Meta-Analysis of Single-Capsule Bismuth-Containing Quadruple Therapy ("Three-in-One" Formulation) for *Helicobacter pylori* Eradication: Efficacy, Safety, and Treatment Compliance

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ABSTRACT

BACKGROUND: Bismuth-containing quadruple therapy has been suggested as first-line and rescue alternative for *Helicobacter pylori* eradication. Our objective was to perform a meta-analysis evaluating the efficacy and safety of single capsule Pylera® (bismuth, metronidazole and tetracycline) plus a proton pump inhibitor (PPI) in any line of treatment.

METHODS: Studies were selected up to October 2018. Outcomes were eradication and adverse events (AEs) rates pooled using the generic inverse variance method.

RESULTS: In total, 30 studies (6,482 patients) were included in the systematic review. The intention-to-treat (ITT) efficacy was 90% (95% CI=87-92%, 21 studies, I^2=88%) in first-line therapy, 89% (95% CI=86-93%, 12 studies, I^2=78%) in second-line and 82%

(95% CI=78-87%, 9 studies, I^2=60%) in third-line; with no differences by the type or dosage of PPI used. For clarithromycin or metronidazole resistant infection, the ITT efficacy as first-line therapy was 94% (95% CI=91-97%, 5 studies, I^2=0%) and 93% (95% CI=90-96%, 6 studies, I^2=0%) respectively. In second-line therapies where patients had been previously treated with clarithromycin, the ITT efficacy was 90% (95% CI=87-93%, 11 studies, I^2=78%). The overall incidence of AEs was 43% (95% CI=35-50%, 24 studies, I^2=92%) and they were mostly mild. In nearly 3% of the cases, treatment was interrupted due to AEs.

CONCLUSIONS: A 10-day treatment with Pylera® represents a highly effective (≥ 90%) therapy against *H. pylori* infection, both as first- and second-line. This applies regardless of the type and dose of the PPI, in patients with clarithromycin or metronidazole resistant strains, and in those previously treated with clarithromycin.

KEY WORDS: *Helicobacter pylori*; bismuth; metronidazole; tetracycline; quadruple therapy; Pylera®.

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is the main cause of gastroduodenal disorders, such as gastritis that may lead to gastroduodenal ulcer disease, preneoplastic lesions, MALT lymphoma and gastric cancer. Over half the population worldwide is thought to be infected with *H. pylori*, making it a global health burden(1-3). Eradication of *H. pylori* is recommended for all patients diagnosed with this infection(1). However, its treatment remains a challenge as there is still no available therapy that achieves a 100% cure rate(4) but expert consensus has dictated a treatment needs to achieve at least a 90% cure rate(2, 3, 5). Indeed, the success rate of standard therapies is declining due to the increased resistance to antibiotics around the globe(6, 7).

When considering *H. pylori* therapies, it is important to differentiate between first-line therapies and rescue regimens, as rescue therapies are usually challenged by selection, or by the acquisition of resistance after prior failed attempts of eradication. In the last two decades, several antibiotic combinations have been proposed to treat this disease, even including quadruple therapies: bismuth-free (sequential, concomitant, hybrid regimens) and bismuth-based therapies.

Historically, Barry Marshall started *H. pylori* treatment with bismuth-based “triple chemotherapy” (bismuth, tetracycline and metronidazole) with the first series published by Thomas Borody et al in 1989(8) suggesting such combination could lead to long-term eradication of *H. pylori* in the majority of patients with and without ulcers. But because this triple “antibiotic therapy” was not backed-up by the pharmaceutical industry, dual therapy (amoxicillin plus a PPI) was introduced.

However, this dual combination did not reach optimal efficacy and triple therapies were suggested as an alternative.

The triple therapy traditionally recommended to eradicate *H. pylori* involves the use of a proton pump inhibitor (PPI) and two antibiotics (clarithromycin and metronidazole or amoxicillin), although this fails in ≥20% of patients due to the increasing worldwide resistance to clarithromycin and/or metronidazole(9, 10). Other first-line regimens including the sequential therapy that involves a dual regimen of a PPI plus amoxicillin for the first five days, followed by a triple regimen of a PPI, clarithromycin and tinidazole for the following five days also have shown efficacy rates below 90%(11). The efficacy of recommended second-line triple therapies has also decreased in recent years, such as that involving administration of a PPI plus amoxicillin and levofloxacin(12).

The classic bismuth quadruple therapy (BQT) involves the use of a PPI, bismuth, metronidazole and tetracycline, and in recent randomised clinical trials (RCTs) it has proven superior to standard triple therapies in eradicating *H. pylori*. Indeed, BQT may be particularly recommendable in areas of high clarithromycin resistance(13, 14). However, the limited availability of bismuth salts and tetracycline in many countries restricts the use of BQT. More recently, interest in BQT was renewed through the development of a novel three-in-one single capsule, Pylera® (Allergan, Inc. Dublin, IE), that contains bismuth, metronidazole and tetracycline. This therapy was first proposed in 2000(15) and it has been used as either a first-line or rescue therapy(1, 16-18). Data on this first prototype of the capsule reported an ITT cure rate of 94% (95% CI 95 – 88) of 53 patients treated and an ITT cure rate of 98% (95% CI 93-100) in 45 patients

treated with metronidazole bacterial sensitivity (19). A European phase-III trial showed that as a first-line therapy, 10-day omeprazole and Pylera® was more effective than a 7-day clarithromycin-based triple therapy(16). These data confirmed the efficacy of 10-day Pylera® reported in the original USA trial that compared it to 10-day clarithromycin-containing triple therapy(20).

