2425] Multiple studies have established an association between advanced (III and IV) OLGA or OLGIM stages with an increased GC risk.[243536] In our cohort, high-risk (III and IV) stages of both systems at baseline were significantly associated with risk of progression to GC.

IM is the most frequently identified gastric precancerous lesion, but the majority of individuals with IM do not develop GC. IM has long been recognised as a heterogeneous lesion,[171837] and multiple studies support IM subtyping in the assessment of GC risk.[3839] Consistent with previous evidence, in our study, the risk of progression to GC was significantly higher for individuals with incomplete-type IM than in those with only complete-type IM at baseline. In addition, similar to the result of another study,[40] we found a significant association between the prevalence of incomplete-type IM and increasing OLGA stages.

The strengths of the current study include its long period of observation and the blinded assessment of histopathology by a consistent group of experienced pathologists. A limitation is that, given that gastric precancerous lesions have a multifocal presentation, we cannot exclude the possibility of diagnosis misclassification due to sampling. Although the *H. pylori* status was determined by combining results from all biopsies (antrum, *incisura angularis* and corpus), low bacterial density and/or its uneven distribution may have led to false-negative results, especially in individuals with extensive IM. Therefore, our finding of significant progression of precancerous lesions in the presence of *H. pylori* infection might be an underestimation. Other limitations include lack of information about environmental factors throughout the course of the study, difficulty in establishing the precise timing of reinfection/recrudescence, the possibility of unreported use of anti-*H. pylori* therapy prescribed out of the trial and the lack of records of anti-*H. pylori* therapy after the 12-year follow-up. Although we did not observe any differences in the demographic features and other available variables between individuals at baseline and 16 years, we cannot rule out that individuals at the 16-year visit may have been more motivated to attend the follow-up, potentially introducing a selection bias. Finally, because the study has been conducted in a region with high incidence of GC in the Andes Mountains, its conclusions may not apply to populations in other regions with different geographical conditions, *H. pylori* strains, microbiome profiles and/or host susceptibility to the disease.

In conclusion, long-term exposure to *H. pylori* was associated with progression of precancerous lesions, with the greatest detrimental effect in individuals with the least advanced baseline lesions. These findings suggest that infected individuals may benefit from successful *H. pylori* eradication, as an adjunct to endoscopic surveillance. In addition, our study supports the presence of incomplete-type IM as a useful marker for the identification of individuals at higher GC risk. Finally, there is a need for a consistent and standardised pathology reporting system of precancerous lesions, including anatomical location and extension as well as subtyping of IM. This information is critical for the improvement of clinical guidelines for the surveillance of individuals at high risk for GC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank all the participants in this study and the members of our research team in Colombia. We dedicate this report to the memory of Dr Guillermo Zarama, primary study endoscopist, who was a devoted professional and a community health worker. We are deeply appreciative of his invaluable contribution.

Funding This work was supported by US National Institutes of Health grants P01CA028842 (KTW, PC and DRM), R01DK053620 and R01AT004821 (KTW), R01CA190612 (KTW and DRM), P01CA116087 (RMP and KTW), R01CA077955 and R01DK058587 (RMP), UL1RR024975 (Vanderbilt CTSA, Pilot Project to KTW) and 5P30 DK058404 (Vanderbilt Digestive Disease Research Center; RMP and KTW); Merit Review Grant I01BX001453 from the Office of Medical Research, Department of Veterans Affairs (KTW); and the Pasto Cancer Registry of the Centro de Estudios en Salud, Universidad de Nariño, Colombia. MCC is supported by funds from the intramural research program of the US National Institutes of Health, National Cancer Institute.

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Significance of this study

What is already known about this subject?

- The multi-step nature of gastric carcinogenesis represents an opportunity for early detection and intervention to prevent progression of premalignant lesions.
- Most randomised trials evaluating the effect of anti-*Helicobacter pylori* therapy on the progression of precancerous lesions have been limited by short follow-up periods.
- Among individuals with intestinal metaplasia (IM), the risk of progression to gastric cancer may vary by IM type.

What are the new findings?

- Sixteen years of cumulative exposure to *H. pylori* led to significant progression of precancerous lesions.
- Benefits of *H. pylori* eradication were greater in individuals with atrophic gastritis (without IM) than in those with IM at baseline.
- In a high gastric cancer risk Latin American population, individuals with incomplete-type IM at baseline had a higher risk of progression to gastric cancer as compared with those with only the complete type.

How might it impact on clinical practice in the foreseeable future?

- The detrimental effect of long-term continuous *H. pylori* exposure provides additional evidence that effective anti-*H. pylori* therapy should be administered, as an adjunct to endoscopic surveillance, in individuals with IM.
- Individuals with incomplete-type IM should be considered at higher risk for gastric cancer than those with only the complete type, and they may require more frequent endoscopic surveillance and more extensive gastric mapping.

