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ORIGINAL ARTICLE

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Personalized therapy for *Helicobacter pylori*: *CYP2C19* genotype effect on first-line triple therapy

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Abstract

Background: Triple therapy efficacy against *Helicobacter pylori* is low worldwide, and thus, alternatives must be sought to improve eradication. The aim of the present study was to determine *CYP2C19* genetic polymorphism effect on *H pylori* eradication.

Methods: A randomized, single-blinded clinical trial including 133 participants was carried out. *H pylori* infection was confirmed by histologic and microbiologic test. Antibiotic susceptibility to amoxicillin and clarithromycin was performed. *CYP2C19* polymorphisms *1, *2, and *3 were analyzed by real-time PCR (Roche ®), and nested PCR for *CYP2C19**17 polymorphisms. Participants were randomized into two groups for different *H pylori* therapies, one with standard omeprazole doses and another with omeprazole doses depending on *CYP2C19* polymorphism. *H pylori* eradication was verified by stool antigen tests (Meridian ®).

Results: The most common *CYP2C19* polymorphism was *1/*1 in 54.9% of the participants followed by *17/*17 in 21.1%. Triple therapy efficacy with standard omeprazole doses versus personalized therapy based on *CYP2C19* polymorphism by ITT analysis was 84% (95% CI: 0.73-0.91) vs 92.2% (95% CI: 0.82-0.97) (P = 0. 14), respectively. The efficacy by PP analysis was 92.1% (95% CI: 0.82-0.97) vs 100% (95% CI: 0.92-0.01) (P = 0.027), respectively.

Conclusions: The most frequent polymorphism was extensive PPI metabolizers (62.4%). Effectiveness of guided therapies by susceptibility test was good, yet they can be further improved by customized therapy based on *CYP* genotype. Therefore, high PPI (80 mg/d) doses are recommended for *H pylori* eradication therapies in Colombia. ClinicalTrials.gov ID: NCT03650543.

KEYWORDS
CYP2C19, *H pylori*, personalized therapy

1 | INTRODUCTION

Helicobacter pylori (H pylori) is a causal agent of chronic gastritis, duodenal ulcer, gastric MALT lymphoma, and gastric cancer. In addition, it is involved in extra-gastric diseases, such as iron deficiency, anemia, and idiopathic thrombocytopenic purpura.[1,2] *H pylori* has been declared and ratified as carcinogen I by the International Agency for Research on Cancer (IARC), which is part of the World Health Organization (WHO).[3] Globally, first-line therapy eradication treatment recommended for *H pylori* combines a

proton-pump inhibitor (PPI) with two antibiotics (amoxicillin and clarithromycin or metronidazole) and is still the most frequently recommended especially in areas with low clarithromycin resistance.[1,4] Nowadays, effectiveness of this therapy is, however, <75%, mainly due to *H pylori* antibiotic resistance.[5-8] Other factors involved in therapeutic failure are treatment compliance and host genetic factors that may affect PPI pharmacokinetics.[9-11] Proton-pump inhibitor plays an essential role in eradication therapies, and it suppresses acid secretion, thus increasing pH above 6.0.[12,13] This allows *H pylori* to replicate more actively than when stomach pH is less than 6.0, and therefore, it improves antibiotic activity promoting greater antimicrobial agent stability and antibiotic concentration in the stomach.[14-17]

The action of proton-pump inhibitors depends on cytochrome P450 (CYP) enzyme metabolism. This enzyme system is comprised of many isoenzymes, the most relevant are CYP3A4 and CYP2C19.[18,19] Recent studies have shown that *CYP2C19* genotype can affect PPIs ability to suppress acid secretion in the stomach, as has been observed for omeprazole.[18,20] Omeprazole is metabolized by CYP2C19 (90%) and by CYP3A4 (10%).[19,21] *CYP2C19* gene has 21 polymorphisms, three of which play an important role in PPIs metabolism. Thus, depending on polymorphism they are designated as extensive metabolizers (EM) (*1/*1), intermediate metabolizers (IM) (*1/*X), and poor metabolizers (PM) (*X/X*), where *1 allele is the wild-type allele ("wild type") and *X corresponds to the mutated allele. Alleles from *2 to *8 and allele *16 have decreased pro-drug metabolizing activity, where alleles *2 and *3 are the most representative of this group.[18,19] Subjects with genotype *CYP2C19* *1/*2 or *1/*3 are IM, and those with genotype *CYP2C19**2/*2 or *CYP2C19**3/*3 are PM.[19,21] The presence of *CYP2C19**2 and *CYP2C19**3 polymorphisms predicts individual metabolizer phenotype. In 2006, *CYP2C19*17* allele was identified, whose function is clinically important because the presence of individuals with two copies of *17 is classified as ultra-rapid metabolizers (UM), with an ability to metabolize PPIs faster than extensive metabolizers.[21-23] While heterozygote individuals with one normal activity allele and one variant allele associated with increased activity (*17), *CYP2C19**1/*17 are considered extensive metabolizers (EM) although this continues to be under study.[21,23] Clinical implication of *CYP2C19**17/*17 polymorphism relies on the following: If PPIs are quickly metabolized, standard PPIs dose fails to adequately suppress acid secretion, and *H pylori* eradication therapy will be less effective if the microorganism is sensitive to antibiotics.[19,21,24,25] A recent work in Colombia to eradicate *H pylori*, using triple therapy with clarithromycin or levofloxacin combined with amoxicillin, reported that although the organism was sensitive to these antibiotics, there was variability with achieved eradication.[26] These results may suggest the possibility that additional factors, different from antimicrobial resistance, can influence treatment effectiveness in our population. In addition, previous reports by Isaza et al found that 83.6% in a population from Pereira, Colombia, corresponded to EM *CYP2C19* genotype.[27] Therefore, the aim of this study was to determine *CYP2C19* genetic polymorphism influence on *H pylori* eradication.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