The effectiveness of rescue therapies depends directly on the prior exposure to antibiotics, with strains surviving any attempt at eradication tending to become more resistant or resilient to further treatment(21). Although there is little data available showing the efficacy of the single capsule as a rescue therapy, there is evidence from a few studies that 10-days of omeprazole and three-in-one bismuth-containing single capsule is an effective rescue therapy in patients infected with antibiotic-resistant strains of *H. pylori*(17, 22). Hence, the aim of this study was to perform a systematic review and meta-analysis of the studies evaluating the efficacy and safety of BQT with three-in-one bismuth-containing single capsule (Pylera®) plus a PPI as any line of treatment to eradicate *H. pylori*.

METHODS

Bibliographic search strategy and the selection of the studies

This meta-analysis was performed in accordance with the guidelines of the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) statement. A systematic literature search was carried out in the Medline, PubMed, ISI Web of Knowledge, EMBASE and, the Cochrane Central Register of Controlled Trials online databases up to October 2018. The search strategy syntax was adapted accordingly in the different platforms using the following keywords (all fields): “pylera” OR “bismuth quadruple”, independently and then combined with “Helicobacter pylori”. Manual searches of additional information in conference abstracts were also performed up to October 2018 from the *International Workshop of the European Helicobacter and Microbiota Study Group*, the *United European Gastroenterology Week* and the *American Digestive Disease Week*. Cross-referencing was implemented for the studies selected from the electronic search and no language restrictions were applied. Searches were performed independently by two researchers (AGM and OPN) and any discordance was resolved by discussion. If a consensus was not reached, a third reviewer (JPG) was consulted.

The articles included in the meta-analysis were comparative and non-comparative trials, randomised or not, assessing either the efficacy or safety of Pylera® (Allergan, Inc. Dublin, IE) treatment for the eradication of *H. pylori* infection in naïve or rescue patients. In addition, they complied to the following criteria: (i) the Pylera® eradication regimens containing bismuth 145 mg, metronidazole 125 mg and tetracycline 125 mg were of any duration but its use had to be combined with any PPI; (ii) any comparison,

such as with a standard triple therapy, bismuth and non-bismuth quadruple therapies (sequential, concomitant or hybrid), and of any treatment duration were considered; (iii) participants could be adults or children, diagnosed by histology, culture, rapid urease test, ^{13}C-urea breath test or the monoclonal stool antigen test; (iv) eradication had to be confirmed at least 4 weeks after treatment with at least one of the aforementioned methods (except culture). Studies were excluded if they did not focus on *H. pylori* treatment, if a diagnostic test of infection was not performed, nor a confirmatory test of eradication after treatment, and if the results of eradication results (in terms of intention-to-treat (ITT)) or safety could not be calculated.

Data Extraction

Data collection was performed independently by two reviewers (OPN and AGM), again resolving discordances through discussion and consulting a third reviewer (JPG) when a consensus could not be reached. The studies identified in the initial search of the relevant bibliography were subjected to a two stage screening process. Prior to screening, duplicates were removed automatically when they were imported into the citation manager and any remaining duplicates were then removed manually during the first screening phase. An initial screening of the titles and abstracts (first screening phase) was undertaken to identify potentially relevant publications that complied with the inclusion criteria. The full text of the citations selected as potentially eligible for inclusion in the first phase were then subjected to a second screening. Finally, when a study selected after both screenings reported data from the same study population as that analysed in another study, data was only collected from the latest study published.

Statistical Methods and Data Pooling

The analyses were pre-planned *a priori* and the primary outcome of efficacy was the eradication rate by ITT. The total number of patients randomised and the number of patients that achieved successful eradication were assessed in each study to compute the eradication rate with its standard error. The estimated effect of each study was later combined as a global effect estimate using the generic inverse variance method. Analyses were performed in Review Manager (version 5.3).

Efficacy sub-analyses were presented for each treatment line of the different subgroup analyses, such as the medical condition at baseline (peptic ulcer disease and non-ulcer dyspepsia), PPI type, prior antibiotic bacterial resistance, or success after prior antibiotic treatment. Safety was evaluated by extracting the following data when reported: percentage of patients with at least one adverse event (AE), distribution of patients according to the intensity of AEs (mild, moderate or severe), and appearance of serious AEs that led to hospitalisation, medical leave, surgery, disability or death. A sub-group analysis also distinguished between the prospective observational studies and RCTs. In such cases, the effect of the estimate chosen to compare the impact of each intervention was the difference in risk and the 95% confidence interval (CI)(23)

A random effect model was used for all the analyses, as the populations evaluated were heterogeneous. The I^2 statistic was used to interpret heterogeneity and as such, an I^2 of <40% was considered as heterogeneity that might not be important, an I^2 between 40–75% as substantial or moderate heterogeneity, and an I^2 >75% indicated that heterogeneity might be considerable. The interpretation of the I^2 was also subject

to the magnitude of the effect and/or the strength of the evidence of heterogeneity (i.e.: a p value <0.1 in the chi-squared test).

