The association of acute aortic dissection with *Helicobacter pylori* virulence specific serotypes: Distinct diversity of systemic antibodies to CagA and VacA genotypes

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Background. *Previous studies reported an association between chronic Helicobacter pylori infection and cardiovascular disease; however, controversy still exists regarding the presence of bacterial genomic material in atherosclerotic plaques. Currently, the genetic polymorphisms of H. pylori have been investigated and many virulence factors have been identified. No one has tried to associate these polymorphisms with aortic dissections. This study evaluated whether more virulent strains of H. pylori represent a risk factor for acute ascending aorta dissections.*
Methods. *The serologic status for H. pylori and type I strains were determined in 100 patients who underwent operative repair of acute, ascending aorta dissection and in 100 population-based control subjects matched fully for clinical, demographic, and socioeconomic characteristics. The specimens from dissected aorta were evaluated to identify the presence of bacterial genomic material in surgical patients.*
Results. *No evidence of genomic material from H. pylori was found in the specimens. The prevalence of positive H. pylori serology was greater in patients than in controls (72 vs 50) with an adjusted odds ratio 2.8 (95% confidence interval, 1.8–4.1; P = .006). Patients with aortic dissection also had a greater prevalence of vacuolating cytotoxin gene subtypes s1m1 (73% vs 31%) with an odds ratio of 6.0 (95% confidence interval, 3.1–11; P < .001). Patients who were positive for vacuolating cytotoxin gene subtypes s1m1 were similar in demographic and clinical features compared with other patients.*
Conclusion. *The findings provide support for the hypothesis that an association exists between the more virulent type I strains of H. pylori (vacuolating cytotoxin gene subtypes s1m1) infection and acute aortic dissection. The mechanism(s) underlying the association remain to be elucidated. (Surgery 2011;149:240-6.)*

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MULTIPLE EXPERIMENTAL AND CLINICAL STUDIES have suggested that infection by *Helicobacter pylori* may have a role in the pathogenesis of cardiovascular diseases.[1-4] The possible mechanistic link between chronic *H. pylori* infection and cardiovascular diseases has been debated intensely. Previous studies investigating the presence of *H. pylori* organisms or genomic material from the pylori bacteria within arterial wall or atherosclerotic plaques provided contradictory results.[5-9] Conversely, other

seroepidemiologic studies suggested that systemic inflammatory diseases caused by chronic infection with *H. pylori* may have a role in the pathogenesis of ischemic heart disease. Mendall et al[1] first reported serologic evidence of a link between *H. pylori* seropositivity and coronary heart diseases.[1] *H. pylori* seemed to be a possible independent risk factor for coronary heart disease. More recent studies produced conflicting findings that were explained, at least in part, by the inclusion of different risk factors and by the genetic polymorphism of *H. pylori*. Currently, evidence suggests that distinct variants of *H. pylori* exist, which may be associated with different aspects of bacterial pathogenicity.[10]

Several *H. pylori* virulence genes that may play a role in its pathogenicity have been identified. Of these, the most important determinants are

vacuolating cytotoxin gene (VacA) and cytotoxin associated gene (CagA). *H. pylori* strains have been divided into 2 broad families, type I and type II, which are based on whether they possess the VacA and CagA genes. Type I strains produce VacA and CagA, whereas type II lacks the CagA gene and presents a nontoxic form of VacA. Type I strains are regarded as having greater pathogenicity and greater potential to cause development of diseases.

The CagA gene codes for CagA protein, which is highly immunogenic and is related to a high level of some cytokines.

The VacA gene encodes a proteinaceous, vacuolating toxin that damages cells by induction of intracellular vacuolar formation. The VacA gene is present in all *H. pylori* strains and includes 2 variable regions. The s region (encoding the signal peptide) exists as an s1 or s2 allele. The m region (middle) occurs as an m1 or m2 allele.[11-13] The mosaic combination of the 2 regions, s- and m-region allelic types, expresses all possible combinations, implies the existence of several subtypes, and determines the production of the cytotoxin associated with pathogenicity of the bacterium.[12,14,15] VacA s1/m1 strains produce large amounts of toxin, s1/m2 strains produce moderate amounts, and s2/m2 strains produce little or no toxin. The VacA s1/m1 strain is thought to be associated with more severe pathologies.[15,16] Different subtypes of type I *H. pylori* can be identified on the basis of the VacA polymorphism, as an expression of mosaic combination.

