Primary antibiotic resistance and effectiveness of *helicobacter pylori* triple therapy in ulcero-inflammatory pathologies of the upper digestive tract

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Abstract

Objectives : **To determine firstly, the rates of primary antimicrobial resistance for *Helicobacter pylori* - associated upper - digestive lesions in relation to the success rate of triple therapy ; and secondly, the performance of HpSA stool antigen detection test for control of eradication after treatment.**

Methods : **Prospective open study of 436 patients who underwent upper - digestive tract endoscopy with biopsies for histological examination and culture between January 1 and July 31, 2002 at a University hospital in Brussels, Belgium. The primary resistance to antibiotics of *H. pylori* isolates was determined by disc diffusion method. Seventy of 164 infected patients agreed to be included in the treatment study with standard triple therapy with amoxicillin + clarithromycin + omeprazole adjusted on the basis of antibiogram results. Control of eradication was tested by ^{14}C-Urea breath test and *H. pylori* Stool Antigen test (HpSA test).**

Results : **Primary resistance to clarithromycin and metronidazole was observed in 3% and 31% of the isolates, respectively. No primary resistance to amoxicillin and tetracycline was observed. By intention to treat analysis, *H. pylori* was eradicated in 56 (80%) patients included in the therapeutic study. Three (4%) patients were lost to follow-up. The rate of eradication failure was 20% (14/70), included 11 cases documented by a positive control test (^{14}C-Urea breath test). In comparison with ^{14}C-Urea breath test, the *H. pylori* Stool Antigen test showed a sensitivity of 100%, a specificity of 91%, PPV of 69%, and NPV of 100%.**

Conclusion : **Standard triple therapy achieved 80% bacterial eradication in this patient population with a low prevalence of *H. pylori* primary antibiotic resistance. Our data confirm that the *H. pylori* Stool Antigen test displays a diagnostic performance similar to the breath test for control of eradication.** (Acta gastroenterol. belg., **2005**, 68, **287-293**).

Key words : *H. pylori*, antibiotic resistance, triple therapy, eradication, stool antigen test.

Introduction

The major role of *H. pylori* in the etiopathogeny of various gastroduodenal diseases (gastritis, gastric and duodenal ulcers, gastric lymphoma) is well established today. The prevalence of infection varies from 30 to 50% of the population in the industrialized countries and 80 to 90% in developping countries (1). The current methods of diagnosis are either invasive (2), requiring an endoscopy and biopsies (urease fast test, histopathological examination, culture and PCR), or non-invasive (2,3-4) (^{13}C or ^{14}C-urea breath test [UBT], serology and stool antigen test). Triple therapy associating a proton pump inhibitor (PPI) with two antibiotics, chosen between amoxicillin, clarithromycin and metronidazole, is currently recommended (5).

Eradication failure after a first treatment course is observed in 10 to 30% of the patients (6). The proportion of failure ascribable to primary resistance to the treatment or to incomplete compliance furthering the selection of secondary resistance varies according to the studies and geographical areas. To evaluate the applicability and efficacy of the therapeutic recommendations in our pratice, we studied the prevalence of *H. pylori* infection in the outpatient population of the Gastroenterology clinic at the Erasme University hospital in Brussels, determined its rate of primary resistance to antimicrobial agents and evaluated the rate of eradication of *H. pylori* by triple therapy. The control of eradication was assessed by two tests : detection of *H. pylori* antigen in stools (HpSA test) and radiolabelled urea breath test (UBT).

Patients and methods

Patients and study design

This prospective cohort study involved the department of digestive endoscopy in collaboration with the departments of bacteriology, histopathology and isotopic imaging from the university hospital Erasme in Brussels from January 1 to July 31 2002.

Patients included in the study were either hospitalized or outpatients and presenting with an indication for upper digestive endoscopy (upper abdominal pain, pyrosis, nausea or vomiting) following the protocol shown in figure 1. Patients were excluded from the study (i) if they had already taken medications against *H. pylori* or other antibiotics in the past 4 weeks, (ii) were allergic to any medication prescribed or (iii) had undergone previous gastric surgery. The nationality of enrolled patients was determined by retrospective review of the medical records.