Risk of Bias and the quality of the Body of Evidence

The studies included were assessed for their quality, and for the potential risk of bias, using a customised version of the Newcastle-Ottawa (NOS) quality assessment scale for studies where only one treatment arm was evaluated or included in the meta-analysis(24). The scale assessed the quality of the study in three main domains: selection of cases and controls, or exposed and unexposed; comparability between cases and controls, or exposed and unexposed; and exposure assessment in the case-control studies or outcome assessment in the cohort studies. For each item there were a few alternative degrees of quality available, ranging from best quality to poor quality or not reported. Randomised studies were evaluated using the Risk of Bias tool, as recommended by the Cochrane Collaboration(25, 26). Resulting poor quality that may compromise the methodology or results of a study was used as a valid exclusion criterion in the present review. Publication bias was assessed by visual inspection of funnel plots.

Sensitivity Analyses

Sensitivity analyses were considered for each meta-analysis to investigate the possible differences in the results. Studies were excluded if they were classified as potentially introducing a serious or critical Risk of Bias according to the variables that could influence the main outcome *a priori*, such as treatment length, PPI type and dose, and the publication format.

RESULTS

Study selection and initial description of the studies included

Overall, of the 57 relevant references initially identified, 30 studies (16-18, 20, 22, 27-48) met the inclusion criteria and were included in the systematic review and meta-analysis (Figure 1). During the full-text review stage, the authors of five primary studies were contacted for data clarification. The characteristics of the selected studies that evaluated the efficacy and safety of three-in-one single capsule bismuth-containing quadruple therapy are reflected in Table 1. These studies, published up to October 2018, presented data from 6,482 infected patients, and all of them were prospective studies, but one(46) which was retrospective and five that were randomised studies (16, 20, 41-43). Twenty-three of them included patients naïve to treatment and 11 reported patients that had previously received clarithromycin therapy.

Risk of Bias in the studies included

Among non-randomised studies, the assessment indicated a low Risk of Bias for each of the items evaluated with the maximum quality score (i.e. score of 9) in 6 studies and a quality score of 8 in 11 studies (Suppl. Table 1). In randomised studies, most of the items assessed were labelled as low risk, except for ‘allocation concealment’ but this item was considered not to influence the outcome in the therapeutic context (Suppl Table 2). No selection or publication biases were evident by visual inspection of the funnel plot (Suppl. Figure 1).

Efficacy of Pylera® in the eradication of H. pylori

Overall efficacy

Among 30 included studies, all reported the efficacy of BQT with the single capsule Pylera® plus a PPI, measured as the eradication rate of *H. pylori* infection in the ITT population. Twenty-five studies reported this efficacy by treatment line and three (18, 30, 39) reported efficacy in the total ITT population treated without specifying eradication rates as a function of the line of treatment and could therefore not be meta-analysed. Overall efficacies in these studies were 82%, 95% and 97% respectively. Two other studies (33, 48) were included in the meta-analysis of safety only as no efficacy data was reported. The overall ITT eradication rate was 88% (95% CI 86−90%), albeit with high heterogeneity (I^2 = 84%, p<0.001: Figure 2). In the sub-group analysis as a function of the treatment line (naïve, second-line, third-line, fourth-line and fifth-line), the eradication rate was 90% (95% CI 87−92%, 21 studies, I^2 =88%) for naïve patients, similar to the second- (89%, 95% CI 86−93%, 12 studies, I^2 =78%) third-line (82%, 95%CI 78−87%, 9 studies, I^2= 60%) and fourth-line (85%, 95% CI 76−94%, 5 studies, I^2= 0%) therapies. By contrast, the fifth-line therapy was less efficacious (67%, 95% CI: 51−82%, 5 studies, I^2= 0%). These differences in the efficacy of BQT (single capsule) plus a PPI were significant for the different lines of treatment evaluated (test for sub-group differences P<0.001 and I^2 = 74%). Almost all studies reported PP eradication rates but in four studies(36, 42, 45, 49), and could not be computed. The overall PP eradication rate was 95% (95% CI 93−96%), with moderate heterogeneity (I^2 = 64%, p<0.001: Suppl Figure 2). The efficacy was significantly different for the different treatment lines (test for sub-group differences P<0.001 and I^2 = 84%).

In all studies the treatment with the BQT (single capsule) plus a PPI lasted 10 days but in the study by Salazar 2012 where patients were treated for 14 days.

Effect of different variables on the efficacy

Medical condition

No differences were seen when the analysis was performed according to the baseline medical condition, either peptic ulcer disease or non-ulcer dyspepsia (Figure 3A and 3B). Pylera® was effective in 86 % of patients with peptic ulcer disease (95% CI 82-90%, I^2 = 70%) and in 87% of patients with non-ulcer dyspepsia (95% CI 82-91%, I^2 = 77%) regardless of the line of treatment. Similar results were found when only first-line treatments were considered, with no differences between patients with non-ulcer dyspepsia relative to those with ulcer disease (88% vs 86%, respectively; p= 0.68: Suppl. Figure 3). There were no significant differences in the efficacy of Pylera® between treatment lines when treating patients with non-ulcer dyspepsia (test for sub-group differences p= 0.68 and I^2=0%: Suppl. Figure 3B).

