Minerva Gastroenterologica e Dietologica
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Title: Management of Helicobacter pylori in Piedmont, Italy
Paper code: Minerva Gastroenterol Dietol-2483
Submission Date: 2018-02-12 18:02:05
Article Type: Review Article

Files:
1): Manuscript
    Version: 1
    Description: manoscritto
    File format: application/msword

Management of *Helicobacter pylori* in Piedmont, Italy

Davide Giuseppe Ribaldone[1 *], Dario Mazzucco[2], Sharmila Fagoonee[3], Lucia Crocellà[4], Alessandro Lavagna[4], Mario Fracchia[4], Gian Paolo Caviglia[1], Daniele Simondi[5], Rodolfo Rocca[2], Marco Astegiano[6]

[1]Department of Medical Sciences, Division of Gastroenterology, University of Torino, Torino, Italy;

[2]Ospedale degli infermi, Division of Gastroenterology, Rivoli, Turin, Italy;

[3]Institute for Biostructures and Bioimages (CNR) c/o Molecular Biotechnology Center, Torino, Italy;

[4]Gastroenterology Unit, Mauriziano Umberto I Hospital, Torino, Italy;

[5]Santa Croce e Carle Hospital, Department of Gastroenterol and Digestive Endoscopy, Cuneo, Italy;

[6]Department of General and Specialistic Medicine, Gastroenterologia-U, Città della Salute e della Scienza di Torino, Torino, Italy

Conflicts of interest: none to declare.

[*]Corresponding author: Davide Giuseppe Ribaldone - Department of Medical Sciences, Division of Gastroenterology, University of Torino, C.so Bramante 88 - 10126 Torino – Italy. E-mail: davrib_1998@yahoo.com Tel: +390116335208, Fax: +390116336752.

Abstract

Helicobacter pylori (*H. pylori)* is a Gram-negative bacterium, usually acquired during childhood, whose natural habitat is the gastric lumen. *H. pylori* is accepted as the most important cause of gastritis and peptic ulcer in humans. Nevertheless, its important role in the pathogenesis of gastric cancer as well as in several extra-gastroduodenal diseases has been confirmed. The aim of this work is to discuss, for the first time in a single article, all publications concerning *H. pylori* infection arising from Piedmont region, Italy, where in 1893 Giulio Bizzozero was the first who observed and described spiral organisms in the stomach of animal models. A systematic review of all publications on the management of *H. pylori* in adults in Piedmont, based on a PubMed and a Scopus research from 1965 to 2017 was performed. The discussed aspects are the epidemiology, the study on gastric and extragastric diseases related to *H. pylori*, the diagnostic methods, the treatment of *H. pylori* infection, and the possibility of reinfection. In conclusions, with almost 70 publications, Piedmont has proudly maintained the tradition of the father of the *H. pylori*.

Key words: Cardiovascular - Cirrhosis – Culture – Diagnosis - Dyspepsia - Extragastric Diseases – Gastritis – Helicobacter - Peptic – Pre-eclampsia - Prevalence - Reinfection – Therapy - Ulcer

Helicobacter pylori (*H. pylori*) is a Gram-negative gastric bacterium "re-discovered" by Marshall and Warren in 1983.[1] Giulio Bizzozero, a former University of Turin' eminent professor, was the first who observed and described spiral organisms in the stomach of dogs and, though he did not realize what actually is known, he considered this finding important enough to publish it in 1893.[2] Bizzozero could not suspect that this spiral-shaped bacterium will be the most widespread infectious agent of gastroduodenal diseases. In fact, he could not imagine that his observation will spark off an explosion of research, diagnostic tools, and developments of treatment protocols.

The aim of this "special article" is to do a systematic review of all original articles including adult patients managed for *H. pylori* infection, arising from Piedmont (the region of Turin). To identify all appropriate publications, a Pubmed and a Scopus search of all studies published from 1965 to 31 December 2017 was conducted. Only original articles and letters were included and discussed.

Epidemiology

Epidemiology of *H. pylori* infection in Piedmont, has been extensively studied in healthy subjects as well as in patients with several gastroduodenal or extra-gastroduodenal diseases.

The first study that reported the *H. pylori* seroprevalence in Turin (Torino), an industrial town in the Northwest of Italy, with a population of almost 1 million people, half of whom were born and lived in the South prior to transfer here, was conducted from April 1995 to July 1995 on 619 consecutive blood

donors attending the Molinette Hospital's Blood Bank (Turin). *H. pylori* seroprevalence was assessed by presence of immunoglobulins G (IgG) against the bacterium in serum, by means of a commercial enzyme-linked immunosorbent assay (ELISA). The overall *H. pylori* seroprevalence was 47%: 51% in males, 27% in females ($P < 0.001$). According to the cohort-effect phenomenon, among subjects between 20-29 years of age, the seroprevalence in males was 29% versus 5% in females while at 40-49 years increases to 56% in males versus 23% in females and at 50-59 years was 65% in males versus 42% in females.[3]

Gastroduodenal diseases

Chronic gastritis and peptic ulcer

In a paper published by Di Napoli *et al*., before treatment all patients infected with *H. pylori* had chronic active gastritis. After eradication, the total score for gastritis decreased significantly (from 7.76 to 3.22; $P < 0.01$, 95% CI = 2.94 to 5.28), while in non-responders the score was only slightly reduced (6.31; $P > 0.1$, CI = 0.57 to 3.32).[4]

In 1993, the examination of serum samples of 82 dyspeptic patients undergoing endoscopy showed that patients with duodenal ulcers (DU) had significantly higher systemic IgG responses to a recombinant fragment of 128 kDa protein (the cytotoxin-associated gene A, CagA antigen) of *H. pylori* than subjects with normal mucosa and chronic gastritis.[5]

In 1996, Bruno *et al*. enrolled 258 consecutive out-patients with active DU and histology-proven *H. pylori* infection. Upper gastrointestinal endoscopies (EGDS) were performed on entering the study, and then at two, six, and twelve months after the completion of the eradicating courses. Eradication produced a significantly higher ulcer healing rate at 2 months and no recurrence at 12 months.[6]

