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Efficacy of a tailored PCR-guided triple therapy in the treatment of *Helicobacter pylori* infection

Efficacité d'un traitement d'éradication guidé par PCR pour le traitement de l'infection par *Helicobacter pylori*

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Contributions of authors

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RÉSUMÉ

Introduction. Nous avons voulu évaluer l'efficacité d'un traitement d'éradication d'*Helicobacter pylori* guidé par le profil de résistance aux antibiotiques versus traitement empirique.

Patients et méthodes. Étude prospective multicentrique randomisée pour rechercher la présence d'*Helicobacter pylori* et le profil de résistance aux antibiotiques par PCR (HelicoDR®). Un traitement d'éradication empirique a été prescrit dans le groupe contrôle (inhibiteur de la pompe à proton [IPP], amoxicilline et clarithromycine pendant 7 jours) et un traitement guidé par la sensibilité aux antibiotiques dans le groupe expérimental (IPP, amoxicilline et clarithromycine ou lévofloxacine ou métronidazole). L'éradication a été évaluée par un test à l'urée ¹³C, au moins 28 jours après la fin du traitement.

Résultats. 526 patients inclus, dont 260 (49,4 %) randomisés dans le groupe de traitement empirique et 266 (50,6 %) dans le groupe expérimental. Le taux de résistance à la clarithromycine et à la lévofloxacine était respectivement de 23,3 % et 12,8 %. Le test respiratoire était disponible pour 415 (78,9 %) patients. Le traitement guidé par la sensibilité aux antibiotiques était supérieur au traitement empirique en termes d'éradication d'*Helicobacter pylori* (85,5 % vs 73,1 %, RR=1,85, IC95 % [1,25-2,78], $p=0,003$). Ces résultats étaient similaires après imputation multiple des données manquantes (RR=1,61, IC95 % [1,14-2,27], $p=0,003$) et en analyse per-protocole (RR=1,89, IC95 % [0,25-2,78], $p=0,003$).

Conclusion. Dans un pays présentant un taux élevé de résistance à la clarithromycine, un traitement guidé par la sensibilité aux antibiotiques permet d'obtenir un taux d'éradication d'*Helicobacter pylori* supérieur à un traitement empirique (ClinicalTrials.gov : NCT01168063).

ABSTRACT

Introduction. Resistance to clarithromycin and fluoroquinolones is increasing in many countries. We aimed to assess the efficacy of a tailored PCR-guided triple therapy versus an empirical triple therapy in the treatment of *H. pylori* infection.

Patients and methods. French multicenter prospective open-label randomized study to assess *H. pylori* and resistance to clarithromycin and levofloxacin with GenoType HelicoDR[®] test. Patients of the control group were treated with empirical therapy of proton pump inhibitor (PPI), amoxicillin, and clarithromycin for 7 days. Patients of the experimental group with clarithromycin-susceptible strains, clarithromycin-resistant/levofloxacin-susceptible strains, and with clarithromycin-resistant/levofloxacin-resistant strains received tailored therapy of PPI, amoxicillin, and clarithromycin for 7 days, PPI, amoxicillin, and levofloxacin for 10 days, and PPI, amoxicillin, and metronidazole for 14 days, respectively. *H. pylori* eradication was assessed by ¹³C urea breath test at least 28 days after the end of treatment.

Results. We included 526 patients: 260 (49.4%) were randomly assigned to empirical triple therapy and 266 (50.6%) to tailored therapy. Clarithromycin and levofloxacin resistances were 23.3% and 12.8%, respectively. Follow-up urea breath test was available for 415 (78.9%) patients. Tailored therapy was superior to empirical therapy in terms of eradication (85.5% vs 73.1%, RR=1.85, 95%CI [1.25-2.78], *p*=0.003). Findings were consistent in the susceptibility analysis using multiple imputation (RR=1.61, 95%CI [1.14-2.27], *p*=0.003) and per-protocol analysis (RR=1.89, 95%CI [0.25-2.78], *p*=0.003).

Conclusion. In a country with a high level of clarithromycin resistance, tailored PCR-guided therapy was superior to empirical triple therapy for *H. pylori* eradication (ClinicalTrials.gov: NCT01168063).

Introduction

Helicobacter pylori infection is one of the most common chronic bacterial infections in the world and has been defined as a major cause of gastritis, peptic ulcer disease, and gastric cancer [1,2]. Clarithromycin resistance is increasing, reaching 20% in many countries such as France [3,4]. Clarithromycin resistance is the main risk factor for treatment failure; resistance was reported to reduce the efficacy of the first-line therapy by up to 70% [5]. Fluoroquinolones, such as levofloxacin, are often used for rescue therapy in second- or third-line treatment. However, levofloxacin resistance has also increased in recent years and has also been shown to be correlated with treatment failure [3,6].