Type of PPI

No significant differences were found in terms of efficacy across treatment lines for either the omeprazole and esomeprazole meta-analyses (test for subgroup differences p=0.35, I^2= 9.5% and p= 0.31, I^2 = 16% for omeprazole and esomeprazole, respectively: Suppl. Figures 4A and 4B). Further information regarding the type and dosage of PPI are reported in Table 1.

a) Omeprazole

When administered with omeprazole, the three-in-one capsule achieved eradication rates between 56 and 99% when any line of treatment was considered. The overall

eradication rate was 89% (95% CI 86−92%), with high heterogeneity (I^2=86%; p<0.001: Suppl. Figure 4A). In a sub-group analysis as a function of the treatment line, eradication rates ranged between 79 and 99% as a first-line therapy when the single capsule was administered with omeprazole, with an overall eradication rate of 90 % (95% CI 86−94%) and significantly high heterogeneity (I^2= 92%; p<0.001). A similar overall eradication rate of 91% was reported as a second-line therapy (95% CI 85−98%; p<0.001), whereas it was less efficacious as a third-line therapy with an efficacy of 77% (95% CI 63-92%; p<0.001). There were only two studies available for the use of the single capsule with omeprazole as a fourth- and fifth-line therapy.

b) Esomeprazole

Fewer studies were available for the use of the three-in-one single capsule together with esomeprazole, for which eradication rates between 67 and 95% were reported when any line of treatment was considered. The overall eradication rate for this combination was 85% (95% CI 81−89%) and with no significant moderate heterogeneity (I^2 = 58%; p = 0.02: Suppl. Figure 4B). In a sub-group analysis as a function of the treatment line, the single capsule administered with esomeprazole achieved an eradication rate of 86% (95% CI 79−93%) as a first-line therapy, with significant moderate heterogeneity (I^2 = 77%; p < 0.001; 6 studies).

c) Omeprazole versus esomeprazole

No significant differences in the efficacy of Pylera® as first-line treatment were found when patients treated with omeprazole were compared to those treated with esomeprazole (90% vs 86%, respectively; I^2 = 0%; p= 0.41: Suppl. Figure 5).

Antibiotic bacterial resistance

In nine studies, information was provided on the proportion of patients with bacterial antibiotic resistance, either to clarithromycin or metronidazole. Such information could not be used in the study by Miehlke 2017(38) as the efficacy of Pylera® in this sub-group of patients was not reported. In the forest plots obtained from the remaining studies (Figure 4A and 4B), the single capsule had a significant curative effect on *H. pylori* infection, despite the clarithromycin and metronidazole resistance.

a) Clarithromycin

In those patients with bacterial infection resistant to clarithromycin, the efficacy of Pylera® reached over 90% in naïve patients, except in the study by Laine 2003(20). In particular, five studies evaluated the efficacy of the single capsule in naïve patients with antibiotic resistance to clarithromycin, obtaining a mean ITT eradication rate of 94% (95% CI 91-97%, I^2 = 0%: Figure 4A).

b) Metronidazole

A total of eight studies evaluated the efficacy of Pylera® in patients with metronidazole resistant bacteria, obtaining an efficacy of 91% (95% CI 88-94, I^2 = 17%; Figure 4B). Treatment was also successful as a second-line therapy in 94% of patients with antibiotic resistance to metronidazole, and as a third-line therapy in 81% of such patients.

c) Dual bacterial resistance

In addition to the above, a single study reported the efficacy of the single capsule in naïve patients with antimicrobial resistance to both clarithromycin and

metronidazole(16). Pylera® successfully overcame this dual antibiotic resistance, with an ITT eradication rate of 91% (95% CI 85-97%; I^2 = 0%; 4 studies; Suppl. Fig 6).

Prior therapy with clarithromycin

There were twelve studies that reported information from patients who failed a prior therapy involving clarithromycin, although the information from one study(18) could not be used as the efficacy of the single capsule in this specific sub-group of patients was not reported. In those patients who did not respond to a prior therapy involving clarithromycin, the overall efficacy was 87% (95% CI 84−91%), with evidence of heterogeneity (I^2 = 85%; p<0.001: Suppl. Figure 7). If only second-line therapeutic treatment was considered, the efficacy was 90% (95% CI 87-93%, I^2 = 78%; p<0.001).

Effects of interventions: Pylera® versus standard triple therapy

Five RCTs were included in the systematic review(16, 20, 41-43), and the data were considered insufficient to pool into a meta-analysis. Laine 2003 and Maltfernheiner 2011 compared the efficacy of BQT (single capsule) plus a PPI with the standard triple therapy (omeprazole, clarithromycin and amoxicillin) lasting 10 and 7 days respectively. Both studies evaluated patients infected with *H. pylori* who were naïve to treatment. In these studies, the efficacy with the single capsule was reported to be 88% (versus 83%) and 98%(versus 68%) (16, 20) (modified ITT), respectively. Differences between the treatment arms were only significant (p=<0.001) in the study by Malfertheiner 2011(16).

Effects of interventions: Pylera® versus quadruple therapy

One RCT(43, 44), compared the efficacy of Pylera® versus two non-bismuth quadruple therapies: a 10 day- sequential therapy (PPI, amoxicillin during 5 days followed by PPI, amoxicillin and tinidazole during the remaining 5 days) and a concomitant therapy (PPI, amoxicillin, clarithromycin and tinidazole altogether during 10 days) in naïve patients. The ITT efficacy achieved with Pylera® (85%) was lower than with concomitant (97%) or sequential (93%) therapies. Similar results were found in the study by Tarhini 2018,(42) where the single capsule achieved a lower cure rate than with sequential therapy (50% vs 80% ITT eradication rate respectively) in first-line therapy.