In 1996, Suriani *et al*. assessed whether *H. pylori* eradication performed after DU healing, could prevent ulcer relapse during a 2-year follow-up. After confirmation of both DU healing and *H. pylori*

infection, all patients were randomized to either the treatment or the placebo group and followed up. At the first endoscopy performed 3 months (± 2 SD) after antibiotic or placebo treatment, 16 patients (100%) in the treatment group and 5 patients (36%) in the placebo group were ulcer-free. After a mean 22.6 months (19-27 months), only four patients (25%) in the treatment group experienced ulcer relapse. In contrast, 13 patients (92.9%) in the placebo group experienced relapse during follow-up. In this group 11 patients had already had a relapse after 6 months.[7]

In another study, 474 patients suffering from DU were recruited. After therapy, endoscopic follow-up provided controls at 6, 12, and 24 months and following controls stoppage in patients with healing *H. pylori* infection and without symptoms. *H. pylori* infection was verified histologically, serologically and, in the last period, with ^{13}C urea breath test (UBT). In patients with DU recurrence, the protocol provided, until 1995-1996, the randomized long-term treatment with famotidine 20 mg/die or ranitidine 150 mg/die; some patients were treated with seasonal modality. Later on, the management changed with the use of 20 mg omeprazole on alternate days or lansoprazole 15 mg/die for patients who did not benefit from the above treatment. Despite bacterial eradication, in this heterogeneous cohort with multiple causes of gastric damage, only 301/474 (63%) did not have DU recurrence or need for long-term PPI assumption.[8]

In a prospective study, Pellicano *et al.* included 84 patients suffering from bleeding DU associated only with *H. pylori* infection. After bacterial eradication the authors observed a recurrence of DU in 3 out of 46 (6.5%) patients who had stopped all medications and in 3 out of 38 (7.9%) patients under continuous long-term therapy with inhibitors of acid secretion. Hemorrhage did not recur during the period of observation in either group. No *H. pylori* reinfection was observed, as confirmed by the UBT, even in the six patients with DU recurrences. This confirmed that eradication of *H. pylori* is mandatory in patients who had an episode of bleeding from DU.[9]

In a prospective study, 41 consecutive patients admitted to the Emergency Care Unit of Molinette Hospital for upper gastrointestinal (GI) bleeding were recruited. Serological diagnosis was performed by measuring antibodies both against *H. pylori*, by a commercial ELISA, and cytotoxic strains (Western Blot for anti-CagA, Vac-A, urease B, urease A). Due to the low sensibility of histology during GI bleeding, the search for *H. pylori* on biopsies was performed only during the follow-up. In case of doubt, either UBT or a stool antigen test were associated. Patients infected by *H. pylori* were treated, after hemodinamic stabilisation, with a triple therapy regimen including clarithromycin, amoxicillin and omeprazole (CAO). *H. pylori* eradication was evaluated after 3 months from the end of the therapy with UBT. Sixteen patients were infected from *H. pylori* (group A), 12 had only a history of non-steroidal antiinflammatory drugs (NSAIDs) consumption (group B), and 13 patients had both factors (group C). Thus, *H. pylori* infection was present in 29 out of 41 patients (71%). This rate of prevalence was higher than their control population, matched by age, admitted to the Emergency Care Unit for other diseases. Furthermore, 20 out of 29 patients (68%) were positive for anti-CagA and anti-VacA antibodies. With respect to rebleeding within 72 h, it occurred more frequently in patients with *H. pylori* infection (groups A and C) than in those with only NSAIDs consumption.[10]

Precancerous lesions and gastric cancer (GC)

Fifty-four patients with DU and *H. pylori* infection received eradication therapy. Endoscopic examination with antral and corporal biopsy was done at baseline and at yearly intervals. The follow-up period ranged from 60 to 144 months. *H. pylori* status was determined using a rapid urease test and modified Giemsa stain. After *H. pylori* eradication, glandular atrophy, detected at baseline in 17/24 patients, disappeared in 5, improved in 2 and worsened in 3 cases. In addition, intestinal metaplasia (IM), presented at baseline in 12/24 patients was undetectable in 2 and persisted in the remaining 10 cases. In the 14 patients in whom *H. pylori* infection persisted, glandular atrophy, disappeared in 2,

worsened in 2 and appeared in 5 patients. IM, detected at baseline in 1 case, appeared in other 5 cases ($P < 0.01$). After *H. pylori* eradication, atrophy grading did not show a significant improvement, although there was a general trend toward improvement in responder patients, while IM improved in 5/12 (41%) patients and was not detectable in 2 cases. In contrast, IM appeared in 5/14 patients in whom *H. pylori* infection persisted. The authors concluded that, in a long-term follow-up, *H. pylori* eradication prevented the appearance of IM, whereas it did not affect glandular atrophy.[11]

Ponzetto *et al*., in 1996, reported the detection of antibodies anti-CagA of *H. pylori* in 96% of 51 patients having surgery for GC versus 18% of the general population (patients admitted to the Hospital's Emergency Care Department).[12]

Twenty patients with GC and *H. pylori* negative histology took part to another study. The control group consisted of *H. pylori* negative subjects, divided in adult patients (N: 19) and asymptomatic children (N: 20) with *H. pylori* negative fecal test. The adult controls suffered from past *H. pylori* infection, eradicated 10 years earlier, and DU. In this group, *H. pylori* negative assessment was performed every year by endoscopy and gastric biopsy specimens (the reason for this interval was not explained). CagA and VacA seropositivity was 90% and 95% in GC, 84% and 84% in DU *H. pylori* negative patients, 25% and 5% in *H. pylori* negative children, respectively. A significant difference was found between adults and children ($P < 0.0001$).[13] The positivity to anti-CagA and anti-VacA, in patients with negativity to other tests, probably reflects an *H. pylori* infection before the appearance of GC, since it has been reported that CagA antibodies persist longer after eradication treatment than IgG anti-*H.pylori*.[14] Similar data were subsequently confirmed by the same group in another study.[15]

In two papers, Suriani *et al*. prospectively evaluated 2360 patients referred to the Endoscopy Unit of Rivoli Hospital. After exclusion of not eligible patients, a total of 1750 patients were studied by endoscopy and multiple biopsies. Type 3 IM was found in 6.7%; 709 patients had *H. pylori* on

histological examination. The presence of *H. pylori* was neither correlated to type 3 IM in the antrum (P = 0.99) nor to gastric atrophy (P = 0.1).[16,17]