The mutations leading to resistance are now well known for clarithromycin and levofloxacin, although they are still unclear for metronidazole and extremely rare for amoxicillin [7]. In routine practice, the detection of clarithromycin and levofloxacin resistance can be based on phenotypic methods performed after culture, but these methods are time-consuming, fastidious and can take up to two weeks to be completed [8]. Several PCR-based assays for the detection of point mutations conferring resistance to clarithromycin or levofloxacin have been developed as alternatives to phenotypic methods, including real-time polymerase chain reaction (PCR), fluorescence *in situ* hybridization, and PCR line probe assays [9–12].

GenoType HelicoDR[®] (Hain Life Science, Nehren, Germany) is a molecular test that combines PCR and reverse hybridization. It allows for the molecular detection of *H. pylori* (specific region of the 23S rRNA gene) and characterization of resistance mechanisms for clarithromycin (mutations A2142G, A2142C, and A2143G) and for levofloxacin (hot spot mutations in the *gyrA* gene at codons 87 and 91) [13,14]. Performances of this test were evaluated in two studies [12,15]. Estimated susceptibility and specificity of clarithromycin resistance detection ranged from 0.94 to 1 and from 0.86 to 0.99, respectively. Estimated

susceptibility and specificity of levofloxacin resistance detection ranged from 0.82 to 0.87 and from 0.95 to 0.98, respectively.

Several studies demonstrated the medical and economic benefits of a phenotypic resistance-guided therapeutic strategy [16–18]. The difficulty in obtaining the phenotypic susceptibility of *H. pylori* and its limited availability make this strategy difficult to apply on a large scale. Molecular techniques that are easier to implement, faster, and more efficient than culture are an interesting alternative that would allow for implementing a therapeutic strategy guided by the results of these molecular tests. Lee *et al.* recently performed a non-randomized study and reported a higher rate of eradication in patients who received a tailored therapy on the basis of PCR detection of clarithromycin resistance as compared with empirical triple therapy with either rabeprazole-amoxicillin-clarithromycin or rabeprazole-amoxicillin-metronidazole [19]. Furthermore, a multicenter retrospective study demonstrated the efficacy of a genotypic resistance-guided sequential therapy in the third-line treatment of refractory *H. pylori* infection in Taiwan [20].

We conducted a prospective multicenter randomized clinical trial to assess the efficacy of a PCR-guided triple therapy in the treatment of *H. pylori* infection in comparison with clarithromycin-based standard triple therapy.

MATERIALS AND METHODS

Study design and participants

We performed an open-label randomized controlled trial in two parallel groups with blinded endpoint assessment, and we prospectively enrolled patients referred for gastroscopy at 13 centers in France (Paris, n=4; Greater Paris area, n=5; Lyon; Bordeaux; Poitiers; Saint-Etienne) from April 2010 to September 2012. Eligible patients (*H. pylori*-positive) were included consecutively in each center to minimize selection bias.

Eligible patients had to be older than 18 years. Patients were not eligible if they had received more than one eradication therapy; had an intolerance to amoxicillin, clarithromycin, metronidazole, or proton pump inhibitor (PPI); or had malignant tumors or severe organ dysfunction. During the gastroscopy all patients underwent two biopsies (one from the body and one from the antrum) that were pooled in the same tube to be sent to the bacteriology laboratory in addition to two biopsies from the body and two biopsies from the antrum for histology. Histology was evaluated according to the Sydney System [21]. The status of *H. pylori* infection was determined according to the results of the GenoType HelicoDR[®] PCR test. Only patients with *H. pylori* infection documented by a positive PCR on gastric biopsies sent to the bacteriology laboratory were included. Positive patients were either randomized in the control arm or in the experimental arm.

The trial protocol was approved by the institutional ethics committee responsible for the participating centers (Comité de Protection des Personnes Ile de France IX - Hôpital Henri Mondor, Créteil, France). Participants provided written informed consent.

Randomization and blinding

Eligible patients with *H. pylori* infection were randomly assigned, on a 1:1 ratio, to receive either a tailored triple therapy guided by the results of the PCR test detecting resistance to clarithromycin and levofloxacin (HelicoDR[®]) or an empirical triple therapy (PPI-amoxicillin-clarithromycin). Centralized randomization was performed using a computer-based randomization list generated by the study statistician at the data center. Randomization, which was performed using CleanWEB[®] (Telemedicine Technologies S.A.–2007), was balanced in blocks of various sizes, with stratification according to the center and previous eradication treatment (yes/no). Patients and investigators were not blinded to treatment assignment. Primary endpoint (urea breath test result) was assessed by physicians who were blinded to the treatment groups.

Study treatments

In the control arm (or empirical therapy group), naive patients were given twice daily a triple therapy of PPI (omeprazole 20 mg or esomeprazole 20 mg, or lansoprazole 30 mg, or pantoprazole 40 mg, or rabeprazole 20 mg), amoxicillin 1 g, and clarithromycin 500 mg for 7 days; patients having failed a first eradication attempt were given twice daily a triple therapy of PPI (omeprazole 20 mg, or esomeprazole 20 mg, or lansoprazole 30 mg, or pantoprazole 40 mg, or rabeprazole 20 mg), amoxicillin 1 g, and metronidazole 500 mg for 14 days.