The RCT by Xie 2018 compared the single capsule versus a bismuth quadruple therapy (bismuth, omeprazole, amoxicillin and clarithromycin during 10 days) in naïve patients. Eradication rates were similar in both arms and reached 87 and 87.5% ITT cure rates respectively(41).

Safety of Pylera®

In 24 studies there was data regarding the adverse events (AEs) (Table 1), with a rate of AEs that ranged between 2% and 73% and 16 studies could be included in the meta-analysis of safety which did not discern by treatment line. The overall rate of AEs was 41% (95% CI 33−49%), with high heterogeneity (I^2 = 92%; p< 0.001) (Figure 5). A sub-group analysis was performed according to the severity of the AE, with a 25% incidence of mild AEs, an 8% of severe AEs and a 1% of serious AEs of the total of AEs

registered. (Suppl. Figures 8, 9 and 10). In almost 3% of the cases treatment was discontinued due to AEs. (16-18, 27, 30-32, 34, 35, 37, 38, 42-45, 47).

Sensitivity analyses

Sensitivity analyses were performed for each meta-analysis (overall and subgroups), excluding those studies considered to be potential sources of risk based on *a priori* variables known to influence the main outcome (Table 2). In particular, the effect of treatment duration, the use of the esomeprazole as a PPI, the dosage of the PPI, the clinical setting of the study and the publication format was evaluated. The ITT results of the overall efficacy of Pylera® were not affected when the studies classified as likely to introduce a Risk of Bias were excluded separately for each of the variables evaluated (Table 2).

DISCUSSION

This is the first systematic review and meta-analysis performed to date that has pooled information from all the studies available to evaluate the efficacy and safety of Pylera® when used as any line of treatment for the eradication of *H. pylori*.

Statement of main findings

The main results obtained indicate that a 10-day treatment with BQT, involving single capsule Pylera® plus a PPI, achieves an overall ITT eradication rate of 90%, reaching the minimum consensus threshold recently established(1, 3). These results are superior in most areas to those reported for triple therapy, such as a PPI, amoxicillin and clarithromycin for 7- to 14-days(50, 51), which still represents the most common treatment in Europe despite being discouraged in most recommendations(1-3). Indeed, current consensus generally recommends the use of quadruple therapies, such as the traditional BQT treatment or the non-bismuth quadruple concomitant treatment with a PPI, amoxicillin, clarithromycin and metronidazole(1, 2).

The use of traditional BQT is often limited by the poor availability of some compounds in many regions, such as bismuth or tetracycline(52). Although the regimen for this 3-in-one bismuth-containing quadruple therapy is still relatively complex, with patients having to take three capsules four times a day for 10 days, in conjunction with a twice-daily standard dose of a PPI, it is simpler than the standard regimen for BQT and a good compliance rate above 95% has been reported(16). In addition, in the concomitant treatment the patient has four different medication boxes whereas with Pylera® only two different boxes and it achieves nearly 90% eradication rates when prescribed for 14 days(1, 52, 53).

Factors influencing the efficacy of Pylera®

Antibiotic resistance is the main factor that affects the success of *H. pylori* treatment(7, 21), with clarithromycin resistance representing a burden to the success of triple therapy, and dual clarithromycin-metronidazole resistance affecting the concomitant regimens(1, 6, 7, 10). In our meta-analysis, the overall eradication rate was high (approximately 90%) even in patients with clarithromycin or metronidazole *H. pylori* resistant strains, as well as in those previously treated with clarithromycin. Hence, it would appear that Pylera® could be effective in most lines of treatment meaning culture and susceptibility testing might be no longer needed leading to subsequent better cost-effectiveness of the 3-in-one capsule treatment.

The type of PPI, the dose of PPI and the duration of the treatment may theoretically influence the effects of BQT (single capsule), yet a sensitivity analysis indicated that the estimated effect of the BQT (single capsule) was robust and none of the variables assessed influenced the main outcome or the overall efficacy of this treatment. Regarding the duration of the treatment regimen, there is only one study evaluating the efficacy of Pylera® administered as a 14-day therapy, with an efficacy rate of 85%. The high success rate achieved with a 10-day administration, reaching the 90% cure rate threshold proposed in different consensus, makes it the only necessary to administer the BQT (single capsule) for this number of days.

Safety

H. pylori eradication therapy with Pylera® was in general safe and moderately tolerated. Antibiotic therapy may be associated with significant adverse reactions, especially gastrointestinal symptoms, yet in our analysis the AEs detected were

generally resolved shortly after completion of the treatment. Mild AEs were not uncommon as 43% of the patients included in the present meta-analysis experienced at least one AE, although only 3% of the individuals that received Pylera® discontinued the treatment due to an adverse reaction. Overall, this antibiotic combination tends to be safe and it falls within the usual tolerance framework of an eradication therapy with three antibiotics(10, 54).