A randomized, single–blinded, clinical trial was conducted from 2012 to 2015 in Bogotá, Colombia. The study was performed in accordance with Good Clinical Practice guidelines and ethical principles of the Declaration of Helsinki.[28,29] Ethics Committee of the participating institutions approved the study protocol, where all subjects signed written informed consent. In addition, the study protocol was registered at Clinical Trials.gov PRS with ID NCT03650543 and protocol number ID 00004554.

After obtaining informed consent, 355 participants were invited to participate in the study. All enrolled participants underwent initial endoscopy, carried out at the upper endoscopy service, Clínica Fundadores in Bogotá, DC, Colombia, to obtain gastric biopsy for histologic, microbiologic, and genotypic test. However, out of the 355 participants only 133 were included in the study.

Monitoring and inspection of the study was done by a biomedical engineer who was present during all the development of study; biomedical engineer accompanied the participants from the beginning of this study, when they were invited to participate, when treatment was assigned and when delivery was performed, and at the end of the therapy. Monitoring was carried out in person during each checkup and by follow-up through telephone calls to supervise treatment adverse events. Additionally, the biomedical engineer certified the quality of all equipment used during this study.

2.2 | Inclusion and Exclusion criteria

Subjects with the following characteristics were included: individuals with functional dyspepsia or peptic ulcers between the ages of 19 and 70 years old, who were referred for upper endoscopy, and they had not received previous *H pylori* eradication treatment within the last six months. Additionally, they had not received antisecreting acid, bismuth, or antibiotics for other diseases 15 days before the endoscopy. For the study, only participants with sensitive isolates of *H pylori* to amoxicillin and clarithromycin were included. The study excluded participants with serious comorbidities, pregnant, or participants allergic to medications used.

2.3 | Endoscopic study

All participants in the study underwent endoscopy of upper digestive tract under aseptic conditions, with a minimum of six hours of fasting.[30] Depending on the participant's tolerance, the procedure was performed with or without sedation. If sedation was required, an anesthesiologist administered it. A gastroenterologist performed the endoscopy with a video endoscope Exera Olympus CV 145. During the endoscopy, five biopsies were collected from the antrum and four biopsies from the body of the stomach for histopathology, microbiology, and molecular analysis. Histopathologic analysis was done according to protocol proposed by Rugge et al[31,32] For these

objectives, five biopsies were collected and were placed in labeled individual vials and sent to pathology for analysis.

For microbiologic and molecular analyses, three antral and three body of the stomach biopsies were collected and used as follows: for rapid urease test: one biopsy from antrum, for *H pylori* culture and susceptibility test two biopsies (one from antrum and one from the body of the stomach). Culture was carried out only when one or both additional tests were positive for *H pylori*. If culture was negative, but any other test positive, microorganism detection was confirmed by molecular analysis (*ureA* gene detection *H pylori*). A participant was considered infected with *H pylori* when two or more tests were positive. Another biopsy was used for DNA extraction and subsequent *CYP2C19* genetic polymorphism analysis. Remaining biopsy samples were stored for future analyses in 20% glycerol (v/v) molecular grade (Invitrogen) at 70°C Brucella® broth (BD). All biopsies were transported in 500 µL Brucella broth with 20% (v/v) glycerol and kept refrigerated until processing.[30]

2.4 | Culture and Susceptibility

Culture was performed from biopsies of participants whose rapid urease test and histology were positive, from a biopsy of antrum and body of the stomach on Brucella agar (BD) enriched with 7% v/v horse blood, Isovitalex (BD), and antibiotic DENT (Oxoid). Following, samples were incubated at 37°C with 11% CO_2 for 3 to 5 days. Identification tests (Gram stain, catalase, oxidase, and urease) were performed on recovered bacteria from culture. Finally, antibiotic susceptibility test from pure colonies was carried out by agar dilution (gold standard method) according to the Clinical and Laboratory Standards Institute (CLSI) to determine amoxicillin and clarithromycin minimum inhibitory concentration (MIC).[33] Participants with resistance to amoxicillin or clarithromycin were excluded to avoid confounding factors in result analysis.

2.5 | *CYP2C19* Genotyping

CYP2C19 polymorphisms *1, *2, *3, and *17 were identified for all 133 participants included in this study. For this purpose, DNA was obtained from gastric biopsies by QUIAmp® kit (QIAGEN), and polymorphisms were then identified.