In the experimental arm (or tailored therapy group), treatment was tailored according to the results of the antimicrobial susceptibility test with HelicoDR[®]. Patients infected with a clarithromycin-susceptible strain were given twice daily a triple therapy of PPI, amoxicillin 1 g, and clarithromycin 500 mg for 7 days. Patients infected with a clarithromycin-resistant strain with susceptibility to levofloxacin were given twice daily a triple therapy of PPI, amoxicillin 1 g, and levofloxacin 250 mg for 10 days. Patients infected with a double resistant strain were given twice daily a triple therapy of PPI, amoxicillin 1 g, and metronidazole 500 mg for 14 days.

Determination of *H. pylori* status and genotypic resistance with GenoType HelicoDR[®]

Before the start of the study, an initial meeting of all 11 laboratories involved in the trial was held to establish standardization for all steps of the PCR detection of *H. pylori* infection and mutations conferring resistance to clarithromycin and levofloxacin using HelicoDR[®]. A protocol for molecular procedures to test included patients as well as external quality controls was designed and followed by each laboratory. Except for the PCR apparatus, all equipment was identical. All reagents came from the same batches. A multicenter external quality control was established to assess the performance of each center involved in the study to extract, amplify, and detect *H. pylori* DNA and mutations conferring resistance to clarithromycin and levofloxacin using HelicoDR[®]. All of the proficiency panels were

prepared in the Poitiers University Hospital laboratory. Three proficiency panels comprised of six bacterial DNA extracts or molecular biology-quality water and, for the first panel, an additional set of four bacterial suspensions to extract, were sent in dry ice to the nine laboratories included in April 2010 and to the 11 laboratories included in November 2010 and in February 2011, representing a total of 222 samples. Controls were performed according to the protocol used for biopsy samples and blinded for all laboratories. Among the 222 controls sent to the laboratories, 216 were analyzable, resulting in a participation rate of 97.3%. The 216 analyzable controls led to 203 expected results yielding a 94% concordance rate. Consequently, PCR results obtained with HelicoDR[®] were validated in concordance with the good implementation of the multicenter external quality control. Molecular procedures are presented in Appendix 1.

Study endpoints

The primary endpoint was the eradication of infection 4 to 12 weeks after the end of the eradication therapy. Eradication was determined by the ¹³C-urea breath test. Patients and investigators were not blinded to treatment allocation, but urea breath tests were assessed by individuals who were blinded. Secondary endpoints included evaluation of resistance to clarithromycin and to fluoroquinolones based on molecular detection with the HelicoDR[®] test performed in the PCR-guided arm, and assessment of the eradication rate of patients infected with clarithromycin-susceptible strains treated with a triple therapy of PPI-amoxicillin-clarithromycin and levofloxacin-susceptible strains treated with a triple therapy of PPI-amoxicillin-levofloxacin. At the end of the eradication therapy, potential adverse reactions were investigated in a standardized way.

Factors affecting *H. pylori* eradication

The type of gastric lesion, age, gender, country of birth, smoking, drinking, body mass index, previous eradication attempt, and completion of the full treatment were examined as factors with a potential impact on *H. pylori* eradication.

Statistical analysis

The sample size calculation was based on the following assumptions: an eradication rate of 70%, a 20% increase in eradication rate in the PCR-guided group, a two-sided α of 0.05, and a power of 95%. Accordingly, 200 patients with a urea breath test follow-up were necessary in each group. Therefore, we planned to recruit 500 patients with bacteriologically documented *H. pylori* infection. Continuous data is presented as a mean (SD) or median (interquartile range) as appropriate. Categorical data is presented as counts and proportions. The statistical analysis followed the intention-to-treat principle, i.e. all patients were analyzed in the group to which they were randomly assigned, for the efficacy endpoint and on the treatment principle for the safety endpoints. We first used a multilevel model, a random-center effect logistic regression, to account for potential clustering [22]. Because neither a center-effect nor an effect modification by center was observed, we first considered clustering ignorable. Therefore, the prevalence of the primary endpoint was compared between the two study groups with the χ^2 test, and the relative risk (RR) and the absolute risk reduction were calculated together with their 95% confidence interval (CI). A susceptibility analysis was performed using a multiple imputation approach which is a standard tool for handling missing data. We used the multiple-multivariate-imputation-by-chained-equations procedure with the missing-at-random assumption. We used all of the predictors together to impute the missing data values, and we independently analyzed 20 copies of the data. A random-center effect multivariate logistic regression model was also fitted to compare the primary endpoint between both groups to take into account center and potential confounders. First, a univariate analysis was performed; variables with $p < 0.20$ were selected for multivariate modeling.

Crude and multivariate odds ratios (ORs) were estimated with their 95% CI. All analyses were performed with the use of R version 2.10.11 (The R Foundation for Statistical Computing; <http://www.R-project.org>) and Stata version 12.1 (StataCorp, College Station, TX, USA). A two-sided p value ≤ 0.05 was considered to indicate statistical significance. No interim analysis was performed. Reporting of the present study was done according to the CONSORT 2010 Statement [23]. This trial is registered with ClinicalTrials.gov, number **NCT01168063**.