Based on this concept, several recent studies provided seroepidemiologic evidence indicating that the *H. pylori* strains expressing the CagA may serve as an independent marker of ischemic heart disease.[17-23] None of these studies, however, which evaluated only 1 specific strain of *H. pylori*, considered the possible pathogenic link between *H. pylori* infection and cardiovascular diseases taking into account its genetic polymorphism.

So far, no data are available in the literature documenting the relationship between both CagA and VacA *H. pylori* strains (and their subtypes) and vascular disease or acute aortic dissection. The current study was designed to evaluate whether virulent CagA and VacA positive *H. pylori* strains are associated with acute, ascending aorta dissections. Therefore, we aimed to confirm the presence of CagA and the VacA, s and m subtypes, in 100 patients who underwent operative repair for acute ascending aorta dissection with an homogeneous group of control subjects. Furthermore, all the specimens from surgical excision were analyzed for the presence of H pylori genomic material.

METHODS

Patient selection. Between January 2007 and May 2008, 100 consecutive patients admitted for surgical treatment of acute dissection of ascending aorta were investigated. To avoid any misleading interference, exclusion criteria were as follows: aortic dissection caused by Marfan Syndrome (diagnosed when the rigorously applied Ghent criteria were fulfilled),[24] aortic dissections related to coarctation of the aorta and/or previous significant aortic valve disease (stenosis, incompetence, with or without calcification), bicuspid aortic valve, aortic root calcification, and cocaine use. Preoperative evaluation by computed tomography or magnetic resonance imaging was performed in all patients. According to the De Bakey classification, 85 patients had a type I aortic dissection and 15 had a type II dissection. Patients with type III aortic dissection were excluded from the study as well, because they usually underwent a different approach. At admission, 27 patients were hemodinamically unstable, and 8 patients had evidence of organ malperfusion. Standardized interviews addressing medical history, hypertension, diabetes, current medication, history of gastritis or peptic ulcers, sociodemographic data, and lifestyle were carried out in all patients and/or their families. Treatment of the dissecting lesion was by operative repair in all patients.

The control group was enrolled after all 100 operative procedures had been performed in the patient group. To avoid any misleading interference, the control group consisted of 100 healthy volunteers selected carefully to be truly comparable with the patient group with the ascending, aortic dissections. From a database of 18,000 patients charged by 18 family doctors of National Health Service who provided their collaboration, 400 subjects were identified as matched fully to patients group regarding geographic area, sex, age, body weight, body mass index, history of myocardial infarction, main risk factors for aortic dissection (hypercholesterolemia, hypertension, diabetes), history of documented peptic ulcer, smoking status, physical activity, and overall social status. Among these eligible subjects, 377 provided informed consent to be enrolled in the study and underwent subsequent selection by standardized interview to obtain 100 controls who were matched fully to patients.

Socioeconomic status was assessed according to the Four Factor Index of Social Status reported by

Hollingshead.[25] This instrument provides an index of socioeconomic level according to 4 factors: marital status, occupation, education, and retirement. The status score of a nuclear family unit is calculated by multiplying the scale value for occupation by a weight of 5 and the scale value for education by a weight of 3 (the overall factor weights were calculated with multiple regression equations). The instrument was enriched by taking the fathers' occupations at the time of birth into account (classified as manual or non manual as indications of childhood living conditions),[26] current patient occupation (categorized as class I professionals, class II managerial and technical occupations, class III subdivided into non-manual and manual skilled workers, and class IV unskilled manual), and education level (identified as none, school, further, or university). The resulting computer scores ranged from 120 (high) to 15 (low).

The history of peptic ulcers was considered only when clinically documented to avoid any possible bias from clinical interview. Subsequent exclusion criteria in both groups were nonsteroidal anti-inflammatory drugs and/or protonpump inhibitors, and/or antibiotics treatment within the previous 4 weeks.

Informed consent was obtained from all patients and the control group, and the protocol was approved by the Hospital Ethic Committee and by the hospital Institutional Review Board.