The study protocol was approved by the ethics committee of Erasme hospital under the reference : 2001/284, N° OM 021.

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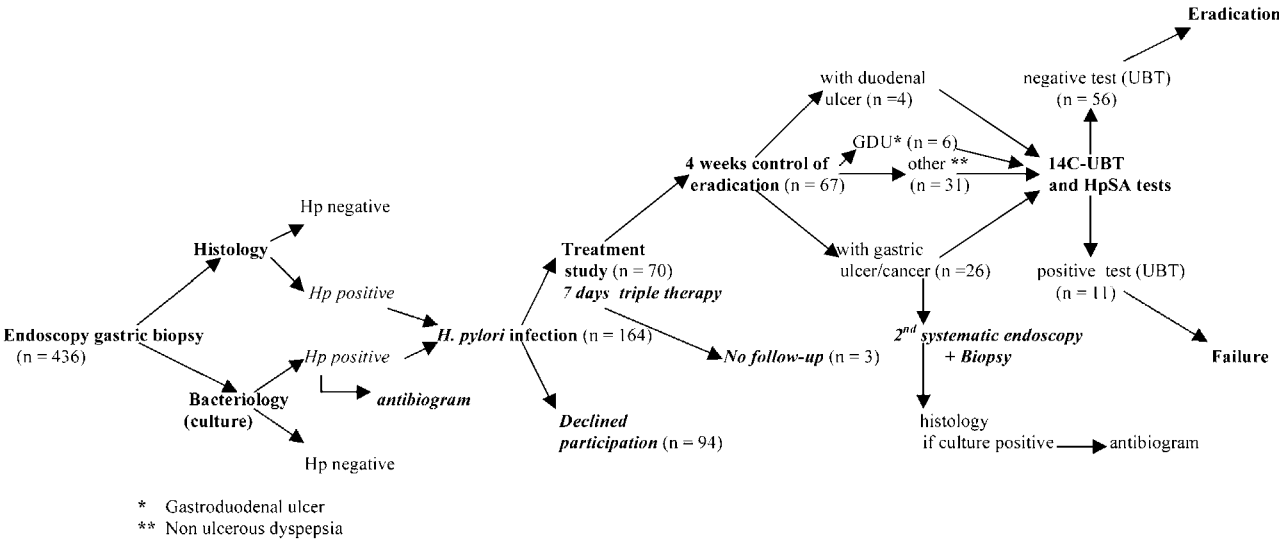

Fig. 1. — Study flow chart

Diagnostic procedures

During the endoscopic examination, four gastric biopsy samples (from the antral area and the corpus) were taken (Fig. 1). Two of them (one of the antrum, one of the fundus) were placed in formaldehyde and sent for histological analyses. Curved bacilli with typical morphology were detected after staining with Cresyl Purple. In presence of spiral bacteria with atypical morphology an immuno-histochemistry stain for *H. pylori* was performed for confirmation. Two other biopsy samples were sent for bacteriological analysis in Stuart's transport medium. On non-working days, samples for bacteriology were frozen at -70° C to ensure bacterial viability. Biopsy samples were homogenised mechanically in a tissue grinder and inoculated on two culture media : a selective medium (Pylori Agar : BioMérieux SA, Marcy l'Etoile, France) supplemented with plasma and polyvitex and with a selective mixture of vancomycin, trimethoprim, amphotericin B and colistin ; and a non-selective medium (Columbia agar) supplemented with peptones and with 5% horse blood. After inoculation, the media were incubated at 37° C in a microaerophilic atmosphere during 10 days. Visual inspection for growth took place on the 3rd day, then every 2 days. When the culture proved positive, the antibiogram was performed by disk diffusion method (Rosco, NeoSensitabs, Taastrup, Denmark) after 3-day incubation on Columbia agar with 5% horse blood. The inhibition zone diameters (mm) used for categorization of strains as resistant or susceptible to the different antibiotics are those recommended by the manufacturer (Rosco) : amoxicillin : 26-32 ; metronidazole : 20-26 ; clarithromycin : 19-23 ; tetracycline : 26-32.