In the context of a multicenter survey, a prospective case-control study of patients who had undergone surgery for GC was performed. Among patients, 82% were seropositive for IgG anti-*H. pylori* compared to 57% of controls (P < 0.0001). Moreover, 84% were seropositive for anti-CagA antibody versus 18% of controls (P < 0.0001). There was no difference between the frequency of *H. pylori* in intestinal type and diffuse type carcinoma.[18]

In a study, conducted in Rivoli and Naples, the authors using the immunohistochemical evaluation of 5-methylcytosine (5-MC), assessed the global DNA methylation of gastric mucosa. The population consisted of 93 patients consecutively referred to the Digestive Endoscopy Unit of Federico II University of Naples because of dyspeptic symptoms. All patients underwent EGDS with eight biopsy specimens, five of which were processed for both histological and immunohistochemical evaluation and three for the rapid urease test. Furthermore, serum samples were collected to determine anti-*H. pylori* and anti-CagA antibodies. Forty-one surgical samples of GC tissues were obtained at the time of surgery and processed for both histological and immunohistochemical evaluation. To validate their results, the authors selected 54 cases of *H. pylori*-positive chronic atrophic gastritis with IM. Finally, they included 10 dyspeptic patients, enrolled at the Endoscopy Unit of Rivoli Hospital, with history of *H. pylori* eradication and followed up endoscopically; these patients had preneoplastic lesions (ie, atrophy and IM) and a family history (first-degree relative) of GC. According to *H. pylori* infection, the 93 dyspeptic patients from Naples were subdivided into 47 *H. pylori*-negative subjects and 46 *H. pylori*-positive patients, 28 of whom were anti-CagA+. In the *H. pylori*-negative group, all subjects had normal gastric mucosa with a minimal infiltration of lymphomonocytes in the lamina propria. The *H. pylori*-positive patients were subdivided into two groups: 21 *H. pylori*-positive chronic gastritis, 10 of whom were CagA+, and 25 *H. pylori*-positive chronic atrophic gastritis, 18 of whom were Cag-A+. In

13/25 *H. pylori*-positive patients, chronic atrophic gastritis, atrophy was mild and restricted to the antrum, while it was moderate and diffuse to both antrum and angulus in the remaining 12 cases, regardless of the Cag-A status. Considering the evaluation of 5-MC, the authors found a gradual decrease in the global DNA methylation from *H. pylori*-negative to *H. pylori*-positive chronic gastritis, *H. pylori*-positive chronic atrophic gastritis and GC cases. These data suggest that DNA hypomethylation could be implicated in *H. pylori*-related gastric carcinogenesis at an early stage. Furthermore, the 5-MC immunopositivity was negatively correlated with markers implicated in cell cycle control such as Ki-67 and p53.[19] Global DNA hypomethylation is generally considered one of the hallmarks of cancer cells because the genes vulnerable to aberrant hypermethylation usually are overlapped by the genes targeted by hypomethylation.[20] During the 10-year follow-up the authors found a progressive decrease in global DNA methylation in patients with chronic atrophic gastritis, despite the successful *H. pylori* eradication. The latter, in subjects with preneoplastic lesions did not halt the gastric carcinogenesis process.[19]

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

The presence of an organized lymphoid infiltrate in the lamina propria of gastric mucosa, so-called MALT, is considered an obligate precursor of MALT lymphoma.[21] In a study published in 1997, the authors followed up 16 patients with B-cell oligoclonality. Nine of them had Sjogren's syndrome, of which seven had primary and two had secondary syndrome associated with rheumatoid arthritis and scleroderma (first group). Two additional patients had rheumatoid arthritis, and one scleroderma (second group). Four patients had dyspepsia without an associated autoimmune disease (third group). Patients were followed up for a minimum of 3 months up to a maximum of 33 months (average 15.3 ± 9.2). In 3 out of 12 patients initially positive for *H. pylori* the bacterium had not been eradicated at the time of the last biopsy. Only in one patient did B-cell clonality seemed to parallel *H. pylori* infection.

In most cases (8 of 10) in the 3 groups, clonality persisted for up to 33 months after bacterial eradication.[22]

Other gastrointestinal conditions

To evaluate the prevalence of *H. pylori* infection in patients with inflammatory bowel disease (IBD) diagnosed for the first time, Pellicano *et al*. performed a pilot case-control study. Twenty patients were compared to 29 controls affected by idiopathic constipation. All were screened for the presence of *H. pylori* by UBT. *H. pylori* infection was shown in 60% of patients with IBD versus 41% among controls (P = 0.2).[23]

In a retrospective case-control study Simondi *et al*. evaluated the prevalence of *H. pylori* infection in patients with celiac disease (CD) or duodenal intraepithelial lymphocytosis (DIL). One hundred and fifty-four patients with a duodenal biopsy and a diagnosis of damage type 1, 2, or 3 according to Marsh-Oberhuber classification were included. As controls, subjects suffering from idiopathic constipation, without CD, were included. *H. pylori* prevalence in CD patients was 36% versus 19% in DIL patients and 41% in controls. Independently from the other variables considered, *H. pylori* prevalence in DIL patients was significantly lower compared to controls (P < 0.05).[24]

In a single-center study, the inclusion criteria were a diagnosis of lymphocytic or collagenous colitis and active *H. pylori* infection. The authors included 50 patients affected by microscopic colitis and in whom *H. pylori* status was assessed by histology (N: 47) or by UBT (N: 3). *H. pylori* resulted positive in 18 patients (36%), of whom 13 affected by lymphocytic colitis and 5 affected by collagenous colitis (P = 0.51). There was no difference in term of *H. pylori* infection comparing cases and controls (P = 0.59). Nevertheless, considering patients with history of *H. pylori* positivity but with eradication before the diagnosis of microscopic colitis as patients with negative *H. pylori* status, there was a statistically significant difference between cases and controls (P = 0.006). The authors concluded that to be *H.*

pylori negative, ab initio or after antibiotic treatment, seemed to be a risk factor for the onset of microscopic colitis.[25]

Extra-gastroduodenal diseases

Since the last decades, several studies have reported on the link between chronic *H. pylori* infection and a variety of extra-gastroduodenal manifestations, based on potential mechanisms involving a low-grade inflammatory state, molecular mimicry patterns, and interferences with the absorption of nutrients.[26] Actually, although the Maastricht V/Florence Consensus Report of the European Helicobacter Study Group has considered causal associations of *H. pylori* only those with unexplained iron-deficiency anemia, idiopathic thrombocytopenic purpura (Grade A of recommendation) and vitamin B12 deficiency (Grade B of recommendation)[27] other relationships could not be excluded. Several studies on this field, have been performed by Piedmont's groups.