CYP2C19 polymorphisms *1, *2, and *3 were determined by real-time polymerase chain reaction (RT-PCR) using LightMix® kit for human *CYP2C19**2 and *CYP2C19**3 (Roche), according to manufacturer's instructions. A LightCycler 1.5 was used, first performing color compensation in reading channels to guarantee good results. Two specific primers synthesizing 133 bp and 164 bp fragments for *CYP2C19* gene were used, corresponding to polymorphisms *2 and *3, respectively. In addition, specific probes labeled with two different fluorochromes at two different wavelengths for each polymorphism were used, to identify polymorphisms by reading in channel 530 for allele 2 and channel 640 for allele 3. Additionally, quality control for each allele (wild type and mutant allele 2 and 3) was included for every test. Results were analyzed according to manufacturer's suggestions. Allelic classification was performed by differences in melting temperatures (Tm) (curves obtained) on Channel 530 for allele 2 and Channel 640 for allele 3.

*CYP2C19**17 polymorphism was performed by nested PCR and RFLP, which was previously standardized in our laboratory at the Pontificia Universidad Javeriana—Bogotá DC Primers and PCR conditions were based on Baldwin et al report.[34] First, PCR mixture used 2C19-1 Forward and Reverse 2C19-1 primer pairs, amplifying a 473 bp fragment, corresponding to *CYP2C19* allele 1. Second, PCR mixture was performed by taking 0.5 µL from the first PCR product and using another set of primers: Forward 2C19-2 and 2C19-2 Reverse. Following, 15 µL of the second PCR product was incubated with 0.8 µL of NsiI restriction enzyme at 37°C for 8 hours. Subsequently, PCRs' digestion product was run on 2% (w/v) agarose gel and stained with Sybr Safe (Invitrogen®) to verify the presence of 116 bp and 143 bp bands, corresponding to *CYP2C19**1 and *CYP2C19**17 alleles, respectively.[34]

2.6 | Randomization of participants, treatment assignment, adverse effects, and verification of *H pylori* eradication

After performing microbiologic test, antibiotic susceptibility, and genotyping, subjects were allocated to a treatment regimen according to a randomized crossover sequence, provided by a computer-generated randomization list. Participants were contacting by telephone for appointment assignment according to the designated group for treatment delivery.

Participants were appointed a treatment (two treatments were established) based on computer-generated randomization. Group I or conventional group received triple standard therapy with standard doses of omeprazole, which consisted of 20 mg omeprazole before breakfast and 20 mg before dinner. Additionally, participants in this group were also prescribed 500 mg clarithromycin after breakfast and after dinner and were also prescribed 1 g amoxicillin after breakfast and after dinner for 10 days. Group II or personalized group received triple standard therapy; however, omeprazole doses were prescribed according to *CYP2C19* genotype as a follows: (a) Participants with *CYP2C19* *1/*1 genotype (EM) were prescribed 40 mg omeprazole before breakfast and before dinner, and 500 mg clarithromycin after breakfast and after dinner, and were also prescribed 1 g amoxicillin after breakfast and 1 g after dinner for 10 days. Ultra-rapid metabolizers were treated as EM. (b) Participants with *CYP2C19* *1/*2 or *1/*3 genotype (IM) were prescribed 20 mg omeprazole before breakfast, 20 mg before lunch, and 20 mg before dinner. Additionally, participants were prescribed 500 mg clarithromycin after breakfast and after dinner and were also prescribed 1g amoxicillin after breakfast and 1 g after dinner for 10 days. (c) Participants with *CYP2C19* *2/*2 (PM) were prescribed 20 mg omeprazole before breakfast and 20 mg before dinner; and 500 mg clarithromycin after breakfast and after dinner; and 1g amoxicillin after breakfast and 1 g after dinner for 10 days.

All participants were given detailed information regarding adverse effects that could occur with different medications. Verification of possible adverse effects was performed by telephone using a validated and precoded questionnaire that included the following symptoms: diarrhea, metallic taste, nausea, bloated feeling, loss of appetite, vomiting, abdominal pain, constipation, and rash. Intensity of each symptom was graded from zero to three: 0: absent, 3: severe, using a Likert scale.

Last, the verification of *H pylori* eradication was performed by (Meridian®) stool antigen test eight weeks post-treatment.[1] For this test, each participant was requested a stool sample. The test was carried out according to manufacturer's indications.

2.7 | Statistical analysis and outcome evaluation

Sample size was calculated using Sample Size 1.0 software, with modified normal approximation by continuity correction. A correction method based on the following criteria was used: 5% Type I error and 20% Type II error, 75% control group proportion, and 95% experimental group proportion. A rate allocation of 1 between groups was assigned. According to this, sample size corresponded to 60 participants per treatment group. Subject assignment to treatments was performed by completely randomized blocks.