Results

Study participants, *H. pylori* status, and genotypic resistance

The flow of participants in this trial is shown in Figure 1. We enrolled 1,384 patients, among whom 532 had a positive HelicoDR[®] test for detection of *H. pylori*; 526 were randomized. Among the 526 randomized patients, 260 (49.4%) were assigned to empirical triple therapy and 266 (50.6%) to tailored therapy. Overall, most of the patients were naive to any *H. pylori* treatment (95.4%); 65% were born outside of France and 16.7% had a peptic ulcer (Table 1). There was no difference between the two groups concerning any of the baseline characteristics. The proportion of patients who were treated *per protocol* did not differ between groups (78.1% versus 75.2%, $p=0.46$).

Attrition

One hundred and eleven patients withdrew from the study before completing the urea breath test after treatment completion, including 52 patients (20%) from the empirical therapy group and 59 (22.2%) from the test group. Characteristics of these 111 patients did not differ from those of the 415 complete cases (data not shown). Comparison of attrition by treatment assignment did not reveal a statistically significant difference ($p=0.38$).

Primary outcome

Using a multilevel model, neither a center-effect ($p=1$) nor an effect modification by center ($p=0.98$) was observed, therefore we first considered clustering ignorable. Analysis of the primary endpoint in the intention-to-treat population (complete cases) showed that 177 of 207 patients (85.5%) in the tailored therapy group were free of *H. pylori* infection, as compared with 152 of the 208 patients (73.1%) in the empirical therapy group (RR=1.85, 95% CI [1.25 to 2.78], $p=0.003$) (Table 2). Use of PCR-guided therapy resulted in an absolute risk reduction of 12.4% (95% CI [4.7 to 20.1]), indicating that 12 treatment failures could have been avoided for every 100 patients treated with PCR-guided therapy. In the total population after multiple imputations (missing at random hypothesis), the estimated RR was 1.61 (95% CI [1.14 to 2.27], $p=0.001$). The *per protocol* analysis showed that 173 of 200 patients (86.5%) in the tailored therapy group were free of *H. pylori* infection, as compared with 151 of the 203 patients (74.4%) in the empirical therapy group with a RR of 1.89 (95% CI [1.25 to 2.78], $p=0.003$).

The OR of PCR-guided therapy estimated by multilevel analysis accounting for potential clustering, was slightly higher than the crude RR (OR 2.17, 95% CI [1.33 to 3.56]) (Table 3). The type of gastric lesion, gender, country of birth, smoking, and drinking did not affect *H. pylori* eradication. Conversely, *H. pylori* eradication decreased significantly with older age, higher body mass index, and first *H. pylori* treatment failure, and increased significantly with the completion of the full treatment. Multivariate analysis adjusted for these variables did not change the association between tailored treatment and eradication rate (Table 3).

Secondary outcomes

Among the 266 patients assigned to PCR-guided triple therapy, the prevalence rate of clarithromycin resistance was 23.3% (95% CI [18.2 to 28.4]) and 12.8% (95% CI [8.7 to 16.8]) for fluoroquinolones.

The eradication rate in patients infected with clarithromycin-susceptible strains and treated with the clarithromycin-based triple therapy was 87% (140/161) (95% CI [81.8 to 92.2%]). It was 80% (32/40) (95% CI [67.6 to 92.4%]) in patients infected with clarithromycin-resistant and levofloxacin-susceptible strains treated with the levofloxacin-based triple therapy, and 83.3% (5/6) (95% CI [35.9 to 99.6%]) in patients infected with both clarithromycin- and levofloxacin-resistant strains treated with the triple therapy PPI-amoxicillin-metronidazole.

Adverse reactions

Mild adverse reactions occurred in 64 patients (26.8%) in the tailored therapy group and 55 patients (23.9%) in the empirical therapy group ($p=0.52$), 24.9% of the patients (102/410) having received clarithromycin-based standard triple therapy, 28.6% (12/42) having received levofloxacin-based triple therapy, and 29.4% (5/17) having received metronidazole-based triple therapy, with no statistically significant difference ($p=0.34$) between the groups (Table 4). General weakness was more frequently observed among patients in the empirical treatment group, and dyspepsia among patients treated with metronidazole ($p<0.05$). Diarrhea and bitter taste were the most common adverse effects.

Discussion

The main novel finding of this prospective open-label randomized controlled multicenter clinical trial was that genotypic resistance-guided triple therapy was able to reach an acceptable level of efficacy as the first-line treatment of *H. pylori* infection in a country with a high level of antibiotic resistance.