Definition of infection and eradication test methods

Any patient having a positive culture for *H. pylori* was considered infected (Fig. 1). The control of eradication was made by simultaneous ^{14}C-UBT and HpSA testing at least 4 weeks after the end of the treatment. Eradication of *H. pylori* was defined on the basis of negative ^{14}C-UBT test (Gold standard). This test was performed in fasting subjects who drank 1 mCi/1 ml of ^{14}C urea dissolved in 25 ml of water. The exhaled air was collected before the ingestion, and 15 mn afterwards. Measurements were evaluated according to the criterion of normality : an exhalation < 1.03% DI % mmole × weight. Detection of the *H. pylori* antigen in stools was performed by ELISA method (HpSA test, Meridian, Cincinnati, Ohio,USA). The stools were sampled on the same day. HpSA test was carried out on fresh specimens or on samples frozen at -20° C after thawing. The absorbance was measured with a double wavelength spectrophotometer (450/630 nM) and evaluated according to the criteria of the manufacturer (positive > 0.120 ; negative < 0.100 and undetermined > 0.100 and < 0.120). The HpSA test was validated in our laboratory and showed a sensitivity of 96.5% and a specificity of 91.2% compared to invasive methods (culture and histology) (7).

Treatment and outcome

A 7-day course associating amoxicillin (2 × 1 g/day) + clarithromycin (2 × 500 mg/day) + omeprazole (2 × 20 mg/day), followed by omeprazole 1 × 20 mg/day for 2 to 6 weeks, was proposed to the infected patients. The prescription of metronidazole (3 × 250 mg/day) for 7 days was the alternative antibiotic used instead of clarithromycin in case of primary resistance documented by the antibiogram.

The eradication failure rate was the ratio (in percentage) of positive patients for any or both of control tests after treatment and patients lost to follow-up divided by the number of patients enrolled in the treatment study. A second endoscopy with gastric biopsy for culture with antibiogram was proposed to patients for whom a

Table 1. — **Characteristics of patients undergoing upper endoscopy by *H. pylori* infection status**

Characteristic	No (%) of Patients (n = 436)	No (%) of Patients		p-value
		Hp+ (n = 164)	*Hp*– (n = 272)	
Age (years)				< 0.05
10-29	52 (11.9)	25 (15.2)	27 (10.0)	
30-49	156 (35.8)	67 (40.9)	89 (32.7)	
50-69	150 (34.4)	52 (31.7)	98 (36.0)	
≥ 70	78 (17.9)	20 (12.2)	58 (21.3)	
Sex				> 0.05
Male	207 (47.5)	81 (49.4)	126 (46.3)	
Female	229 (52.5)	83 (50.6)	146 (53.7)	
Type of care				> 0.05
Outpatient	294 (67.4)	111 (67.7)	183 (67.3)	
Hospitalised	142 (32.6)	53 (32.3)	89 (32.7)	
Endoscopic findings				< 0.01
Normal mucosa	28 (6.4)	11 (6.7)	17 (6.3)	
Gastritis*	83 (19.0)	43 (26.2)	40 (14.7)	
Gastric ulcer	112 (25.7)	39 (23.8)	73 (26.8)	
Duodenal ulcer	40 (9.2)	17 (10.4)	23 (8.5)	
Gastroduodenal ulcer	28 (6.4)	14 (8.5)	14 (5.1)	
Oesophagitis	145 (33.3)	40 (24.2)	105 (38.6)	

Hp : *H. pylori ;* * erythematous, atrophic or hemorragic gastritis. The figures between brackets correspond to percentages by category for each characteristic ; p-values based on 2 by n Chi-square test for comparing the proportion of patients with characteristics between infected and non-infected patients.

treatment of second intention was considered after initial treatment failure.

Statistical analysis

The statistical analysis was carried out using Epi-info 6.04 version software. The statistical tests used in univaried analysis included : the Pearson c^2 test or the Fisher exact test for the comparison of proportions and the Student t-test for the comparison of means. The threshold of significance was a = 5%. A stratified analysis was made for age, sex, nationality, type of pathology, patient's history of prior *H. pylori* infection.