Limitations of the study

Although the results obtained are encouraging, there are some limitations to this meta-analysis that should be taken into account. In the first place, there were only five RCTs included comparing the efficacy of Pylera® versus other treatments in naïve patients, which may be thought to reduce the overall quality of the reports evaluated as randomised designs are preferred when in search of the best medical evidence. However, *H. pylori* is a solid hard endpoint (when compared to other endpoints such as "death") and furthermore, in this systematic review confirmation of eradication was considered as an exclusion criterion if not properly performed. Therefore, the design of the included studies in this specific circumstance is clearly reduced and even open-label non-randomised studies can be trusted.

On the other hand, two of the RCTs(16, 20) compared the single capsule to the standard triple therapy (PPI, amoxicillin, clarithromycin) during 10 and 7 days respectively, differences between arms were only significant ($p<0.001$) in the study by Malfertheiner 2011 in which the 7-days triple therapy achieved a 68% eradication rate by ITT.

Additionally, two other RCTs compared the efficacy of Pylera® versus a non-bismuth quadruple therapy (sequential and concomitant therapies).(42, 43) In both studies, ITT cure rates where reported higher with the non-bismuth quadruple therapy (both sequential and concomitant), although differences between treatments where only statistically significant (p= 0.001) in the study by Tarhini 2018 (80% versus 50%). The last RCT included compared Pylera® versus a bismuth quadruple and no differences between treatment arms were reported (p=0.07)(41).

Furthermore, there were relatively few studies available on which such a sub-group meta-analyses could be performed, limiting the strength of the conclusions drawn from these sub-analyses. In addition, some of the studies lacked some relevant information regarding antibiotic-resistance, prior exposure to antibiotics or prior treatment regimens.

An additional limitation is the significant heterogeneity found in some of the analyses, implying that the efficacy in real clinical practice may vary depending on the context and target population. Thus, clinicians should not only re-evaluate treatment efficacy to ensure that the recommended thresholds (≥90%) are met but should also first and foremost stress the importance of a good compliance with any antibiotic treatment in their own practice.

A final factor that may limit the applicability of these results, and should be taken in consideration when pondering the use of this treatment in clinical practice, is that there are no available cost-effectiveness models regarding the use of Pylera®, but clinicians must bear in mind the treatment with the highest cure rate is usually also the most cost-effective therapy when costs associated with re-treatment are taken in

consideration. This needs also to be taken into account apart from drugs' local prices prior to a general shift to this regimen formulation.

Unfortunately, the aforementioned limitations are commonplace in *H. pylori* literature therapy and consequently, they will influence any meta-analysis of such studies, especially with respect to novel treatments like single capsule BQT. However, in our review no selection or publication biases were seen that might potentially compromise the robustness of the analysis. In depth revision of all the prospective studies partially compensated for the lack of randomised studies, controlling the results by analysing sub-groups, and performing sensitivity analysis that highlight the general consistency and coherence of the results.

Conclusion

In summary, the present study is the first meta-analysis carried out to date that pools and summarises the evidence available regarding the use of Pylera® to eradicate *H. pylori* infection. The results show that treatment with the single Pylera® capsule plus a PPI for 10 days represents an effective (≥90% eradication success by ITT), safe and moderately well tolerated alternative to eradicate *H. pylori* in first- and second-line treatments, irrespective of the dose or type of PPI. This high efficacy was confirmed even in those patients with clarithromycin or metronidazole resistant strains of the bacteria, and in individuals previously treated with clarithromycin.

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Statement of Interests

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Table 1. Studies evaluating the efficacy and safety of 3-in-one single capsule bismuth-containing quadruple therapy (Pylera®).

First author, Year	Publication type	Country	Design	Age	Medical condition	Treatment line	PPI[a]	Previous treatment scheme	Prior clarithromycin therapy	Efficacy (%)*	Incidence of AEs (%)
O'Morain 2003	Full article	Australia, Canada, Ireland, the Netherlands and USA	Single arm	Adults	Dyspepsia with or without ulcer disease	Naïve	O 20mg bid	NA	NA	158/170 (93)	130/177 (73)
Laine 2003	Full article	USA	Randomised	Adults	Active duodenal ulcer or a history of duodenal ulcer	Naïve	O 20mg bid	NA	NA	121/138 (88)	86/147 (59)
Malfertheiner 2011	Full article	France, Germany, Ireland, Italy, Poland, Spain and UK	Randomised	Adults	Upper gastrointestinal symptoms	Naïve	O 20mg bid	NA	NA	174/178 (98)	101/216 (47)
Salazar 2012	Full article	USA	Single arm	Adults	Asymptomatic/ mildly dyspeptic	Naïve	O	NA	NA	40/47 (85)	35/47 (75)
Delchier 2014	Full article	France, Germany, Italy and Spain	Single arm	Adults	NR	Second-line	O 20mg bid	Triple	Yes	44/47 (94)	33/49 (67)
McNicholl 2016	Abstract	Europe (31 countries)	Multiple arm	Adults	NR	Naïve, second-line and third-line	NR	NR	NR	NA[b]	24/175 (14)