Various studies demonstrated that standard triple therapy is unable to reach the acceptable threshold of 80% efficacy in countries with a primary resistance level to clarithromycin >15% and the efficacy of second-line treatment with levofloxacin-based triple

therapy is also decreased in the case of resistance against this antibiotic [2]. The high levels of clarithromycin and levofloxacin resistance reported in France (21% and 17%, respectively) indicate that empirical triple therapy should no longer be used [3,4]. Therefore, the Maastricht IV Consensus report recommends empirical quadruple therapies (sequential, hybrid, non-bismuth concomitant and bismuth quadruple) as first-line treatments in regions or populations of high clarithromycin resistance in spite of obvious ecological consequences on the gastroenterological microflora and adverse effects.

Nonetheless, empirical first-line treatment with PPI-amoxicillin-clarithromycin for 7 days and empirical second-line treatment with PPI-amoxicillin-metronidazole for 14 days continued to be recommended in France until 2013 [24].

The eradication rate with triple therapy may be increased by selecting antibiotics after testing for resistance [25]. However, in routine practice there are many limitations to this strategy. *H. pylori* culture and antimicrobial susceptibility testing are expensive, time consuming, and may lead to false negative results due to a loss of strain viability during transport from the endoscopy unit to the laboratory.

Molecular test availability could reactivate a therapeutic strategy based on bacterial susceptibility testing. A previous study showed that GenoType HelicoDR[®] could simultaneously detect *H. pylori* and clarithromycin and fluoroquinolone resistance in gastric biopsies [12] This test is easier to perform than culture and is not affected by the loss of strain viability linked to the gastric biopsy transport.

The present study demonstrates the feasibility of applying HelicoDR[®] in clinical practice with a rapid diffusion of the technique in the 11 laboratories involved in the trial, including a majority of laboratories lacking expertise in *H. pylori* molecular detection, and satisfactory results for the external quality control.

The strengths of this first random prospective trial were: the inclusion of a large number of patients randomized into two groups (260 and 266) enrolled from a large number of centers (13) distributed over the entire French territory; and the assessment of a pre-defined strategy in selecting antibiotics according to the determination of genotypic resistance for two of them (clarithromycin and levofloxacin).

The main limitation of the study was the high number of patients lost to follow up (111, 20%). This was due to our desire to be in a routine clinical practice situation. Consequently, gastroenterologists from the 13 centers had given patients included in the study a prescription to have the urea breath test performed in the laboratory of their choice to follow the usual practice and had not centralized analyses to avoid any bias. However, a comparison of attrition by treatment assignment did not reveal a statistically significant difference ($p=0.38$).

Results in the empirical arm confirmed the negative impact of high-level clarithromycin resistance. It should be pointed out that the resistance rates obtained with HelicoDR[®], 23.3% and 12.8% for clarithromycin and levofloxacin respectively, were in accordance with the expected resistance rates observed in France using culture and antimicrobial susceptibility testing during the same period [3,4].

Our results are in agreement with a recent study carried out in Asia. Lee *et al.*, in a retrospective study in Korea, reported a better eradication rate in patients treated with PPI-amoxicillin-clarithromycin or PPI-amoxicillin-metronidazole following a PCR test to detect clarithromycin resistance (91.2%) than in those with an empirical treatment with either PPI-amoxicillin-clarithromycin (75.9%) or PPI-amoxicillin-metronidazole (75.9%) [19]. The absolute reduction risk of failure in Lee's study was similar to ours (15.3% vs 12.4%, respectively) in spite of lower eradication in the tailored treatment group in our study as compared with the study by Lee *et al.*

It should be noted that in our study and even in susceptible strains, eradication rates with clarithromycin-based triple therapy and levofloxacin-based triple therapy were below 90% (respectively 87% and 81%). These results suggest that the regimens administered were not optimal. As proposed by several authors, clarithromycin-based triple therapy could probably be improved by 1) increasing treatment duration to 10 or 14 days, 2) increasing the PPI dose, or 3) administering amoxicillin three or four times daily [26,27].

Quadruple therapies (sequential, hybrid, non-bismuth concomitant and bismuth quadruple) are becoming commonly used in clinical practice. Sequential therapy is the most popular regimen worldwide and is recommended as a first-line option in France despite the absence of local studies. Recent studies demonstrated that the efficacy of sequential therapy seems to be decreasing over time and that the impact of clarithromycin resistance may have been underestimated [28,29]. Liou *et al.* proposed a genotypic resistance-guided sequential therapy to improve management of the third-line treatment of refractory *H. pylori* infection [20]. Finally, bismuth quadruple therapy and non-bismuth concomitant quadruple therapy seem to be the most efficient empirical options [28,30]. However, they raise questions about tolerance and impact on bacterial ecology.

Conclusion

Tailored treatment according to systematic antimicrobial susceptibility testing is the best means to limit the emergence of antibiotic resistance worldwide. Our study, including 13 centers, demonstrates the feasibility of using molecular tests in routine practice and shows that optimal eradication rates could be provided by PCR-guided triple therapy.

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Figure 1. Flow diagram of a trial treatment of *Helicobacter pylori* infection comparing PCR-guided vs. empirical triple therapy.

Figure 1. Organigramme de l'essai clinique HELICOSTIC comparant le taux d'éradication d'*Helicobacter pylori* avec un traitement empirique ou guidé par PCR.