The primary and secondary outcomes were the *H pylori* eradication rate in which the effectiveness of each therapy analysis was carried out by per-protocol (PP) and intention-to-treat (ITT) analyses. Additional prespecified outcome such as characteristics of the population, state of *H pylori* infection, distribution of antibiotic resistance, and genetic *CYP2C*19* polymorphisms frequencies was performed using descriptive statistics, employing SPSS v.24 Statistics program.

3 | RESULTS

3.1 | Population characteristics

From 2012 to 2015, 355 participants were recruited, of which 68.2% (242/355) were positive for *H pylori* infection. Nevertheless, 109 *H pylori*-positive participants were excluded, due to *H pylori* antibiotic resistance in 46 participants. From the remaining population, 35 participants decided to receive treatment with a personal physician, two subjects became pregnant, and 26 changed contact information, thus could not be followed up. In addition, 113 participants were negative for *H pylori* infection; therefore, a total of 222 participants were excluded from the study and only 133 participants met with established inclusion criteria. Out of the 133 participants studied, 69.2% (92/133) were women, and 30.8% (41/133) were men. Participants had an average age (years) of 45.8 ± 12.3 (women 46 ± 11 and men 44 ± 14), weighed 67.1 ± 14.9 (women 64 ± 16 kg and men 73 ± 11). Their height in cm was 161.1 ± 12.4 (women 156 ± 12 and men 172 ± 5), with an average body mass index (Kg/m^2) of 25.3 ± 4.5 (women 25.6 ± 5 and men 24.7 ± 3.5). None of the participants smoked or drank alcoholic beverages. Colombian geographic distribution for participants was as follows: 123 (92.5%) from the Andean region, 3 (2.3%) from the Pacific region, 2 (1.5%) from the West and the Caribbean region. Participants consulted for dyspepsia (44%, 59/133), reflux (30%, 40/133), weight loss (3.7% 5/133), anemia (2.3% 3/133), and other symptoms (19.5%, 26/133). For all 133 participants after endoscopic examination, the main finding was chronic gastritis 99.2% (132/133). Never the less, they presented additional conditions such as esophagitis 70.7% (94/133), hiatal hernia 21.8% (29/133), intestinal metaplasia 9% (12/133), duodenal ulcer 3.7% (5/133), and gastric ulcer 3% (4/133). For each participant, gastric atrophy was evaluated using OLGA staging system, where histopathologic findings evidenced 84.2% (122/133) did not present gastric atrophy (OLGA score = 0), 12.8% (17/133) presented mild atrophy (OLGA score = I), 2.3% (3/133) presented moderate atrophy (OLGA score = II), and 0.8% (1/133) presented severe atrophy (OLGA score = III). None of the participants were graded for severe atrophy with OLGA score = IV (Table 1).

All 133 participants included in the study were randomized in two groups for assigned treatment. Group I or conventional group included 69 participants and group II or personalized group included 64 participants. Participants of the conventional group consisted of 67% (46/69) women and 33% (23/69) men with an average age of 45.7 ± 13, weighing 68.3 ± 16, and height in cm of 160.9 ± 15 and body mass index of 25.4 ± 3.9. The main reason for medical consultation in this group was dyspepsia 46.4% (32/69) and reflux 27.5% (19/69). Less frequent consultation reasons are detailed in Table 1. The main endoscopic finding was gastritis in 98.5% (68/69) of the participants, without atrophy (histopathologic finding).

On the other hand, participants of the personalized group included 72% (46/64) women and 28% (18/64) men with average age of 45.9 ± 11.5, and weighing 65.7 ± 13, with an average height of 161.5 ± 7.6 and body mass index of 25.3 ± 3.9. The primary reason for medical consultation in this group was dyspepsia and reflux in 42.2% (27/64) and 33% (21/64) of the participants, respectively. Other less frequent symptoms are detailed in Table 1. Endoscopic analysis evidenced gastritis in 100% (64/64) of the participants, some of them with additional less frequent findings detailed in Table 1, without histopathologic atrophy. No significant differences between the two groups were observed (Table 1).

3.2 | *CYP2C19* polymorphism distribution

CYP2C19 polymorphisms *1, *2, *3, and *17 were analyzed for all 133 subjects included in the present study. *CYP2C19* polymorphism distribution was 54.9% (73/133) for genotype*1/*1, followed by *17/*17 for 21.1% (28/133) of the participant's studied, 7.5% (10/133) for *1/*17, 9.8% (13/133) for *1/*2, 6% (8/133) for *2/*17, and 0.8% (1/133) for *2/*2. Based on these polymorphisms, 133 participants were classified into different omeprazole metabolizer phenotypes as follows: extensive 62.4% (83/133), ultra-rapid 21% (28/133), intermediate 15.8% (21/133), and poor metabolizers 0.8% (1/133) (Figure 1 and Table 1).