First author, Year	Publication type	Country	Design	Age	Medical condition	Treatment line	PPI[a]	Previous treatment scheme	Prior clarithro mycin therapy	Efficacy (%)*	Incidence of AEs (%)
McNicholl 2016b	Full-text	Europe (31 countries)	Multiple arm	Adults	Any indication	Naïve, second-line and third-line	NR	NR	NR	376/422 (89) 259/280 (90) 323/404 (80)	NA
Muller 2016	Full article	France	Single arm	Adults	Peptic ulcer disease, non-ulcer dyspepsia	Overall, second (NR), third (NR) and fourth (NR)	O 20mg bid	Triple	Yes	85/103 (82)	56/103 (54)
Alcedo 2017	Abstract	Spain	Multiple arm	Adults	Functional dyspepsia, gastroduodenal ulcer and non-investigated dyspepsia	Naïve	O 20mg bid	NA	NA	97/113 (86)	NR
Gómez-Rodríguez 2017	Full article	Spain	Single arm	Adults	Upper gastrointestinal symptoms	Overall, Naïve and second-line	O 40mg bid	Triple or quadruple	Yes	54/58 (93) 40/41 (98) 14/17 (82)	28/58 (48)
Miehlke 2017[c]	Full article	Germany	Single arm	Adults	Non-ulcer dyspepsia and peptic ulcer disease	Overall, naïve, second and third-line	O 20mg bid	NR	NR	266/322 (83) 197/238 (83) 47/54 (87) 22/30 (73)	NR
Pérez-Arellano 2017	Full article	Spain	Single arm	Adults	Upper gastrointestinal symptoms	Overall, naïve and second-line	E 40mg bid	Triple	Yes	88/100 (88) 68/75 (91) 20/25 (80)	18/100 (18)
Fiorini 2017	Abstract	Italy	Single arm	Adults	NR	Näive	NR	NA	NA	223/245 (91)	131/245 (53)
Fiorini 2017b	Full article	Italy	Single arm	Adults	Peptic ulcer disease	Overall, second,	E 20mg bid	Triple or quadruple	Yes	94/116 (81) 39/45 (87)	71/116 (61)

First author, Year	Publication type	Country	Design	Age	Medical condition	Treatment line	PPI[a]	Previous treatment scheme	Prior clarithro mycin therapy	Efficacy (%)*	Incidence of AEs (%)
						third, fourth and ≥fifth-line				23/29 (79) 18/21 (86) 14/21 (66)	
Di Ciaula 2017	Full article	Italy	Multiple arm	Adults	Asymptomatic subjects, functional dyspepsia, previous history of peptic ulcer	Overall, naïve, second, third, fourth and fifth-line	O 20mg bid	Triple, sequential and Pylera	Yes	220/227 (97) 85/85 (100) 116/118 (98) 12/13 (92) 5/9 (56) 2/2 (100)	NR
Rodriguez de Santiago 2017	Full article	Spain	Single arm	Adults	Non-ulcerous dyspepsia, peptic ulcer disease	Third-line	E 40mg bid/ O 40mg bid	Triple	Yes	81/101 (80)	68/101 (97)
Di Mario 2017	Abstract	Italy	Single arm	NR	Dyspepsia	Overall, naïve (NR) and second-line (NR)	NR	NR	NR	124/131 (95)	35/131 (27)
Zullo 2017	Full article	Italy	Multiple arm	Adults	Non-ulcerous dyspepsia, peptic ulcer disease	Overall, Second, third and fourth and fifth-line	E 20mg bid	NR	NR	180/208 (86.5) 74/87 (85) 79/88 (90) 21/24 (87.5) 6/9 (67)	97/208 (47)
Gravina 2018	Abstract	Italy	Multiple arm	Adults	Dyspepsia	Naïve	E 40mg bid	NR	NR	53/60 (88)	21/60 (35)
Franceschi 2018	Abstract	Italy	Multiple arm	Adults	Dyspepsia	Naïve (NR) and second-line (NR)	NR	NR	NR	NR[b]	less than 10%

First author, Year	Publication type	Country	Design	Age	Medical condition	Treatment line	PPI[a]	Previous treatment scheme	Prior clarithromycin therapy	Efficacy (%)*	Incidence of AEs (%)
Ciccaglione 2018	Abstract	Italy	Multiple arm	Adults	NR	Naïve	E 40mg bid	NA	NA	10/10 (100)	0/10 (0)
Zagari 2018	Full article	Italy	Single arm	Adults	Dyspepsia, gastroesophageal reflux disease, peptic ulcer disease	Overall, naïve, second, third, fourth and fifth-line	O and R 20mg bid, L 30mg bid, E and P 40mg bid	Triple, Sequential and concomitant	Yes	339/376 (90) 202/221 (91) 112/128 (87.5) 22/24 (92) 2/2 (100) 1/1 (100)	122/376 (32)
Xie 2018	Full article	China	Randomised	Adults	Peptic ulcer disease	Naïve	O 20mg	NA	NA	166/192 (68.5)	76/186 (41)
Tursi 2018	Full article	Italy	Multiple arm	Adults	Peptic ulcer disease, gastritis	Overall, Naïve and second-line	O 20mg bid and E 40mg bid	Triple and sequential	Yes	316/349 (90.5) 136/149 (91) 180/200 (90)	55/339(16)
Fiorini 2018	Full article	Italy	Multiple arm	Adults	Dyspepsia	Naïve	E 20mg bid	NA	NA	223/245 (91)	131/230 (57)
Tarhini 2018	Full article	Lebanon	Randomised	Adults	Peptic ulcer disease, gastritis	Naïve	E 40mg bid	NA	NA	15/30 (50)	NR
De Francesco 2018	Full article	Italy	Randomised	Adults	Non-ulcerous dyspepsia, peptic ulcer disease	Naïve	E 20mg bid	NA	NA	52/61 (85)	15/61 (25)