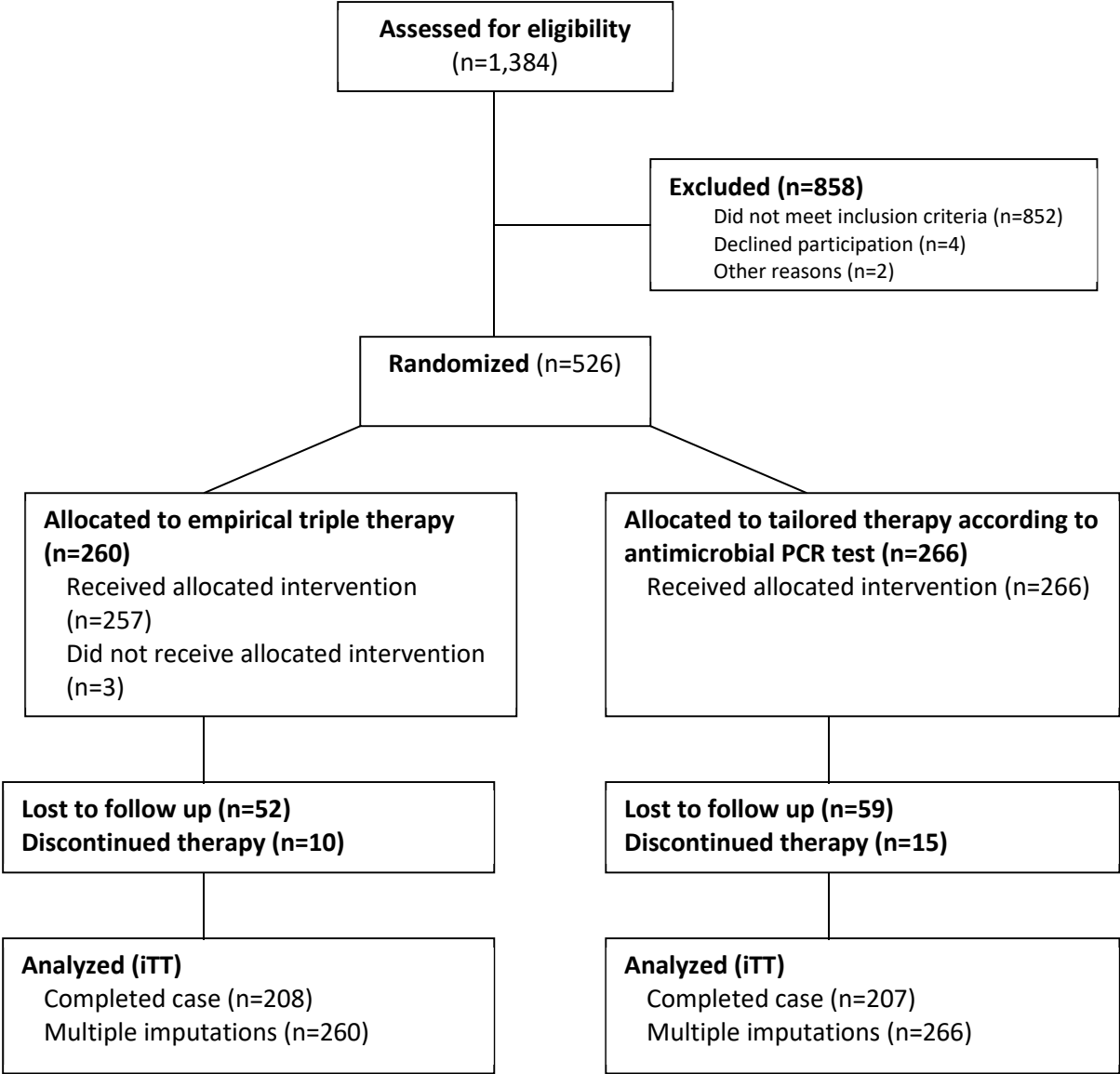


Table 1. Patient's baseline characteristics**Tableau 1.** Caractéristiques des patients à l'inclusion

	Overall population (N=526)	Empirical therapy* (N=260)	Tailored therapy (N=266)
Age, mean (\pm 1 SD), years	46.49 (14)	47.2 (15)	45.79 (13)
Male sex	274 (52.1)	133 (51.2)	141 (53.0)
Birth location			
France	184 (35)	98 (37.7)	86 (32.3)
Other European countries	37 (7)	12 (4.6)	25 (9.4)
North Africa	155 (29.5)	77 (29.6)	78 (29.3)
Sub-Saharan Africa	89 (16.9)	47 (18.1)	42 (15.8)
Central and South America	34 (6.5)	15 (5.8)	19 (7.1)
Near, Middle, and Far East	28 (5.2)	11 (4.2)	16 (6.2)
Body mass index, mean (\pm 1 SD)	27 (7.5)	26.6 (8)	27.39 (7)
Daily alcohol intake	60 (11.4)	30 (11.5)	30 (11.3)
Current smoker	138 (26.2)	65 (25)	73 (27.4)
Naive to any <i>H. pylori</i> eradication	502 (95.4)	249 (95.8)	253 (95.1)
Gastroscopy result			
Normal	195 (37)	94 (36.2)	101 (37.8)
Peptic ulcer	88 (16.7)	37 (14.2)	51 (19.1)
Gastric superficial lesion	191 (36.3)	106 (40.8)	85 (32)
Esophageal lesion	49 (9.3)	22 (8.5)	27 (10.1)
Non-malignant tumor	3 (0.6)	1 (0.4)	2 (0.8)