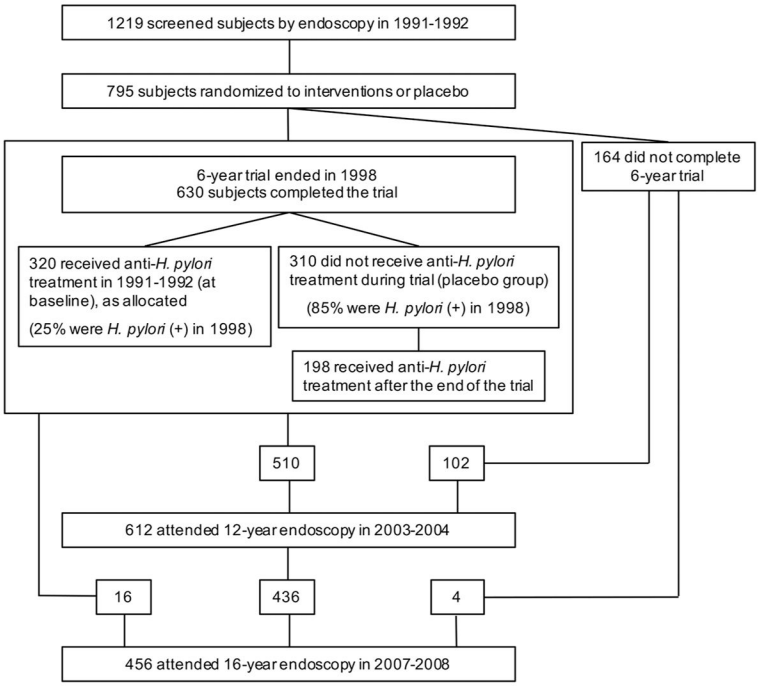

Figure 1.
Flow chart of study participants. *H. pylori, Helicobacter pylori.*

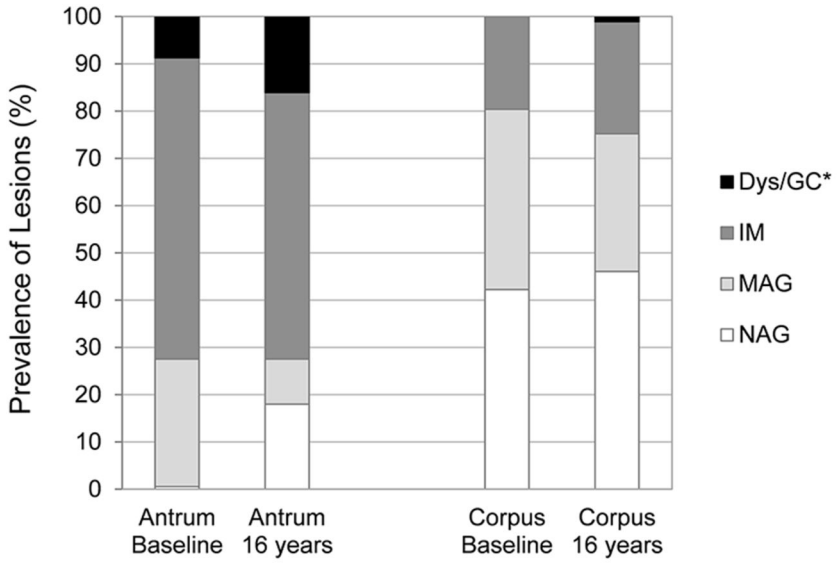

Figure 2.

Prevalence of the most advanced histological lesion by anatomic subsite at baseline and 16 years of follow-up. *Only one GC (located in the antrum) was diagnosed at the 16-year follow-up. Test for trend in ordered diagnostic categories comparing baseline versus 16 years: antrum, $p<0.001$; corpus, $p<0.001$. Dys, dysplasia; GC, gastric cancer; IM, intestinal metaplasia; MAG, multifocal atrophic gastritis without intestinal metaplasia; NAG, non-atrophic gastritis.

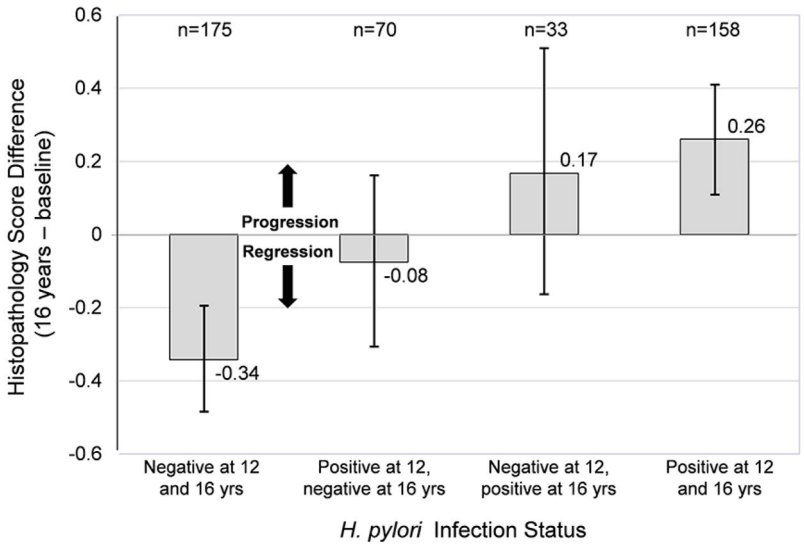

Figure 3.