Results

Between January 1 and July 31, 2002, 436 patients, including 142 (32.6%) hospitalized at the Erasme hospital, underwent an upper digestive endoscopy with biopsy for histology and culture of *H. pylori*. Among the subjects enrolled in the study, 376 were identified as Belgian nationals and 60 were of other nationalities. Their clinical symptomatology was diverse, consisting in (i) dyspeptic symptoms (nausea, heavy stomach, slow digestion), (ii) epigastric pain (burn, painful hunger, cramp), (iii) digestive haemorrhages (mainly maelena). Most patients (74%) had a good general health status but 16% had a past history of ulcer, of which 51 (72%) were gastric ulcer, 15 (21%) duodenal and 5 (7%) gastroduodenal. Forty-five (10%) patients had been carrying *H. pylori* 8 weeks or more before study, among whom 43 patients had been treated for this infection. The mean age of the patients was 51 (SD ± 17) years with extremes varying from 14 to 90 years and 229 (52.5%) were women (Table 1).

Analysis of gastric biopsies detected 164 (37.6%) patients with *H. pylori* infection documented by culture. Among the 164 *H. pylori* infected patients, 26 (16%) were already infected 8 weeks or more before study. Among Belgian patients, 35.4% (133/376) were *H. pylori* culture positive versus 51.7% (31/60) non-Belgian patients ($p < 0.01$).

Endoscopic data showed that 180 patients (41.3%) had ulcerous lesions of the upper digestive tract. Infected patients tended to be younger and had more frequently gastritis and less frequently oesophagitis than non infected patients (Table 1). Moderate and severe chronic gastritis were more frequently observed in *H. pylori* positive than negative patients ($p < 0.001$; Table 2). Patients presenting ulcerous lesions at the endoscopic examination showed no statistical difference in the proportion with *H. pylori* infection by anatomic location of ulcer (Table 3).

Diagnostic findings

Histological examination established the presence of *H. pylori* in 163 subjects (37.4%) including 7 (4.3%) by immuno-histo-chemistry and 156 (95.7%) by Cresyl Purple staining.

Histological analysis was positive for *H. pylori* in 99% (162/163) antrum and 96% (156/163) fundus biopsies. Culture positivity was 91% (149/164) in antrum and 89% (146/164) in fundus biopsies. Results of culture and histology were *H. pylori* positive for both methods in 89% (143 cases) antrum samples and 87% (137 cases) fundus samples. Overall, the sensitivity of histology compared to culture was 95.7% and its specificity was 97.8%. Six patients had histology positive and culture negative specimens among whom three cases

Table 2. — **Distribution of histologic lesions by *H. pylori* infection status**

Histology	No (%) of Patients (n = 436)	No (%) of Patients		p-value**
		Hp+ (n = 163)	*Hp*– (n = 273)	
Normal mucosa	8 (1.8)	1 (0.6)	7 (2.6)	
Reactive gastritis	113 (25.9)	0 (0.0)	113 (41.4)	
Mild chronic gastritis	167 (38.3)	40 (24.5)	127 (46.5)	
Moderate chronic gastritis	105 (24.1)	97 (59.5)	8 (2.9)	
Severe chronic gastritis	14 (3.2)	12 (7.4)	2 (0.7)	
Gastritis + metaplasic lesions	19 (4.4)	5 (3.1)	14 (5.1)	
Gastritis + duodenitis	10 (2.3)	8 (4.9)	2(0.7)	

** based on 2 by 7 Chi-Square test ($p < 0.001$).

Table 3. — ***H. pylori* infection status in patients with ulcerous lesions at endoscopy (n = 180) by age category and localization of ulcer**

	Endoscopic localisation of ulcer			P- value
	gastric n (%)	duodenal n (%)	gastroduodenal n (%)	
Age < 60 years				> 0.05
Infected	28 (38.9)	12 (52.2)	10 (52.6)	
Non infected	44 (61.1)	11 (47.8)	9 (47.4)	
Age ≥ 60 years				> 0.05
Infected	11 (27.5)	5 (29.4)	4 (44.4)	
Non infected	29 (72.5)	12 (70.6)	5 (55.6)	

showed cytologic examination typical of *Gastrospirillum hominis* (*H. helmannii*) (Table 4).