TABLE 1 Characteristics of participants enrolled in the study

Characteristics	Global study participants (n = 133)	Conventional Therapy Group I (n = 69)	Tailored Therapy Group II (n = 64)	Differences Groups I and II ($P < 0.05$)
General Characteristics				
Gender (Male:Female)	41:92	23:46	18:46	0.21
Age (y)	45.8 ± 12.3	45.7 ± 13	45.9 ± 11.5	0.93
Weight (Kg)	67.1 ± 14.9	68.3 ± 16	65.7 ± 13	0.33
Height (cm)	161.1 ± 12.4	160.9 ± 15	161.5 ± 7.6	0.88
Body mass index (Kg/m^2)	25.3 ± 4.5	25.4 ± 3.9	25.3 ± 3.9	0.92
Reason for medical consultation				
Dyspepsia	59	32	27	0.62
Reflux	40	19	21	0.51
Weight loss	5	1	4	0.15
Anemia	3	0	3	0.07
Other symptoms	26	17	9	0.12
Endoscopic findings				
Gastritis	132	68	64	0.33
Esophagitis	94	52	42	0.22
Hiatal hernia	29	15	14	0.98
Metaplasia	12	4	8	0.18
Duodenal ulcer	5	3	2	0.71
Gastric ulcer	4	1	3	0.28
Grade of atrophy—OLGA score				
No atrophy—score 0	122	60	52	0.38
Mild atrophy—score I	17	8	9	0.66
Moderate atrophy—score II	3	0	3	0.06
Severe atrophy—score III	1	1	0	0.33
CYP2C19 Polymorphisms				
*1/*1	73	37	36	0.89
*17/*17	28	15	13	0.99
*1/*17	10	7	3	0.39
*1/*2	13	6	7	0.88
*2/*17	8	4	4	0.79
*2/*2	1	0	1	0.97

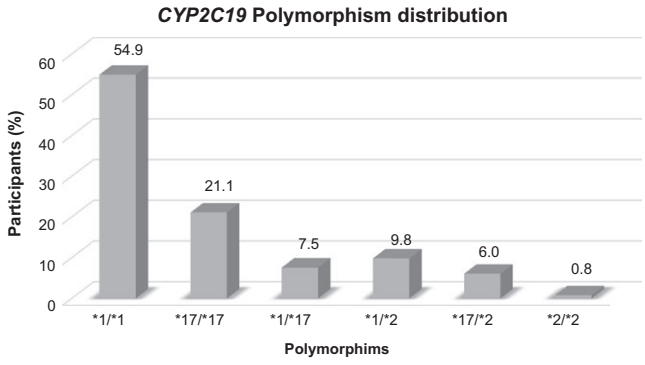


FIGURE 1 *CYP2C19* polymorphism distribution in 133 participants enrolled in the study

3.3 | Effectiveness of conventional therapy vs tailored therapy according to *CYP2C19* status

Eradication rates in groups I vs II are shown in terms of analysis by intention-to-treat (ITT) and per-protocol (PP) as follows: ITT = 84% (95% CI: 0.73-0.91) vs 92.2% (95% CI: 0.82-0.97) ($P = 0.14$), respectively, RR = 0.49 (95% CI: 0.18-1.33). The analysis by PP for conventional therapy vs personalized therapy was PP = 92.1% (95% CI: 0.82-0.97) vs 100% (95% CI: 0.92-0.01) ($P = 0.027$), respectively, RR = 0.92 (95% CI: 0.86-0.99) (Figure 2).

Results showed that according to an intention-to-treat basis proposed by Dr Graham, *H pylori* infection therapy outcome score for personalized therapy was good with respect to conventional therapy, which even in the absence of antibiotic resistance was poor (Figure 3A).