Average differences in histopathology scores between baseline and 16 years of follow-up by *H. pylori* status at 12 and 16 years. Bars indicate 95% CIs. *H. pylori, Helicobacter pylori.*

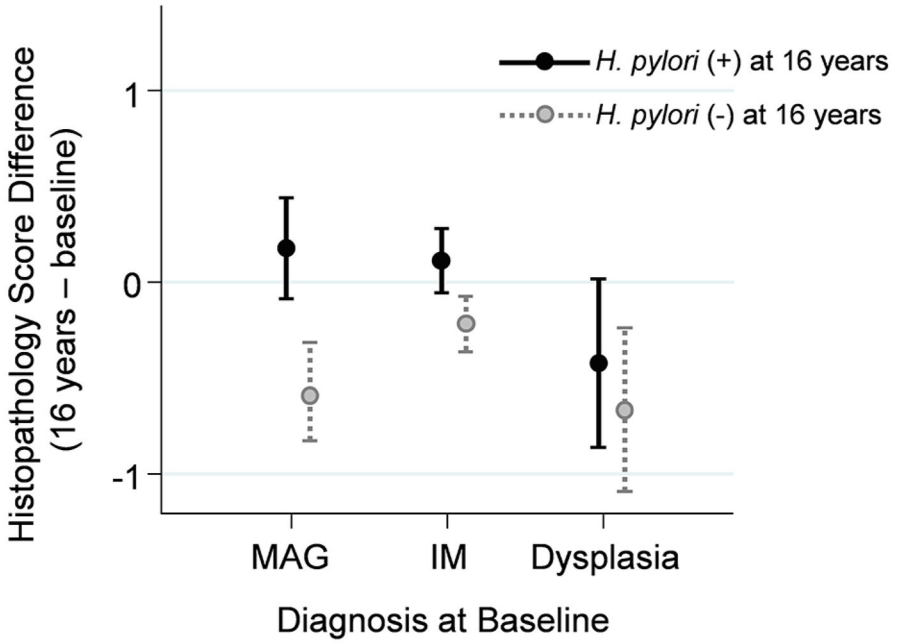

Figure 4.

Average differences in histopathology scores between baseline and 16 years of follow-up by histological diagnosis at baseline and *H. pylori* status at 16 years (76% of dysplasias at baseline were in the category indefinite for dysplasia, 23% were low grade and 1% were high grade). Bars indicate 95% CIs. *H. pylori, Helicobacter pylori;* IM, intestinal metaplasia; MAG, multifocal atrophic gastritis without intestinal metaplasia.

Table 1

Correa Histopathology Scoring System values according to the histological diagnosis categories

Histological diagnosis	Correa Histopathology Scores (range)
1. Normal	1
2. NAG	2
3. MAG	3.25–4.00 *
4. IM	4.30–5.00 *
5. Dysplasia	5.25–5.75 *
6. Gastric Cancer	6

* Assignment of values within these ranges is described in Methods and in online supplementary table 1.

IM, intestinal metaplasia; MAG, multifocal atrophic gastritis without intestinal metaplasia; NAG, non-atrophic gastritis.

Table 2

Characteristics of the participants at baseline and during follow-up

Characteristics	Baseline n=795	6 years n=630	12 years n=612	16 years* n=456
Age in years, mean±SD	51±9	57±8	62±8	67±8
Males, n (%)	362 (46)	284 (45)	274 (45)	205 (45)
H. pylori positive, n (%)	773 (97)	345 (55)	322 (53)	201 (44)
Anti-*H. pylori* therapy†, n (%)	394 (50)	198 (31)	n/a	n/a

*p Value was not significant for age and gender comparisons between baseline and 16 years.

†50% of all individuals were randomised to receive eradication therapy at baseline and the other 50% were offered it at the end of the 6-year trial.

H. pylori, Helicobacter pylori.

Table 3

Incidence rates and 95% CIs of *H. pylori* reinfection/recrudescence and clearance

Incidence rates	Baseline to 6 years	6 to 12 years	12 to 16 years
Reinfection/recrudescence	5.4 per 100 PY* (3.7 to 7.8)	5.8 per 100 PY† (4.5 to 7.4)	4.0 per 100 PY‡ (2.8 to 5.6)
Clearance	2.0 per 100 PY§ (1.5 to 2.8)	4.2 per 100 PY¶ (2.5 to 6.9)	7.4 per 100 PY** (5.8 to 9.4)

*,†,‡Rate among those negative at 3, 6 and 12 years, respectively.

§Rate among those positive at baseline and not treated for *H. pylori* at baseline.

¶Rate among those positive at 6 years and not treated for *H. pylori* at 6 years.

**Rate among those positive at 12 years. Treatment after the 12 year endoscopy was not part of the protocol study and therefore not recorded. This rate includes individuals that may have received treatment between the 12-year and 16-year visits.

H. pylori, Helicobacter pylori; PY, person-years.