<i>H. pylori</i> on histology	464 (88.7)	229 (88.1)	235 (89.4)
Resistant strain to clarithromycin			62 (23.3)
Resistant strain to quinolones			34 (12.8)

SD, standard deviation

*Empirical therapy consisted of PPI-amoxicillin-clarithromycin for 7 days for naive patients and PPI-amoxicillin-metronidazole for 14 days for patients with failure after the first eradication attempt. Other European countries include Bosnia, Italy, Kosovo, Moldavia, Portugal, Spain, and Russia.

Data is expressed as N (%), except otherwise stated

Table 2. Rates of *Helicobacter pylori* eradication in the intention-to-treat and per-protocol populations

Tableau 2. Taux d'éradication d'*Helicobacter pylori* en intention de traiter et en analyse per-protocole

	Empirical therapy		p value	RR [95% CI]	ARR [95% CI]
		Tailored therapy			
Intention-to-treat analysis					
Complete case	N=208	N=207			
Eradication, N (%)	152 (73.1)	177 (85.5)	0.003	1.85 [1.25-2.78]	12.4 [4.7 to 20.1]
Multiple imputation	N=260	N=266	0.001	1.61 [1.14-2.27]	9.7 [2.8 to 16.6]
Per protocol analysis					
	N=203	N=200			
Eradication, N (%)	151 (74.4)	173 (86.5)	0.003	1.89 [1.25-2.78]	12.6 [4.7 to 12.6]

RR, relative risk; CI, confidence interval; ARR, absolute reduction risk

Table 3. Factors affecting *Helicobacter pylori* eradication, univariate and multivariate analyses using multilevel random-center effect logistic regression models

Tableau 3. Facteurs affectant le taux d'éradication d'*Helicobacter pylori* en analyse univariée et multivariée (régression logistique par modèles à effets aléatoires)

	Eradication		Univariate analysis		Multivariate analysis*	
	Yes (N=329)	No (N=86)	OR [95% CI]	p value	OR [95% CI]	p value
Tailored therapy	177 (53.8)	30 (34.9)	2.17 [1.33-3.56]	0.002	2.29 [1.36-3.84]	0.001
Age, median (Q1 – Q3) †	46 [36- 56]	48.5 [40-59]	0.98 [0.97-1.00]	0.043	0.98 [0.96-0.997]	0.02
Male gender	175 (53.2)	41 (47.7)	0.81 [0.50-1.29]	0.36	-	
Type of gastric lesions			-	0.61	-	
None	129 (39.2)	28 (32.6)				
Peptic ulcer	51 (15.5)	13 (15.1)				
Gastric superficial lesion	116 (35.3)	38 (44.2)				
Esophageal lesion	30 (9.1)	7 (8.1)				
Non-malignant tumor	3 (0.9)	0 (-)				
Birth location			-	0.99	-	

France	112 (34)	29 (33.7)				
Other European countries ‡	25 (7.6)	7 (8.1)				
Africa	155 (47.1)	41 (47.7)				
Other †	37 (11.3)	9 (10.5)				
Body mass index, median (Q1 – Q3) §	25.1 [22.8-28.3]	27.3 [23.7-30.1]	0.96 [0.94-0.99]	0.01	0.96 [0.93-0.99]	0.01
Daily alcohol intake	40 (12.2)	8 (9.3)	1.35 [0.61-2.01]	0.46	-	
Current smoker	88 (26.8)	18 (20.9)	1.38 [0.77-2.31]	0.28	-	
First <i>H. pylori</i> treatment failure	12 (3.5)	7 (8.1)	0.43 [0.16-1.12]	0.08	0.15 [0.12-0.89]	0.03
Full treatment	326 (99.1)	80 (93.0)	8.15 [2.0-33.29]	0.003	10.3 [2.46-43.21]	0.001

OR, odds ratio; CI: confidence interval; Q1-Q3: first to third quartiles

Data is expressed as N (%), except otherwise stated.