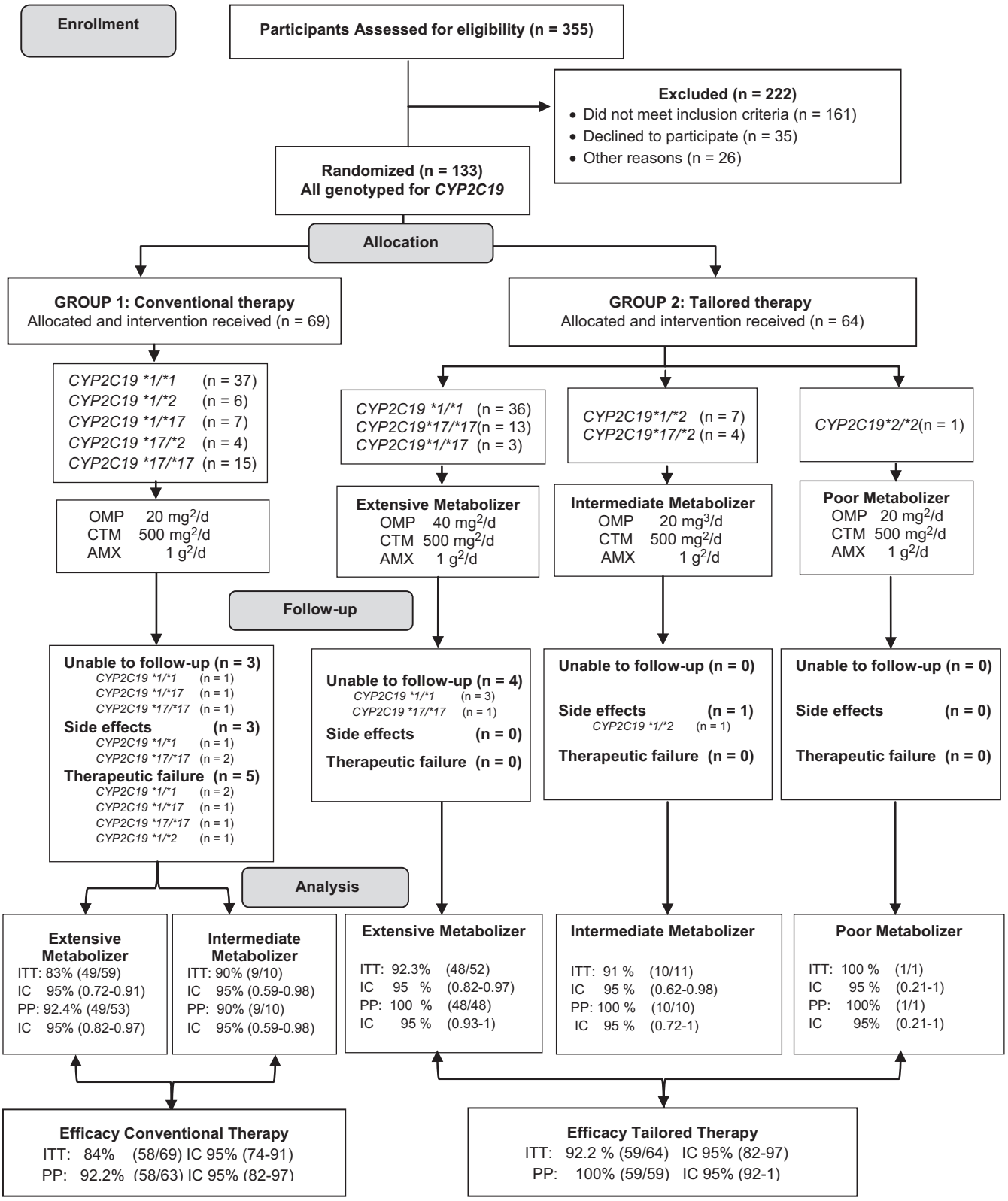
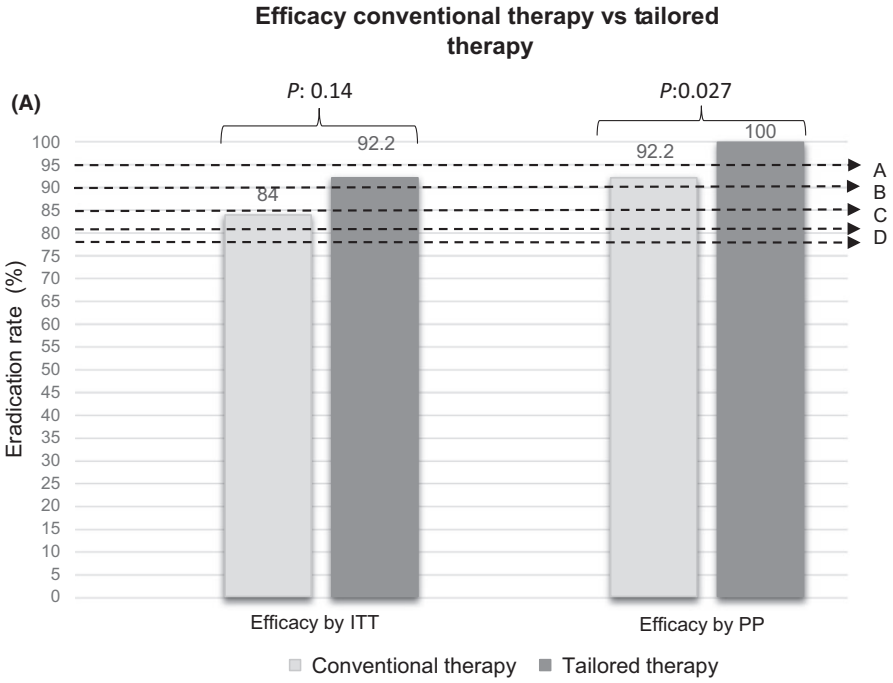


FIGURE 2 Consort Diagram: It shows enrolled participants in the study, allocation of therapies in group I—conventional therapy (participants who received triple standard therapy with clarithromycin (CTM) and amoxicillin (AMX)) and group II—tailored therapy (participants who received triple standard therapy with omeprazole doses according to *CYP2C19* genotype). Participant follow-up and each therapy efficacy are illustrated. Verification of *H pylori* eradication was performed by fecal antigen test