Heart diseases

In 1995 Morgando *et al*. recruited 35 male and 7 female patients admitted to the Emergency Unit of Molinette Hospital in Turin with acute myocardial infarction (AMI). They used a commercial ELISA to measure *H. pylori* specific antibodies. The 198 controls were obtained from a cohort of 619 consecutive patients admitted to the same Emergency Unit for other reasons. All 7 AMI patients aged 50-59 years were positive to *H. pylori* compared with 50% of controls. However, at age 60 or more the association seemed less striking (86% versus 67% for men, 71 % versus 52% for women).[28] The same group confirmed these findings in a series of studies.[29-31] In a study performed in Turin and Sondalo (Northern Italy), 223 consecutive male patients admitted for AMI at the Coronary Care Unit were compared with 223 age-matched male patients admitted to the Emergency Care Unit. Cases and controls came from the geographical area of Northern Italy and had a similar socioeconomic status as

based on occupation and level of education. The seroprevalence of *H. pylori* was significantly higher among patients with AMI (84%) as compared to the control population (62%; P < 0.0001). The anti-CagA antibodies were detected in 34% of the patients versus 27% of the controls (P = 0.17).[32]

Pellicano *et al*. investigated if *H. pylori* infection was associated with unstable angina (UA). Thirty-two patients consecutively admitted for UA to the Coronary Care Unit of the Novi Ligure and Rivoli Hospitals were included. As controls, the authors selected 64 subjects admitted to the Emergency Care Unit for any reason (excluding cardiological causes), age and sex-matched (2 controls for each case). Cases and controls were from the geographical area of Northwestern Italy and had similar socioeconomic status judged on work type and on the level of instruction. They found that 81% of the patients with UA were seropositive, as compared with 53% among the control population (P = 0.007). Serum levels of cholesterol, glucose, and fibrinogen in plasma, presence of hypertension and socioeconomic status were comparable in both *H. pylori* seronegative and seropositive patients.[33]

To investigate the possible influence of *H. pylori* eradication on parameters involved in cardiovascular diseases, 496 patients with *H. pylori*-positive dyspepsia and/or peptic ulcer (DU, N: 80; gastric ulcer, N: 51) were studied up to five years. After *H. pylori* eradication, a significant trend towards increase for high-density lipoprotein cholesterol (from 48 to 52 mg/dL; P= 0.02) was observed. C-reactive protein (from 0.22 to 0.19 mg/dL) and fibrinogen (from 257 to 222 mg/dL) levels decreased in a significant manner (P < 0.0001). Body mass index (BMI)(from 25 to 27 kg/m^2, P= 0.03) and diastolic blood pressure (from 85 to 89 mmHg, P = 0.04) increased gradually compared to baseline.[34]

In 2014, a multicenter study including Ponzetto A, reported data on 103 consecutive patients admitted during the year 2005 for ischemic heart disorders. *H. pylori* infection was determined serologically using an ELISA test and confirmed by western blotting. The latter was also used to detect the presence of anti-CagA antibodies in serum samples. A total of 41 patients (39.8%) were *H. pylori*

positive, and 19 of these (46.3%) had serum antibody to CagA. B-type natriuretic peptide median levels in *H. pylori* infected and uninfected patients were 601 pg/mL and 325 pg/mL, respectively (P = 0.18); median levels in infected CagA+ and CagA− patients were 781 pg/mL and 305 pg/mL (P < 0.01). Interleukin (IL)-6 median levels among infected and uninfected patients were 17 pg/mL and 7.7 pg/mL (P = 0.03), respectively. Tumor necrosis factor-α (TNF- α) median levels in infected and uninfected patients were 2.9 and 2.5, respectively (P = 0.01).[35]

Liver diseases

In an initial study on cirrhotic patients, an ELISA method was used to assess the seroprevalence of anti-*H. pylori* IgG antibodies in 70 consecutive male hepatitis C virus (HCV) positive cirrhotic patients, and in a control population of 310 male age-matched blood donors from the same area. An immunoblot assay was also used to detect antibodies against the CagA antigen in sera. The prevalences of *H. pylori*

were 77% and 59% in patients and controls (P = 0.004), respectively. Cag-A positivity was observed in 95% (57/60) of the patients versus 18% of the controls. The authors also analyzed resected liver tissues from 25 hepatocellular carcinomas (HCC): *Helicobacter* genomic sequences were detected in 23/25 cases (92%). PCR with primers specific for the conserved region of the *cag A* gene of *H. pylori* was positive in 81%.[36] This was the first worldwide report to show Helicobacter sequences in the liver of patients with HCC, published contemporarily with another paper of the Bordeaux' group.[37]

Following this work, the same group showed that the prevalence of antibodies against *H. pylori* in cirrhotic hepatitis B virus patients was significantly higher than in controls (P < 0.001) and that there was no relationship between this prevalence and Child-Turcotte-Pugh's class.[38] Similar results was reported considering cirrhotic HCV-positive patients.[39] On the contrary, the seroprevalence of antibodies against *H. pylori* in patients with chronic autoimmune hepatitis[40] or with the formerly known primary biliary cirrhosis, now primary biliary cholangitis, was similar to that of controls.[41]

The presence of anti-*H. pylori* antibodies was evaluated in 46 consecutive cirrhotic HCV-positive patients and superimposed HCC, attending the Department of Gastro-Hepatology, Molinette Hospital of Turin. The prevalence of antibodies to *H. pylori* was significantly higher (78.2%) in patients with HCC than (54%) in controls.[40] To confirm this initial result, the same group analyzed liver samples obtained during the surgical excision from 23 patients operated upon for HCC superimposed on HCV-related cirrhosis and from 6 patients suffering from metastatic cancers to the liver, in whom surgery might obtain remission of the disease. The authors found DNA sequences typical of *Helicobacter spp.* in 85% livers from patients operated for HCC using *Helicobacter* genus-specific 16S rRNA primers. Two of 6 liver samples obtained from patients whose livers were resected for metastasis due to colon cancer, contained sequences typical of *Helicobacter spp.*. Ninety-four percent of liver tissues from positive patients, when tested for *Helicobacter genus*, were also found positive when tested for the presence of a sequence that was typical for *H. pylori*, i.e., the *cag A* gene.[43]