Biochemical measurements. Venous blood samples were collected in surgical patients at hospital admission and in control subjects at the time of enrollment. All blood samples were cooled immediately to 4°C and centrifuged (3,000 g for 10 min at 4°C). The plasma was separated and stored at –70°C until assay. Total anti-*H. pylori* antibodies were determined using an enzyme-linked immunosorbent assay commercial kit (Helicobacter pylori immunoglobulin G; Diamedix Co., Miami, FL) according to the manufacturer's instructions. Titers were judged positive when >24 U/mL (sensitivity and specificity >98%). Immunoglobulin G antibody profiles were determined by Helicoblot versions 2.1 (Genelabs Diagnostics, Singapore) according to the manufacturer's instructions (96% sensitivity and 95% specificity). The Helicoblot assays were based on a Western blot analysis of whole-cell *H. pylori* antigens. The interpretation of the serologic reactivity was restricted to antigens of various molecular masses. Version 2.1 contained antigens of 19.5, 30, 35, 37, 89 (VacA), and 116 (CagA) kDa. For Helicoblot 2.1, the criteria for *H. pylori* seropositivity were as follows: (1) positive result for the 116-kDa (CagA) band, where CagA has to be present with 1 or more bands at the positions 89 (VacA), 37, 35, 30 (UreA), and 19.5 kDa together, or with current infection marker (CIM); (2) the presence of any 1 band at 89, 37, or 35 kDa, with or without the CIM; and (3) the presence of both the 30- and 19.5-kDa bands, with or without the CIM. This kit allows rapid visualization of full serologic profiles of various antigens that belong to *H. pylori* and standardization for international comparisons.[27]

Fasting total serum cholesterol and high density lipoprotein (HDL) concentrations were also measured. Hypercholesterolemia was defined as a total serum cholesterol >200 mg/dL, and HDL values were considered normal when >80 mg/dL. We did not take into account leukocyte count, fibrinogen, or other markers of inflammatory process because of their natural activation in all cases of acute aortic root dissection.

Detection of *H. pylori* DNA. After operative resection of the aneurysm, aortic wall specimens collected from the anterior and posterior wall of the ascending aorta were separated with sterile scalpel blades into multiple sections of about 0.3 cm^2 each and frozen at –20°C. For each patient, 1 sample from the anterior aortic wall and 1 from the posterior wall were analyzed. The tissue samples were digested by incubation for 3 h at 52°C in 500 μL of digestion buffer (10 mmol/L Tris pH, 8.5), 1 mmol/L ethylenediaminetetraacetic acid, and 0.5% sodium dodecyl sulfate containing 300 μg/mL of proteinase K. DNA purification from digested tissues using a spin-column DNA purification kit (Invitek GmbH, Berlin, Germany). *H. pylori* DNA in the samples was detected by using real-time, fluorometric, polymerase chain reaction (PCR), directed to amplify the 23S rRNA gene. PCR was performed in an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). The amplification protocol included 10 min incubation at 95°C for denaturation, and enzyme activation and 48 cycles of denaturation at 95°C for 15 s and extension at 60°C for 90 s. DNA extracted from cultured *H. pylori* J99 cells was used as positive amplification control. To exclude the possibility of the presence of Taq polymerase inhibitors, H pylori was added to negative samples that were then reanalyzed. No evidence of inhibition was found. The results were analyzed with the software of the device. Additionally, all specimens were examined microscopically for evidence of *H. pylori* organisms.

End points. The primary end point of this study was the relationship between type I strains of *H. pylori* and acute ascending aorta dissection. Secondary

Table I. Important clinical and demographic characteristics of patients and control subjects

	Patients (n = 100)	Controls (n = 100)	P value
Age, years	64 ± 9	64 ± 9	Matched
Males	90	90	Matched
BMI (kg/m^2)	27 ± 2	27 ± 2	Matched
Previous MI	3	3	Matched
Total cholesterol (mg/dL)	190 ± 31	194 ± 29	.3
HDL (mg/dL)	68 ± 23	62 ± 32	.1
Creatinine (mg/dL)	1.4 ± 0.6	1.0 ± 0.3	.05
History of hypertension	50	50	Matched
History of diabetes	21	22	<.001
History of peptic ulcer	5	4	1
Smoking status			
Ex-smokers	16	16	Matched
Current smokers	32	32	Matched
Socioeconomic status	61 ± 19	59 ± 17	.4
Physical activity	33	33	Matched

Values are mean ± SD or numbers (percentages). Socioeconomic status is assessed by Hollingshead modified index from 120 to 15 (low, 15–45; medium, 46–80; and high, 81–120).
BMI, Body mass index; *MI*, myocardial infarction.