*Multivariate analysis was adjusted for the five variables listed in the table

†Odds ratios (95% CI) per one-year increase

‡Other European countries included Bosnia, Italy, Kosovo, Moldavia, Portugal, Spain, and Russia

‡Other included countries of Central and South America and of Near, Middle, and Far East

§Odds ratios (95% CI) per one-point increase

Table 4. Prevalence rate of adverse effects according to the treatment arm and to the therapy received (469 patients with adverse effect status available)

Tableau 4. Prévalence des événements indésirables dans le bras contrôle et le bras expérimental

	Treatment arm		<i>p</i> value	Eradication with PPI-amoxicillin plus			<i>p</i> value
	Empirical	Tailored		clarithromycin	levofloxacin	metronidazole	
	(N=239)	(N=230)		(N=410)	(N=42)	(N=17)	
Any adverse effect reported	64 (26.8)	55 (23.9)	0.52	102 (24.9)	12 (28.6)	5 (29.4)	0.74
Adverse effects leading to discontinuation	7 (2.9)	8 (3.5)	0.80	14 (3.4)	1 (2.4)	0 (-)	1
Type							
Bitter taste	22 (9.1)	23 (10)	0.87	42 (10.2)	2 (4.8)	1 (5.9)	0.64
Diarrhea	30 (12.6)	27 (11.7)	0.89	47 (11.5)	7 (16.7)	3 (17.7)	0.43
Nausea/vomiting	8 (3.4)	2 (0.9)	0.11	10 (24)	0 (-)	0 (-)	0.73
Dyspepsia	4 (1.7)	2 (0.9)	0.69	4 (1)	0 (-)	2 (11.8)	0.03
Abdominal pain	26 (10.9)	15 (6.5)	0.10	36 (8.8)	3 (7.1)	2 (11.8)	0.85
General weakness	12 (5)	3 (1.3)	0.04	15 (3.7)	0 (-)	0 (-)	0.65
Dizziness/vertigo	1 (0.4)	2 (0.9)	0.62	2 (0.5)	0	1 (5.9)	0.13

Tendon pain	8 (3.4)	4 (1.7)	0.38	10 (2.4)	2 (4.8)	0 (-)	0.56
Pruritus/cutaneous rash	5 (2.1)	4 (1.7)	1	6 (1.5)	2 (4.8)	1 (5.9)	0.11
Oral mycosis	2 (0.8)	3 (1.3)	0.68	3 (0.7)	2 (4.8)	0 (-)	0.12
Lower limb edema	0	1 (0.5)	0.21	0 (-)	1 (2.4)	0 (-)	0.13

Data is expressed as N (%), except otherwise stated.

p value of the Chi² test or Fisher's exact test as appropriate.

Appendix 1. Molecular procedure

Annexe 1. Procédure de réalisation de l'HELICO-DR®

Annex 1.

For patients assessed for eligibility in the study, one antrum and one body biopsy specimen obtained during endoscopy were pooled together for PCR detection, either sent within two hours in a sterile tube containing 1 mL of saline or, in case of longer time, conserved in a dry tube immediately immersed in liquid nitrogen. Biopsies were ground in 200 µL of 10% glycerol brain heart infusion broth with a micro-pestle (Labo-Moderne, Paris, France).

Tissue lysis of biopsy specimens was performed on 100 µL of the ground material in 400 µL of ATL buffer (Qiagen, Courtaboeuf, France) containing 1 mg/mL proteinase K (Sigma Aldrich, Saint Quentin Fallavier, France) for one to three hours at 56°C. DNA extraction was performed in each center on an automatic Arrow extraction instrument (Norddiag, Oslo, Norway) according to the manufacturer's instructions. The isolated DNA was eluted in a volume of 200 µL and stored at 4°C until use.

Amplification of the bacterial DNA was done using Taq DNA polymerase (Bioron, Ludwigshafen, Germany). Biotinylated primers were provided in the amplification kit. PCRs were performed in a final volume of 50 µL containing 35 µL of primer/nucleotide mix (PNM), 5 µL of 10x polymerase incubation buffer, 4.8 µL of nuclease free water, 0.2 µL of Taq polymerase (2 units), and 5 µL of DNA template. Amplification cycles consisted of an initial denaturation at 95°C for 5 minutes, followed by 10 cycles at 95°C for 30 seconds and 58°C for 2 minutes. Twenty-five cycles were then run for a first step at 95°C for 25 seconds, a second step at 53°C for 40 seconds, and a third step at 70°C for 40 seconds. The PCR ended with 8 minutes at 70°C. Hybridization was performed using a TwinCubator (Hain Lifescience, Nehren, Germany) at 45°C. The denaturation solution was mixed

with 20 µl of the amplified sample and submitted to the recommended protocol for hybridization (Hain Lifescience).

A positive and a negative control were included in each run. The positive control consisted of 150 ng of the bacterial DNA extracted from a reference *H. pylori* strain resistant to both clarithromycin (A2143G mutation in the 23S rRNA gene) and levofloxacin (C261A mutation at 87 AA position in the *gyrA* gene) [14]. The negative control consisted of nuclease free water.

To assess positive and negative strips, the DNA strips were stuck on an evaluation sheet after the hybridization, and a template was aligned side by side with the conjugate control band of the respective strip. Control strips that should appear positive to validate the test were strips corresponding to the conjugate control and the amplification control, an identification strip for *H. pylori* (the PCR fragment was from the *rrl* gene, which codes for the 23S rRNA, as stated above), and amplification controls of the *rrl* and *gyrA* genes.