Neurological diseases

One hundred three consecutive patients with migraine attending the Headache Center of the University of Turin, for the first visit, were enrolled in a study. A group of 103 healthy subjects attending the Molinette Hospital's Blood Bank, matched for sex and age, served as controls. *H. pylori* infection was assessed by means of both UBT and IgG against the bacterium in serum. *H. pylori* infection was present in 30.1% of the patients with migraine and 31.1% of the controls (P = NS). Morevover, this infection was not associated with any significant variation in the clinical features of the disease.[44]

Eighty consecutive patients aged less than 65 years, suffering from ischemic stroke presenting to the Neurological Ward of three Hospitals in Turin were recruited. Sex and age-matched blood donors (4 controls for each case) served as controls. *H. pylori* infection was demonstrated in 80% of patients as compared to 59.4% of controls (P<0.001). The authors concluded that these results support the association between *H. pylori* infection and stroke.[45]

Metabolism

Forty-one consecutive type 2 diabetic subjects attending an out-patient clinic in Turin participated in a study. Thirty-one healthy subjects matched for sex, age, and geographical area of residence served as controls. The UBT showed gastric *H. pylori* infection in 66% of diabetics and in 48% of controls (P < 0.05). Nevertheless, microangiopathy was significantly more prevalent (P < 0.05) in *H. pylori* negative patients (85%) than in *H. pylori* positive patients (48%).[46]

Twelve patients with primary autonomic failure were studied. The results were compared with those of 31 healthy controls and 31 patients affected by type 2 diabetes without autonomic neuropathy. To assess the presence of *H. pylori* infection, all patients and controls underwent a UBT. *H. pylori*

infection was detected in 100% of autonomic failure patients, in 48% controls and in 71% diabetic patients; this difference was significant (P = 0.02).[47]

Blood disease and systemic autoimmunity

Thirty-seven consecutive polycythemia vera (PV) patients, followed at the Hematological Unit of the Institute of Medical Science, were included. Seventy-three controls were selected from consecutive out-patients referred to the Department of Internal Medicine and to the Emergency Department for endoscopic examination, matched to patients (1 patient : 2 controls) for sex and age. All patients underwent EGDS with multiple gastric biopsies. The operator was blinded as to whether the patient had PV or belonged to the control group. Blood samples of patients and controls were obtained for serum anti-CagA and IgG antibody determination. *H. pylori* infection was more frequent in PV patients (83%) than in controls (57%) (P < 0.01). The prevalence of anti-CagA antibodies was also significantly higher in PV patients than in controls (66% versus 37%, P < 0.01).[48]

The presence of non-organ-specific autoantibodies (NOSAs) was evaluated in 49 consecutive patients suffering from DU and *H. pylori* infection (Group A) and 38 consecutive subjects affected with DU related to the consumption of NSAIDs, but not to the presence of *H. pylori* (Group B) and resident in the same area. A serum sample from each patient was tested to detect antinuclear (ANA), anti-smooth muscle (SMA), and anti-liver/kidney microsomal-1 (LKM-1) antibodies. ANA, SMA, and anti-LKM-1 antibodies were present, respectively, in 10%, 4%, and none in Group A. In Group B, ANA was present in 8%, SMA in 8%, and anti-LKM-1 in none. The difference was not statistically significant.[49] A recent study, with a similar design, including patients with gastric ulcer, obtained similar results.[50]

Pregnancy

A total of 167 serum samples from patients (118 male) with reproductive disorders, seen at two clinics for infertility in Turin and Siena were examined. As controls, sera from blood donors of similar

range of age and proportion male/female as the patients, living in Turin or Siena (N: 837) were examined. The *H. pylori status* was determined serologically using two tests, a commercially available ELISA and a Western blotting assay. The authors determined the presence of anti-*H. pylori* IgG, and that of specific IgA in 11 specimens of follicular fluid, 16 specimens of vaginal secretions, and 28 specimens of semen obtained from some of the above-reported patients chosen at random. Seropositivity for *H. pylori* was significantly more common in patients than in age- and gender-matched controls (49.1% and 33.5%, respectively, P = 0.0001).[51] Despite these results, it is premature to state that the association between *H. pylori* infection and infertility is not coincidental.

In a very interesting study, pregnant women delivering at the Maternal–Fetal Medicine Unit of the University of Turin were recruited. Maternal blood samples were collected before delivery from 47 consecutive pregnant women with diagnosis of pre-eclampsia (PE), and from 47 women with uneventful pregnancies, matched for maternal age. Twenty placentas were obtained from 10 of the PE pregnancies and 10 of the controls delivered by Cesarean section. Serum anti-*H. pylori* IgG and anti-CagA were assessed. Detection of *H. pylori* DNA was carried out by a nested PCR. The two groups were comparable for maternal age, smoking habit, pre-pregnancy BMI, gestational weight gain, treatment with antibiotics and risk factors for PE. Among PE women, there were significantly more *H. pylori* seropositive subjects (51.1%) than in normal pregnant women (31.9%) (P = 0.03). Anti-CagA antibodies were significantly more common among PE mothers (80.9%) than among normal pregnant women (14.9%) (OR, 26.0; 95% CI, 8.19–82.7; P < 0.001). All samples, from each of the placentas tested (cases and controls), were negative for the presence of *H. pylori* DNA. The association was stronger in cases of CagA+ strains.[52] This study presents direct evidence of an association between *H. pylori* infection and PE in Italian. A possible bias could arise from the lacking data of ethnic background of all women. The fact that all placentas were negative for *H. pylori* DNA indicates that the pathogenic mechanism would not be local. This finding, along with the knowledge that *H. pylori* is

predominantly acquired during childhood and that the infection is lifelong when untreated, elicits the speculation that *H. pylori* positive women may have underlying vascular damage; such subclinical dysfunction might augment the inflammatory changes of pregnancy, thus contributing to the symptoms of PE. CagA+ strains are associated with increased TNF-α levels.[53] PE is equally characterized by an exaggerated inflammatory response: high leucocyte and neutrophil count, high levels of C-reactive protein and high levels of proinflammatory cytokines, such as TNF- α.[54] These data were substantially confirmed by the same group in a subsequent study.[55]