First author, Year	Publication type	Country	Design	Age	Medical condition	Treatment line	PPI[a]	Previous treatment scheme	Prior clarithromycin therapy	Efficacy (%)*	Incidence of AEs (%)
Agudo-Fernández 2018	Full article	Spain	Multiple arm	Adults	Dyspepsia	Naïve, second, third, fourth and fifth-line	O 20mg bid	Triple	Yes	84/107 (78) 35/41 (85) 19/31 (61) 5/5 (100) 1/1 (100)	4/107 (4) 1/41 (2) 4/31 (13) 0/5 (0) 0/1 (0)
McNicholl 2018	Abstract	European countries	Multiple arm	Adults	Non-ulcerous dyspepsia, peptic ulcer disease	Naïve, second and third	NR	NR	NR	610/666 (92) 243/273 (89) 124/151 (82)	NR

*Efficacy was measured (by intention to treat) as the number of patients that achieved successful eradication relative to the number of patients treated. [a] Proton-pump-inhibitor type and dosage. O: omeprazole. E: esomeprazole. [b]Studies included in safety analysis only. [c]Miehlke et al used modified ITT for 1st, 2nd and 3rd lines respectively but initial ITT populations have been accounted within this systematic review for consistency with the remaining included studies. AE: Adverse events. NR: not reported. NA: not applicable.

Table 2: Sensitivity Analyses

Variables evaluated	Risk Difference (95% CI)
14 day treatment (n=1 study excluded)[a]	0.88 (0.86 - 0.90)
PPI at double and high doses (n=11 studies excluded)[b]	0.91 (0.86, 0.95)
PPI high-dosage only (n=7 studies excluded)[c]	0.90 (0.86, 0.93)
Clinical practice studies (non-randomised) in naïve patients	0.91 (0.88, 0.93)
Published as abstracts (n=6 excluded studies)[d]	0.88 (0.85, 0.90)

[a]The study by Salazar 2012 was the only study evaluating Pylera® for 14 days. [b]Fiorini 2017b, Zullo 2017, De Francesco 2018 and Fiorini 2018 used double doses PPI (esomeprazole 20mg bid); Gómez-Rodríguez 2017 and Rodríguez de Santiago 2017 used double dosis PPI (omeprazole 40mg bid); Rodriguez de Santiago 2017, Perez-Arellano 2017, Gravina 2018, Tarhini 2018 and Ciccaglione 2018 used high-dosage PPI (esomeprazole 40mg bid) and were also excluded from the sensitivity analyses. [c] Rodriguez de Santiago 2017, Pérez Arellano 2017, Gravina 2018, Tarhini 2018 and Ciccaglione 2018 used esomeprazole 40mg bid. Salazar 2012 and Zagari 2018 were excluded from sensitivity analyses as no dosage of the PPI given was reported [d]The studies by Alcedo 2012, McNicholl 2016b, Fiorini 2017, Gravina 2018, MchNicholl 2018, Ciccaglione 2018 were abstracts and excluded from the sensitivity analyses.

FIGURES' LEGENDS

Figure 1. PRISMA diagram

Figure 2. Overall efficacy of Pylera® in the intention-to-treat population. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Figure 3. Efficacy of Pylera® according to the medical condition: (A) peptic ulcer disease, (B) non-ulcer dyspepsia. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Figure 4. Efficacy of Pylera® in patients with bacterial antibiotic resistance to: (A) clarithromycin, (B) metronidazole. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Figure 5. Overall incidence of adverse events in Pylera®-treated patients. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. New-Castle Ottawa scale Quality assessment for non-randomized included studies.

Supplementary Table 2. Cochrane Risk of bias (RoB) tool for quality assessment in randomized included studies.

Supplementary Figure 1. Funnel plot of the overall intention-to-treat efficacy of Pylera® in any treatment line. SE, standard error.

Supplementary Figure 2. Overall efficacy of Pylera® in the per protocol population. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Supplementary Figure 3. Efficacy of Pylera® according to the medical condition: peptic ulcer disease vs non-ulcer dyspepsia. PUD, peptic ulcer disease; NUD, non-ulcer disease; CI, confidence interval; IV, inverse variance method; SE, standard error.

Supplementary Figure 4. Efficacy of Pylera® according to the type of PPI. Figure 4A. Omeprazole. Figure 4B. Esomeprazole. CI, confidence interval; IV, inverse variance method; SE, standard error.

Supplementary Figure 5. Efficacy of Pylera® when omeprazole was compared to esomeprazole in naïve patients. CI, confidence interval; IV, inverse variance method; SE, standard error.

Supplementary Figure 6. Efficacy of Pylera® in patients with dual bacterial antibiotic resistance. CI, confidence interval; IV, inverse variance method; SE, standard error.

Supplementary Figure 7. Efficacy of Pylera® as a rescue therapy after prior treatment with clarithromycin. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Supplementary Figure 8. Incidence of mild adverse events in patients treated with Pylera®. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Supplementary Figure 9. Incidence of severe adverse events in patients treated with Pylera®. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.

Supplementary Figure 10. Incidence of serious adverse events in patients treated with Pylera®. CI, Confidence interval; IV, Inverse variance method; SE, Standard error.