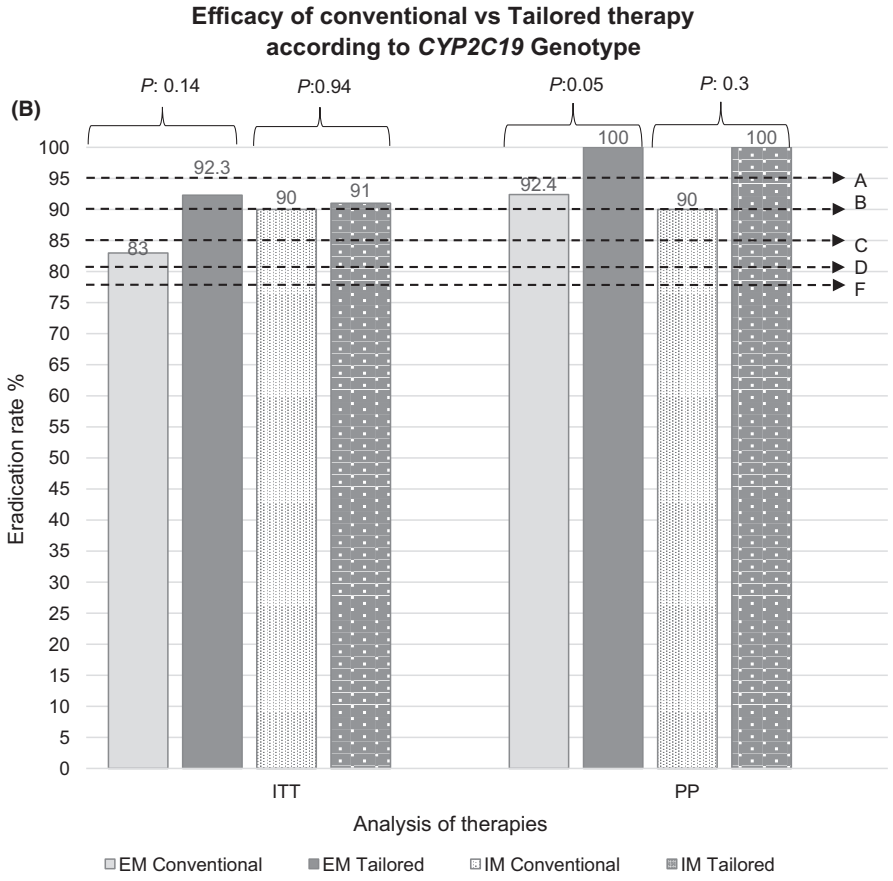


FIGURE 3 Efficacy of therapies: Figure 3A shows the efficacy of conventional versus personalized therapy by intention-to-treat (ITT) and per-protocol (PP) analyses with respective *H pylori* infection therapy outcome score for an intention-to-treat basis. (A, Excellent [≥ 95%], B, Good [90%-94%], C, Acceptable [85%-89%], D, Poor [81%-84%], F, Unacceptable [≤80%]). Efficacies in tailored therapy group were superior to conventional therapy. B, shows therapy efficacy in each study arm discriminated by *CYP2C19* genotype (EM, extensive metabolizer; IM, intermediate metabolizer). An important difference between conventional and tailored therapy was observed for extensive *CYP2C19* metabolizers, evidencing ITT, and particularly, PP analyses for tailored therapy were better in comparison with conventional therapy

To analyze each therapy performance, the effect of *CYP2C19* genotype on eradication rate was evaluated in groups I and II, noting that although eradication rates were better in group II than in group I, no significant differences in the eradication rates between different *CYP2C19* genotypes in both groups were observed (Figure 3B). Nevertheless, no participant receiving personalized therapy presented therapeutic failure (group II). In contrast, in group I five participants receiving conventional *H pylori* therapy had therapeutic failure. It is noteworthy that participants with therapeutic failure in group I were characterized as extensive, ultra-rapid, and intermediate PPI metabolizers (Figure 2).

3.4 | Adverse effects

Side effects were analyzed for all 133 participants by treatment. In general, 63.2% (84/133) of participants referred having side effects; thus, 3% (4/133) suspended treatment, three from conventional therapy and one from personalized. According to therapy, 60.9% (42/69) of participants from conventional therapy referred having side effects, while from personalized therapy 66.4% (42.5/64) participants described experiencing side effects. Hence, there were no significant adverse effect differences between conventional and tailored therapies ($P = 0.211$). The most frequent adverse effects were altered taste (metallic taste) as described by 17.4% (12/69) from the conventional therapy group and 17.2% (11/64) from participants undergoing personalized therapy. A similar percentage suffered from diarrhea, 17.4% (6/69) conventional therapy participants and 17.2% (9/64) personalized therapy participants.

4 | DISCUSSION

This study carried out a randomized, single-blinded clinical trial using a 10-day treatment regimen for *H pylori* in clarithromycin- and amoxicillin-sensitive bacteria. To this end, omeprazole doses were assigned according to *CYP2C19* polymorphism with 40 mg twice a day omeprazole doses for extensive metabolizers, 20 mg three times a day for intermediate metabolizers, and 20 mg twice a day for poor metabolizers. Under these omeprazole doses, *H pylori* eradication rate by ITT was 92.2% and PP 100%, with greater significant efficacies ($P = 0.027$) in comparison with standard omeprazole doses per-protocol (conventional therapy), with eradication rates of 84% (ITT) and 92.1% (PP). These results were probably due to the profound gastric acid suppression, which increases *H pylori* eradication by favoring *H pylori* replication and improving antibiotic activity.[14-17] Efficacies observed in this study thus far are the highest reported in comparison with other studies (ITT 92.2% vs 74.7% to 86.6%) also describing *CYP2C19* polymorphism influence on *H pylori* treatment when triple standard therapy with omeprazole is used.[35-43] Our findings might be accounted by the methodological design used, since all subjects were antibiotic sensitive.