Diagnostic methods

A total of 125 consecutive patients, either dyspeptic or with a previous history of peptic disease referring to GI Unit of Mauriziano Hospital of Turin, for *H. pylori* assessment by means of UBT, were included in a study. Fresh stool specimens were collected within 1 week of ^{13}C-UBT. Fecal *H. pylori* detection was performed by means of a commercially available kit that uses polyclonal anti-*H. pylori* antibodies (HpSA). Overall UBT was positive in 40 cases and negative in 85, while HpSA was positive in 71 cases and negative in 54. Hence, conflicting results were found in 30% of subjects; 34 out of 37 cases presented a positive HpSA test in the presence of negative UBT. The discrepancy was mostly found in the group of "treated" patients. While a negative HpSA test correlates well with UBT, a certain degree of discrepancy can be appreciated in the presence of positive results, mainly in subjects evaluated after eradication treatment.[56] This confirm the importance to use monoclonal tests when diagnosis of *H. pylori* infection is performed with a fecal test.[21]

UBT was administered to 91 patients attending the out-patient clinic of the Department of Gastro-Hepatology (Molinette Hospital, Turin). The presence of antibodies against *H. pylori* was tested both in serum and the saliva. A patient was considered infected when had at least two positive tests (gold standard). Saliva (Simplex™ H. pylori RAPID test, Analyte Diagnostics, Inc. Hallandale, Florida

USA) was collected by a sterile absorbent pad placed in the mouth and assayed immediately for *H. pylori* antibodies by an immunochromatographic method. There were 37 out of 91 gold standard positive patients, of which Simplex™ *H. pylori* RAPID test identified 29. Of 54 gold standard negative test, the salivary test falsely identified 13 as positive. Statistical analysis revealed a sensitivity of 78.3%, a specificity of 75.9% and an accuracy of 76.9%.[57]

The first study in Piedmont that analyzed by culture the prevalence of *H. pylori* antibiotic resistance was published by Franzin *et al*. The methods used were the agar disk diffusion test and E-test, a technique for the quantitative determination of susceptibility to antimicrobial agents. Forty-nine patients were included. All the strains tested were susceptible to amoxicillin by both methods. Two strains were resistant to clarithromycin by the disk diffusion test and by E-test, and one strain was resistant only by E-test. In this study two strains were found to be clarithromycin-resistant by the disk diffusion method and three by E-test. Resistant pretreatment strains were found in two patients (5.9%) and a resistant posttreatment strain in one (2.9%), indicating acquired resistance.[58] In a European multicentre study, including data from Turin, the primary rate of *H. pylori* clarithromycin resistance in Italy (2008-9) in adult patients resulted 26.7%.[59]

To evaluate if in a clinical setting culture can be performed with a good accuracy, bioptic samples of patients who failed at least 3 courses of treatment were considered before to made the antibiogram for antibiotic resistance assessment. Out of 30 positive patients, culture correctly identified 29. In 1 case, no growth of micro-organisms was observed for up to 12 days. On the contrary, histology, serology and UBT gave a positive result. In addition, searching for antibodies against CagA in circulation, the strain of the bacterium was considered more virulent. Among specimens obtained from the control group, bacterial culture accurately identified all negative samples. Statistical analysis revealed sensitivity of 97%, specificity of 100%, and accuracy of 98% for bacterial culture. Positive and negative predictive

values were 100% and 94%, respectively.[60] Thus, also in a clinical setting this procedure provides a good level of diagnostic accuracy.

In a multicentric study including patients with functional dyspepsia, the authors prospectively evaluated the symptomatic benefit of treatment with a food supplement composed of sodium alginate, carbonate calcium, pineapple, papaya, ginger, α-galactosidase and fennel (Perdiges, Bioten Snc, Turin, Italy), after *H. pylori* treatment. The primary goal was to establish the percentage of patients who continued to abstain from PPI as they waited to carry out the UBT, differentiating between patients who were treated (N: 55) with Perdiges and those who were not (N: 36). All the patients treated with Perdiges (100%) and 86% of the patients who were not (P = 0.008) continued to abstain from PPI in the period awaiting the UBT.[61] This could allow to reduce the need for antisecretive drugs.

Treatment of *H. pylori* infection

In 1992, Di Napoli *et al*. treated for 10 days 50 consecutive patients with *H. pylori* positive non-ulcer dyspepsia, with colloidal bismuth subcitrate (CBS), tinidazole and amoxicillin. Infection was confirmed by histological evaluation of four antral biopsy specimens taken during endoscopy, and by a positive urease test. The same evaluations were performed six weeks after treatment. At the second endoscopy 33 patients out of 48 (69%) gave a negative result to the urea test and there was no histological evidence of *H. pylori* infection.[4]

Bologna *et al*. compared the ability of 4 different regimens of CBS in combination with one or two antibiotics to eradicate *H. pylori*. A total of 140 consecutive patients with histological evidence of *H. pylori* infection on antral biopsies were included in the study. The treatment groups were: a) CBS with amoxicillin and tinidazole b) CBS with ofloxacin c) CBS with ofloxacin and amoxicillin d) CBS with metronidazole. Although higher eradication rates (Table 1) were achieved in group d, the difference did not reach statistical significance.[62]

Suriani *et al.* treated with ranitidine, metronidazole, doxycycline, and CBS for 2 weeks 16 patients with a history of DU healed by H_2-receptor antagonist therapy and with *H. pylori* positivity at histological tests: the therapy eradicated *H. pylori* in 81% of cases.[7]

In another study 126 patients shown infected by *H. pylori* were treated with CAO or MAO (metronidazole 250 mg q.i.d., amoxicillin 500 mg q.i.d and omeprazole 20 mg b.i.d.) regimens for 10 or 14 days. Diagnosis of *H. pylori* infection was assessed by histology and serological evidence of raised levels of IgG against the bacterium at 3, 6, 12, 24 months (Table 1). No statistical difference was demonstrated between the two regimens as well as between the two periods.[63]

In 1999, 37 naïve patients with *H. pylori* positive gastritis were treated with lansoprazole, amoxicillin, plus azithromycin for 3 days starting from the second day of treatment (Table 1). *H. pylori* eradication rate on per-protocol (PP) basis was 54%.[64]

In the Fanzin' study 10 patients were treated with bismuth salts (CBS: 240 mg four times a day) and omeprazole (20 mg a day) (CBSO) for 2 weeks. Other 39 subjects were treated with the triple therapy CAO for 1 week. PP eradication rate was 90% (28/31) for patients treated with CAO and 67% (4/6) for patients who received CBSO.[58]