H. pylori susceptibility to antibiotics

Among the 164 infected patients, no primary or secondary resistance of *H. pylori* isolated from gastric biopsies was observed to amoxicillin and tetracycline, but 31% of the strains had a primary resistance to metronidazole and 3% to clarithromycin. The 70 patients included in the therapeutic follow-up showed a primary resistance of 33% to metronidazole and 3% to clarithromycin.

Stool antigen test

In comparison with UBT, the HpSA test presented a sensitivity of 100%, a specificity of 91%, a PPV of 69% and a NPV of 100% (Table 5). Five patients with a false positive HpSA test, including two which presented an "undetermined result", had a control assessment. Histological analysis of biopsies of these five patients were negative for *H. pylori* but no culture was carried out.

Effectiveness of the treatment

Among the 164 infected patients, 70 gave written consent to be included in the therapeutic study. Whereas 94 patients declined to take part for various reasons, including long distance from their place of residence to the hospital and preference for treatment by their general practitioner. At follow-up visit 4 to 8 weeks after the end of treatment, stool samples of the 70 treated patients were analysed and 67 (95.7%) of these patients underwent UBT test. Three patients did not undergo UBT test at the time of stool sampling (HpSA test positive for all three) and did not test later in spite of a written recall.

The rate of eradication failure in intention to treat analysis was 20% (14/70) including 11 cases of failure documented by a positive control test (^{14}C-UBT). The patients showing a documented failure claimed to have taken the treatment correctly. They presented a gastric ulcer (2 patients), a duodenal ulcer (3 patients) or a non-ulcerous dyspepsia (6 patients). Two of these patients had received a previous treatment for *H. pylori* more than 8 weeks before inclusion.

Of the 67 evaluable patients included in the study, 56 (83.6%) received a first intention treatment and presented a rate of eradication of 84% (47/56). Among 11 (16.4%) patients who had received a treatment against *H. pylori* more than 8 weeks before inclusion, the rate of eradication was 82% (9/11). No statistically significant difference between these two rates of eradication ($p = 0.86$). Treatment failure was not statistically associated with the type of pathology, age category, sex, or the timing of the follow-up control tests. None of the 11 patients with documented failure was infected by a strain of *H. pylori* resistant to clarithromycin. For nine of them, a second endoscopy allowed to isolate *H. pylori* and to perform a second antibiogram. Among these isolates, only one showed a secondary resistance to clarithromycin.

Discussion

The prevalence of *H. pylori* infection in this patient population in Brussels was 37.6%. This prevalence is

Table 4. — **Diagnosis of infection with *H. pylori* on upper digestive biopsy by culture and histology**

Histology	Culture		Total
	+	–	
+	157	6	163 (37.4%)
–	7	266	273 (62.6%)
Total	164 (37.6%)	272 (62.4%)	436 (100%)

Table 5. — **Results of ^{14}C-UBT and of HpSA tests for the control of *H. pylori* eradication in treatment follow-up visit (n = 67)**

HpSA test	^{14}C–UBT		Total
	Positive	Negative	
Positive	11	5	16 (23.9%)
Negative	0	51	51 (76.1%)

similar to prevalence of 30 to 40% observed in Western European countries (1,8-9).

Infection was more frequent in patients of non-Belgian origin.

We observed infection rates of 35% for patients with gastric ulcer, 43% for duodenal ulcer, and 50% for gastroduodenal ulcer (Table 1). These rates are comparable with those obtained in Nothern American studies (10) where *H. pylori* was identified in 32 to 73% of the cases, with an average of 69% for duodenal ulcer and a variation of 30 to 83% (average of 43%) for gastric ulcer. These rates are lower than those from a Belgian multicentric study (11) in 2000 which showed infection rates of 38%, 68%, and 58% for gastric, duodenal and gastroduodenal ulcer respectively. This lower rate could be explained by the time interval between studies owing to the progressive decrease in the prevalence of infection in industrialized countries or by a different age distribution in these series or by difference of geographic origin (lower proportion of patients of non-Belgian origin). This difference might be even larger because in the Belgian multicentric study (11), most centres were relying on histology alone to make the diagnosis of *H. pylori* infection. Indeed a stratified analysis by age in the present study showed rates of infection in patients < 60 years comparable with those in the previous multicentric study (11). The 28 to 44% rates of infection in the group of patients older than 60 years (Table 3) are lower than rates of 60 to 80% reported from Italy (12). The frequent consumption of drugs by elderly Belgian patients, especially antibiotics or non steroid anti-inflammatory drugs, could explain the lower infection rate observed in our study.