Table II. Important clinical and demographic characteristics of patients and controls positive for type I *H. pylori* infection

	Patients (n = 61)	Controls (n = 29)	P value
Age, years	64 ± 7	65 ± 4	.5
Males	55 (90%)	26 (90%)	1
BMI (kg/m^2)	28 ± 3	27 ± 2	.1
Previous MI	2 (3%)	1 (3%)	1
Total cholesterol (mg/dL)	182 ± 32	189 ± 25	.3
HDL (mg/dL)	72 ± 23	69 ± 31	.8
Creatinine (mg/dL)	1.3 ± 0.5	1.0 ± 0.3	.03
History of hypertension	31 (50%)	15 (51%)	.8
History of diabetes	11 (18%)	4 (14%)	.8
History of peptic ulcer	4 (6.5%)	3 (10.3%)	.8
Smoking status			
Ex-smokers	10 (16%)	7 (24%)	.2
Current smokers	25 (41%)	12 (41%)	.8
Socioeconomic status	49 ± 18	56 ± 19	.09
Physical activity	20 (32%)	9 (31%)	.9

Values are mean ± SD or numbers (percentages). Socioeconomic status is assessed by Hollingshead modified index from 120 to 15 (low, 15–45; medium, 46–80; and high, 81–120).
BMI, Body mass index; *MI*, myocardial infarction.

end points were the identification of the most aggressive subtypes of type I *H. pylori* among to VacA strains and the assessment of presence of *H pylori* genomic material in dissected aorta.

Statistical analysis. As reported previously, evidence of CagA and VacA strains of *H. pylori* in asymptomatic healthy subjects is about 30–40%.[28] Serologic evidence of more virulent *H. pylori* strains was found in 50% of patients with documented coronary heart disease.[20,23] Thus, a sample of 100 patients and 100 controls would provide up to 95% power to detect the difference (30–50%) with a α of .05. Continuous variables were expressed as mean ± standard deviation (SD), and categorical data as proportion. A comparison between continuous variables in the 2 groups was made by means of the Student *t* test for normally distributed features values. The Mann-Whitney *U* test was used for variables not normally distributed. The categorical variables were analyzed with the χ^2-test or Fisher exact test. Variables with *P* values less than .05 were considered significant. Odds ratio (OR) and 95% confidence intervals (CI) assessing the risk of aortic dissection associated with infection or by CagA and/or VacA strains positive *H. pylori* were evaluated by multiple logistic regression, when necessary adjusted for age, sex, body mass index, history of hypertension, smoking or diabetes, presence of hypertension or hypercholesterolemia,

and socioeconomic status. An α value of <.05 (2-tailed) was considered significant. Both univariate (independent sample *t* test and χ^2) and multivariate analyses (multivariate regression and logistic regression) were performed using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Study population. The general and clinical features of patients and controls are summarized in Table I. Because of matching, both groups were similar in all important clinical and demographic features. The only significant difference between patients and controls were in creatinine values which were >2.2 mg/dL in malperfused patients.

Detection of *H. pylori* genomic material. In all 100 patients evaluated, PCR showed no evidence of the presence of *H. pylori* genomic material or infections in aortic aneurysm specimens.