In two studies, patients referred to the Unit of Gastroenterology at Mauriziano Hospital (Turin) for the assessment of *H. pylori* status were evaluated to analyze eradication rate after treatments prescribed by generalists. The eradication rates for first-line *H. pylori* eradication therapies adopted by family doctors are reported in Table 1.[65,66]

In the study by Rocco *et al*. 54 consecutive patients with DU and *H. pylori* infection were treated with CAO regimen: sixteen patients dropped out during the follow-up, 24 responded to eradication therapy, whereas *H. pylori* persisted in the remaining 14.[11]

A total of 172 patients were randomly treated with a triple therapy regimen comprising proton pump inhibitor (PPI), amoxicillin and clarithromycin: 66 patients received a 1-week triple therapy (group I),

42 subjects a 10-day triple therapy (group II) and 64 a 14-day triple therapy (group III). *H. pylori* infection and the outcome of eradication treatment, were assessed by UBT and histology. The overall *H. pylori* eradication rate was 68% in group I, 76% in group II and 72% in group III, without any statistically significant difference.[67]

A total of 159 patients, with a documented history of recurrent DU, were randomly treated with a triple MAO regimen. In detail, 53 patients received a one-week course (Group I), 53 subjects a 10-day course (Group II) and 53 others a 14-day course (Group III). *H. pylori* infection was assessed by UBT and histology. At the end of the course of treatment, the overall *H. pylori* eradication rate in the PP analysis showed no statistical differences, with an eradication rate of 74% in group I, 76% in group II and 78% in group III.[68]

In a randomized study the efficacy of eradication treatment, using metronidazole versus tinidazole, in subjects never treated for *H. pylori,* was evaluated. Diagnosis and eradication of *H. pylori* infection were assessed by UBT. A total of 171 patients was treated: 91 of them with a MAO regimen and 80 with tinidazole, amoxicillin and omeprazole regimen for 7, 10 or 14 days. No difference was found between the two regimens as well as between the two periods.[69]

Reviewing the charts of naïve patients underwent UBT in the outpatient clinic of Unit of Gastroenterology of Molinette Hospital (with a mean of 2000 test performed per year[70]), several interesting data were reported. First, an azithromycin-based triple therapy is not different from a clarithromycin-based triple therapy.[71] Second, a clarithromycin plus metronidazole-based triple therapy give similar results than a triple therapy based on a standard dose of PPI, amoxicillin and clarithromycin or metro- or tinidazole.[72] Third, the use of amoxicillin plus clavulanic acid-based triple therapy is not different of a CAO regimen.[73] Fourth, considering a triple therapy based on PPI, amoxicillin and tetracycline, the overall *H. pylori* eradication rate was 53%.[73] When compared with the amoxicillin plus clarithromycin or plus metronidazole or tinidazole regimens, a significant

disadvantage was observed versus the metronidazole- (P = 0.04) but not versus the tinidazole- (P = 0.08) and the clarithromycin-based regimens (P = 0.11).[74] Fifth, the efficacy of a triple therapy based on ranitidine bismuth citrate, amoxicillin and clarithromycin, is not more effective than a CAO regimen.[75] Sixth, the efficacy of the ampicillin-based triple therapy for *H. pylori* eradication remains unclear.[76] Seventh, a one-week PPI, cefixime plus metronidazole based triple therapy gave similar results than a MAO regimen.[77] Eighth, the success rate of a one-week cefixime plus clarithromycin based triple therapy was not different from that of the CAO regimen.[78]

In 2015 Ribaldone *et al*., to asses the change over time of antibiotic efficacy, prospectively evaluated the *H. pylori* eradication rate of consecutive naïve patients, treated for *H. pylori* with a standard dose of PPI, amoxicillin 1 g and clarithromycin 500 mg twice daily.[79] Furthermore, results were compared with the randomized prospective study conducted 10 years earlier with the same schedule.[66] Eradication of *H. pylori* infection was assessed by UBT. The cohort included 182 patients, 99 of them received a 1-week regimen (group I) and 83 were treated with a 10-day regimen (group II). The overall *H. pylori* eradication rate was 71% in group I and 73% in group II, without significant difference between the 2 regimens.[78] When compared with the prospective study published in the year 2002, no difference was observed between the groups (P = 0.87 and P = 0.9, respectively).[69] Thus, in Piedmont, a clarithromycin-based treatment regimen was equally effective than 10 years earlier. Nevertheless, these eradication rates were significantly inferior than those reported 17 years before.[63] In the same context, the authors evaluated prospectively the *H. pylori* eradication rate of consecutive naïve patients, treated with a triple therapy comprising a standard dose of PPI, amoxicillin 1 g and metronidazole 500 mg twice daily. The cohort included 39 patients treated with a a 1-week regimen (group I) and 27 treated with a 10-day regimen (group II). The *H. pylori* eradication rate was 69% in group I and 70% in group II (P = 0.96).[80] When compared with the prospective study published in the year 2002 no differences were observed in the effectiveness of therapy (P = 0.81 for 7 days and P = 0.95 for 10 days).[68] Thus, in

Piedmont, a metronidazole-based treatment regimen for *H. pylori* eradication, although unsatisfactory, is as effective as 10 years ago.

Table 1

Relapse and reinfection

In 1996, Bruno *et al*. in a cohort of patients with DU in whom *H. pylori* had been eradicated, showed that reinfection occurred in 14.8% of patients between month 2 and month 6, in 5.4% between month 6 and month 12, and in 11.1% between month 12 and month 24. Active antral gastritis at month 2 was found in 19% of the patients converting to *H. pylori* positivity at month 6, but only in 3.4% of the patients in whom eradication still persisted at month 6 ($P = 0.02$).[81] Although limited by the tests used (urease test and histology), this study has the advantage of a regular follow-up.

In another study, using histology, serology and UBT, the authors showed that none of the 108 patients with history of DU, followed up during the average period of 24 months, had a reinfection. Five patients (4.6%) had a DU recurrence (none due to NSAIDs).[82] These findings provided further evidence that the cure of *H. pylori* infection allows for a dramatic reduction in the frequency of DU recurrences, and that the reinfection in adults is unlikely in their population. The different results of these two studies[81,82] could be explained by the potential relapse risk in the former and the exclusion of reinfection in the latter. The robustness of the second is witnessed by the fact that reinfection should be confirmed by the positivity of at least two tests.