Concerning the diagnostic approach, our results confirm that the histology has a good sensitivity and a good specificity compared to the culture, in agreement with previous studies (11-12). Three cases (1.8%) of infection by *Gastrospirillum hominis* were identified by histology only. This gram negative spiraled bacillus, producer of urease, which is considered as a cause of moderate chronic gastritis (13-14) exhibits a distinct cellular morphology from *H. pylori* but is not easily cultivable.

In the present study, *H. pylori* showed a primary resistance of 31% to metronidazole whereas no resistance to amoxicillin nor to tetracycline was detected. A multicentric study done in Western Europe (15) in 1998 and a national survey (16) in France in 1999-2000, gave similar results. Other authors (17) found a lower primary resistance rate of 26% to metronidazole during the period 1995-2000 in Germany. We observed a primary resistance rate of 3% to clarithromycin, which is similar with that reported in Germany (17) (2.2%) and Italy (18) (5.7%), but markedly lower than those (10 to 15%) reported in France (19-20) and from other centres in Brussels and Belgium (15-20%) (21-23). The lower rate of resistance to clarithromycin observed in our study could be explained by less exposure to macrolides in this population. Although large differences in resistance rates observed between our study and others may reflect true differences in the study populations, this might also be due to difference in selection criteria of the patients, such as the high proportion of first intention treatment in our study.

It appears unlikely that this low frequency of resistance is due to lack of sensitivity of the disc diffusion method because resistance to clarithromycin is most usually expressed at high level (MIC > 1mg/L), and is easily detectable (24). The nearly absence of secondary resistance **(**only one patient among 9 failures) is more surprising. This rate is usually > 50% of the cases of failure of first intention treatment. This discrepancy suggests that our retrospective interview method over-estimated the degree of treatment compliance. In the future, regular bacteriological samplings during gastric endoscopies appears warranted to follow the local evolution of resistance of *H. pylori* to antibiotics.

The search for an alternative test for the control of the eradication of *H. pylori*, led us to evaluate stool antigen test in comparison with the breath test. Our findings confirm that this simple test is reliable, as previously reported (25-29). This test is therefore an efficient alternative for therapeutic follow-up of the infected patients, and its reimbursement by social security appears warranted. A few false positive results observed could have been due to the presence of degenerated forms of *H. pylori*, which are non-cultivable but still detectable by the HpSA test. According to the manufacturer, this test would detect such coccoïd bacterial forms which no longer produce a ureasic activity and are therefore also non detectable by the breath test.

We obtained a rate of eradication of 80% with a currently recommended triple therapy regimen, similar to those reported of 65 to 83% cure for combination of two antibiotics with omeprazole, 74 to 87% with lansopra-

zole and 80 to 87% with pantozole (30-31). This eradication rate is higher than rates of 73-74% (32) in recent reports by French authors in spite of good therapeutic compliance. No variable such as age, sex, treated pathology, time of control of eradication, had a significant influence on eradication in our study, in contrast with the observations made in a French study (16) which showed an excess of risk of failure depending on age and smoking.

In conclusion, the present study confirms the results reported previously on the prevalence of the infection with *H. pylori* in Western Europe. Histology is nearly as sensitive and specific as culture for diagnosis. The histologic analysis of biopsies remains however essential for the diagnosis of the lesions whereas culture is required for antibiotic susceptibility testing. Primary resistance to antibiotics was low in our patient population and this finding supports the use of amoxicillin- clarithromycin-IPP therapy as first line treatment, which achieved an acceptable rate of eradication of 80%. However, monitoring of resistance should be done regularly to validate this therapeutic scheme. The HpSA test (detection of the antigen in the stools) is an attractive alternative for the control of eradication and possibly for diagnosis, in case of counter- indication of an endoscopy.

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