Serological status. Seventy-two surgical patients and 50 control subjects were seropositive for *H. pylori* (OR, 2.5; 95% CI, 1.3–4.8; *P* = .002). The association remains significant after adjustment for age, sex, social class, and risk factors for aortic dissection (OR, 2.8; 95% CI, 1.8–4.1; *P* = .006). Patients who were either seropositive or seronegative for *H. pylori* infection were similar in age, sex, body mass index, and prevalence of risk
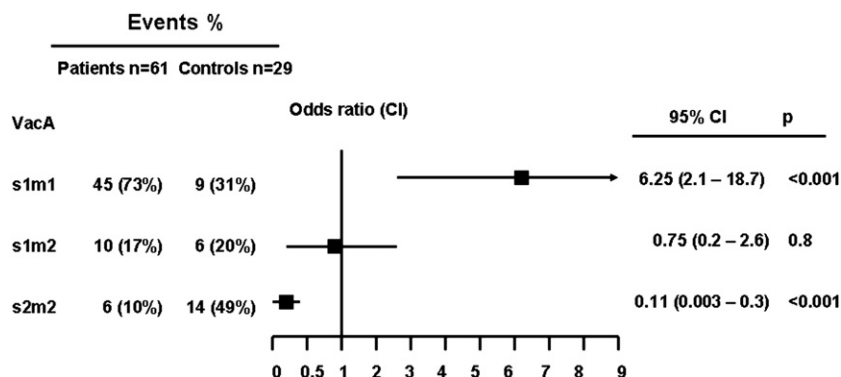
Events %

Patients n=61 Controls n=29

Figure. Results of multivariate analysis for more virulent strains of *H. pylori* in patients and controls.

factors for aortic dissection; however, seropositive patients tended to come from a lower socioeconomic status. Type I *H. pylori* (CagA+ and VacA+) was identified in 61 of 72 seropositive patients (85%) and in 29 of 50 control subjects (58%; OR, 4.1; 95% CI, 1.5–10.3; $P = .001$). The association remained significant after adjustment for age, sex, social class, body mass index, and risk factors for aortic dissection (OR, 3.8; 95% CI, 1.3–11.5; $P < .001$). No significant differences were recorded between the 2 groups in the important clinical or demographic features (Table II). When we considered the s and m subtypes of the VacA strains, 90% (55/61) of the patients in the surgical group were s1 and 38% (23/61) were m2. The combinations s1-m1, s1-m2, and s2-m2 were present in 73%, 17%, and 10% of strains in the surgical group, respectively, and in 31%, 20%, and 49%, respectively, in the control group. The more frequent (and considered the most virulent) combination s1-m1 in the surgical patient group compared with control subjects had an OR of 6.25 (95% CI, 2.1–18.7; $P < .001$; Fig). The association remained significant after adjustment for age, sex, body mass index, prevalence of risk factors for aortic dissection, and socioeconomic status (OR, 5.9; 95% CI, 1.9–16.9; $P < .001$). In the patients group, patients who were VacA s1m1 strains positive were similar for clinical and demographic features compared with patients who were VacA s1m1 strains negative. Patients who were VacA s1m1 strains positive were older ($P = .001$), more frequently male ($P = .04$), and came from a lower socioeconomic status ($P = .008$) compared with other patients (Table III). A multivariate analysis of patients who were VacA s1m1 stain positive confirmed that only age >65 years (OR, 1.61; 95% CI, 1.12–2.51; $P = .02$) and Hollingshead modified index <50 (OR, 2.58; 95% CI,

1.61–4.61; $P = .006$) were associated with seropositivity for the VacA s1m1 positive strains.

Seropositivity for *H. pylori* was detected in 4 of 5 patients and 3 of 4 controls with history of peptic ulcers ($P = .5$). All patients and controls seropositive for *H. pylori* with history of peptic ulcers were type I positive and VacA s1m1 strains positive. There was no prevalence of serologic status regarding the type of dissection; the combination s1-m1 was present in 75% and in 64% ($P = .6$) of patients with type I and type II aortic dissection, respectively.

DISCUSSION

This case-control study provides strong evidence that infection by the more virulent strains of *H. pylori* is associated with acute ascending aorta dissection. Systematic reviews of epidemiologic studies suggested the existence of a strong positive relationship between cardiovascular disease and chronic *H. pylori* infection.[1-7] Several reports showed clearly that cytotoxic strains of bacterium bearing the CagA play a key role in the pathogenesis of cardiovascular diseases.[17-23] Currently, different *H. pylori* strains can be distinguished by genotyping of virulence-associated gene, such as CagA and VacA. The gene for VacA is polymorphic and encodes several subtypes of *H. pylori* strains that have different roles in gastroduodenal diseases.[10-16]

Our study demonstrates a possible linkage of these more virulent strains with acute aortic dissection. We identified the prevalence of type I *H. pylori* strains in patients who underwent repair of an acute aortic dissection compared with a tightly matched homogeneous population of control subjects. We provided evidence that seropositivity for the more virulent CagA and VacA s1m1 subtypes is an independent risk marker in acute