Conclusions

In Piedmont, in the last 25 years an intense research on clinical aspects of *H.pylori* infection has been conducted. Nevertheless, several aspects of basic research have been approached, with the first

description of Helicobacter sequences in the liver of patients with HCC. The actual practical challenge, based on data of antibiotic resistance, is the application of the Maastricht V/Florence Guidelines, with in Countries with high clarithromycin-resistance rates, as in our region, recommended the bismuth-containing quadruple therapy as first choice of treatment.[27,83]

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Table 1. Studies evaluating efficacy of *H. pylori* therapy in Piedmont

Author	Year of publication	Origin of patients	Diagnosis	Treatment	Days	# patients	Eradication diagnosis	Eradication
Di Napoli et al.[4]	1992	S. Vito Hospital Turin	G	CBS 120 mg/6 h + Ti 500 mg/12 h + Am 1g/12 h	10	48	H	69%

Bologna et al.[62]	1992	Molinette / Cuorgnè	DU, Dy	CBS 120 mg/6 h +	28		H		
				Am 1 g/12 h + Ti 500 mg/12 h	13	22			59%
				Of 300 mg/12 h	11	27			59%
				Of 300 mg/12 h + Am 500 mg/12 h	11	31			58%
				M 500 mg/8 h	11	43			72%
Suriani et al.[7]	1996	Rivoli / Valdese	DU	R 150 mg/24 h + M 500 mg/6 h + Do 100/24 h + CBS 300 mg/6 h	14	16	H		81%
Pellicano et al.[63]	1997	Molinette	DU	Cl 250 mg/8 h + Am 500 mg/6 h + Om 20 mg/12 h	10	16	H, IgG		93%
					14	39			95%
				M 250 mg/8 h + Am 500 mg/6 h +	10	26			88%

				Om 20 mg/12 h	14	45			89%
				RBC 400 mg/12 h + Cl 500 mg/12 h	28 7	40			80%
				RBC 400 mg/12 h + Cl 500 mg/12 h + Am 1 g/12 h	28 7	40			88%
Lombardo et al.[64]	1999	Maurizi ano	G	L 30 mg/24 h + Am 1 g/12 h + Az 500 mg/24 h	7 3	37	UBT		54%
Franzin et al.[59]	2000	SGAS	GU, DU	CBS 240 mg/6 h + Om 20 mg/24 h	14	10	H		67%
				Om 20 mg/12 h + Am 1 g/12 h + Cl 500 mg/12 h	7	39			90%
Della Monica et al.[65, 66]	2002	Primary care	Dy, GERD, PUD, ?	Am + Cl + PPI	7 14	812 188	UBT		72% 78%
				Am + M + PPI	7 14	23 7			61% 86%

				Am + Ti + PPI	7	62		71%
					14	14		79%
				Cl + M + PPI	7	46		61%
					14	4		50%
				Cl + Ti + PPI	7	91		76%
					14	8		75%
Rocco et al.[11]	2002	Rivoli	DU	Om 40 mg/12 h + Am 1 g/12 h + Cl 500 mg/12 h	?	38		63%
Palmas et al.[67]	2002	Molinette	DU	PPI 20 mg/12 h + Cl 500 mg/12 h + Am 1 g/12 h	7	66	H, UBT	68%
					10	42		76%
					14	64		72%
Pellicano et al.[68]	2002	Molinette	DU	PPI 20 mg/12 h + Am 1 g/12 h + M 500 mg/12 h	7	53	H, UBT	74%
					10	50		76%
					14	50		78%
Berrutti et al.[69]	2008	Molinette	Indications as recommended by Maastricht 2	PPI 20 mg/12 h +	7		UBT, H	
				Am 1 g/12 h + M 250 mg/8	7	30		77%
					10	26		77%
					14	32		78%
				Am 1 g/12 h + Ti 500	7	27		74%
					10	26		77%

				mg/12 h	14	27		78%
Pellicano et al.[71]	2008	Primary care	PUD, G	PPI + Am 1 g/12 h + Az 500 mg/24 h	7	13	UBT	54%
					10	11		64%
					14	23		70%
					15	12		67%
Berrutti et al.[72]	2010	Primary care	PUD, G	PPI 20 mg/12 h + M 250 mg/8 + Cl 500 mg/12 h	7	20	UBT	70%
					14	42		74%
Berrutti et al[73]	2010	Primary care	PUD, G	PPI 20 mg/12 h + Cl 500 mg/12 h + AmC 1 g/12 h	7	28	UBT	79%
					14	14		79%
Pellicano et al.[74]	2011	Primary care	?	PPI 20 mg/12 h + Am 1 g/12 h + Te 500 mg/8 h	7	40	UBT	53%
Cisarò et al.[75]	2011	Primary care	?	RBC 400 mg/12 h + Cl 500 mg/12 h + Am 1 g/12 h	7	67	UBT	67%
Adriani et al.[77]	2013	Primary care	?	M + PPI + Ce 200	7	24	UBT	

				mg/12 h				63%
				Ce 400 mg/12 h		7		71%
Fagoonee et al.[78]	2013	Primary care	?	Cl + PPI + Ce 200 mg/12 h	7	34	UBT	71%
				Ce 400 mg/12 h		15		73%
Ribaldone et al.[79]	2015	?	?	PPI + Am 1 g/12 h + Cl 500 mg/12 h	7	99	UBT	71%
					10	83		73%
Ribaldone et al.[80]	2017	?	?	PPI + Am 1 g /12 h + M 500 mg/12 h	7	39	UBT	69%
					10	27		70%

DU, duodenal ulcer; Dy, dyspepsia; CBS, colloidal bismuth subcitrate; Am, amoxicillin; Ti, tinidazole; Of, ofloxacin; M, metronidazole; H, histology; R, ranitidine; Do, doxycycline; Te, tetracycline; F, floxacine; Om, omeprazole; G, gastritis; Cl, clarithromycin; IgG anti-H. pylori; PUD, peptic ulcer disease; Du, duodenitis; UBT, urea breath test; RBC, ranitidine bismuth subcitrate; L, lansoprazole; Az, azithromycin; RUT, rapid urease test; Cu, culture; SGAS, San Giovanni Antica Sede; GU, gastric ulcer; PPI, Proton Pump Inhibitors; GERD, gastroesophageal reflux disease; ?, unreported; ATB, antibiotic; AmC, amoxicillin and clavulanic acid; Ce, cefixime.