Actually, international guidelines recommend 14-day regimens, to reach different *H pylori* niches, persistent and dormant state of bacteria.[1,44-47] However, the present study was conducted using a 10-day regimen, because in Colombia and Latin America there is no evidence of efficacy during this time in the absence of antibiotic resistance, and using different doses of PPI based on *CYP2C19* polymorphism. Therefore, it is important to know the local behavior of the triple therapy under these particular conditions before adopting any recommendation.[1] Our results with a 10-day in personalized regimen for *H pylori* eradication based on *CYP2C19* polymorphism and without antibiotic resistance with rates of 92.1% by ITT and 100% by PP analyses raise the need to re-thinking on 10-day therapies based on high PPI doses before ruling out its use. For now, we consider 14-day therapies should be prescribed when empirical therapies are employed, and when local resistance to clarithromycin is below 15%, as recommended by experts and international consensus.[1,44,45]

Additionally, it was demonstrated first-line therapies, which had greater efficacy based on susceptibility tests in comparison with empirical as has been previous reported.[48,49] The goal of medicine precision is identify which regimen is best for an individual patient.[53] Thus, our results suggest in the future that personalized therapy could be ideal for *H pylori* eradication under controlled conditions, considering personalized medicine could avoid an increase in adverse effects, such as lack of patient's compliance and increase in antibiotic resistance.

Furthermore, in the present study we identified omeprazole at high doses (40 mg/twice a day). It was not only efficient in extensive metabolizers, but it was also efficient in ultra-rapid metabolizers (*17/*17). This last finding is in agreement with Sugimoto et al,[54] results which are relevant for populations with these type of prevalence (subjects with extensive and ultra-rapid).

These findings demonstrate that the action of extensive and ultra-rapid metabolizers on omeprazole can be overcome with omeprazole doses described in this study. However, it can be solved using other PPIs with low CYP metabolism, such as esomeprazole or rabeprazole at adequate doses.[55,56] An example is a recent study described that rabeprazole-based hybrid therapy was used to surpass *CYP2C19* genotype's effect and *H pylori* sensitivity to antibiotics. Efficacy at 14-day regimen was similar to 10-day regimen found in this study (ITT 92.94%),[57] another alternative would be to use vonoprazan, whose metabolism is independent of *CYP2C19*.[58] One main obstacle is not available in Colombia.

In addition, no significant differences were observed for *CYP2C19* genotype subgroups in regard to eradication rates, probably undetectable due to sample size.

In the present study, 62.4% of the subjects were extensive and 21.1% were ultra-rapid metabolizers. These results are similar to those previously described by Isaza et al. in 2007 in Neiva-Colombia,[13] who reported 83.6% extensive metabolizers. Differences between Isaza et al. results and the present study can be explained by detection of ultra-rapid metabolizers (*17/*17) in the later one. Moreover, its determination could be masked by *1/*1 polymorphism, establishing this study as the first to detect ultra-rapid metabolizers in our country.

Less frequent polymorphisms were 9.8% (13/133) intermediate metabolizers *1/*2 and 0.8% (1/133) poor metabolizers *2/*2. Their frequencies are in agreement with those previously reported by Isaza in Colombia: 15.3% and 1.1%, respectively.[27] In general, *CYP2C19* polymorphism founder effect distribution in our environment is different from those reported in other parts of the world. This might be related to heritable traits, since polymorphisms vary depending on ancestors. Nevertheless, the frequencies herein reported are similar to previous reports in Caucasians, where prevalence of alleles *2 and *3 is low.

We consider extensive and ultra-rapid metabolizers high prevalence could have an impact in our population on treatments for other

illnesses, such as gastroesophageal reflux disease, since it would require higher doses than those currently prescribed. Nonetheless, other studies are needed to confirm this.

In conclusion, this work demonstrated that high omeprazole doses are required to eradicate *H pylori*, even in antibiotic-sensitive subjects. In addition, these high doses can overcome extensive and ultra-rapid PPIs metabolizer's effects. Findings in the present study propose to evaluate 10-day regimens and recommend personalized treatments. Even though in the Maastricht V Consensus report a 14-day regimen is recommended,[1] it is also emphasized therapies may be of shorter duration, if on local populations evidence demonstrates so, as was the case for this study.

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ETHICAL APPROVAL

Ethical approval was endorsed by the participating institutions.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

AAT-R and WO designed the study; WO supplied samples; AA-G performed all experiments, collected, and analyzed data; WO and AA-G follow the participants; AA-G, WO, and AAT-R drafted and revised the manuscript. All authors read and approved the final manuscript.

PROTOCOL STUDY REGISTER

Study protocol is registered at Clinical Trials.gov PRS with ID NCT03650543 and protocol number ID 00004554.

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