Table III. Important clinical and demographic characteristics of patients with and without type I s1m1 *H. pylori* strains

	$s1m1^+$ (n = 45)	$s1m1^-$ (n = 55)	P value
Age, years	66 ± 4	63 ± 5	.001
Males	45 (82%)	44 (98%)	.04
BMI (kg/m^2)	28 ± 2	28 ± 3	1
Previous MI	1 (2%)	2 (3%)	.8
Total cholesterol (mg/dL)	195 ± 30	188 ± 28	.2
HDL (mg/dL)	68 ± 25	74 ± 30	.2
Creatinine (mg/dL)	1.3 ± 0.7	1.3 ± 0.5	.09
History of hypertension	21 (46%)	29 (53%)	.6
History of diabetes	9 (20%)	12 (22%)	.9
History of peptic ulcer	4 (8.8%)	—	.07
Smoking status			
Ex-smokers	8 (18%)	8 (14%)	.8
Current smokers	14 (31%)	18 (33%)	.9
Socioeconomic status	44 ± 18	54 ± 19	.008
Physical activity	17 (38%)	16 (29%)	.4

Values are mean ± SD or numbers (percentages). Socioeconomic status is assessed by Hollingshead modified index from 120 to 15 (low, 15–45; medium, 46–80; and high, 81–120).
BMI, Body mass index; *MI*, myocardial infarction.

aortic dissection. The mechanisms by which this association is made possible are unclear and cannot be deduced from this study.

Many reports evaluated the presence of *H. pylori* in atherosclerotic plaques or in aortic aneurism specimens with controversial results.[5-9] Our results seem to exclude the involvement of *H. pylori* in acute aortic dissection based on a direct interaction of the bacteria with the arterial wall. We failed to detect any bacterial genomic material directly in the specimens of resected aortic wall, leaving open the question of how the infectious agents might affect the aortic wall. A plausible explanation has been put forward, suggesting that the enhanced immunologic response evoked by these *H. pylori* strains may influence atherogenesis and its evolution.[18] Indeed, bacterial cytotoxins induce the production of several cytokines (interleukin 1 (IL1) and IL2) that may activate the vascular endothelium to change its own hemostatic control systems.[29] Recently, a cross-mimicry between CagA and VacA proteins and antigens of the endothelial wall has been demonstrated and has been postulated to induce endothelial damage.[30] In addition, it has been hypothesized that type I strains of *H. pylori* predisposing to gastric atrophy may induce vitamin malabsorption and, therefore, hyperhomocystinemia, which is a well-documented risk factor for vascular disease. Finally, the vacuoling

cytotoxin, encoded by VacA gene, when released into the bloodstream might induce degenerative vacuolization of the endothelium with the same mechanism described in gastric epithelium.

This study could be limited by the cross-sectional evaluations in the recruitment of an appropriate control group. To avoid all misleading interferences by confounding variables and subsequent statistical adjustments, we chose preliminarily to enroll a control group selected carefully and matched fully with the patient group according to demographic and clinical features and risk factors for acute aortic dissection. The socioeconomic status, which is considered by many investigators to be the most important multivariable misleading factor, was assessed carefully by giving a score that embedded several variables. The additional limitation of our study is relatively low number of patients. Nevertheless, our sample size has enough statistical power to demonstrate an association, albeit not a causal relationship, between VacA and CagA-positive strains and aortic dissection. Indeed, our preliminary results are hypothesis generating and require future wider and definitive evaluations.

In conclusion, we report that chronic infection by CagA-VacA s1m1 subtypes of *H. pylori* is related to acute ascending aorta dissections. Many functional aspects of the various *H. pylori* subtypes, however, must be elucidated to explain the molecular mechanisms underlying their action in the arterial wall. Our results may support the idea that chronic infection by these virulent bacterial strains may become a new risk factor in the etiopathogenesis of acute aortic dissections. Strategies aimed at prevent *H. pylori* infection and eradication, which have already been used successfully to decrease the risk of peptic ulcer disease and gastric cancer, may have some impact on the incidence of acute aortic dissection. Before trials of antibiotics against *H. pylori* for the prevention of acute aortic dissection can be recommended, however, subsequent data from prospective studies are necessary.

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