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CME

REVIEW

Sequential Therapy or Triple Therapy for *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis of Randomized Controlled Trials in Adults and Children

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OBJECTIVES: **Eradication rates with triple therapy (TT) for *Helicobacter pylori* infection have declined to unacceptable levels. Sequential therapy (ST) is a novel treatment that has shown promise in several controlled trials. Our aim was to assess the efficacy of ST in adults and children compared with that of TT by performing a systematic review and meta-analysis.**

METHODS: **We performed an electronic search of the following: Cochrane Trial Register (until Issue 4, 2008), MEDLINE (1966 to 21 October 2008), EMBASE (1980 to 21 October 2008), and abstracts from the major US, European, and Asian gastroenterology conferences. Randomized controlled trials (RCTs) and controlled clinical trials with a parallel group design comparing the ST with a TT lasting at least 7 days were used.**

RESULTS: **Ten RCTs enrolled 3,006 adult patients and the odds ratio (OR) for eradication of *H. pylori* with ST compared with TT was 2.99 (95% confidence interval (CI): 2.47–3.62), giving a number needed to treat (NNT) of 6 (95% CI: 5–7) favoring ST. There was no publication bias. The OR for eradication with ST compared with 10-day TT was 2.92 (95% CI: 1.95–4.38), yielding an NNT of 8 (95% CI: 6–12), favoring ST. In patients with clarithromycin resistance, the OR for eradication with ST was 10.21 (95% CI: 3.01–34.58) compared with TT, but the numbers studied are small. Three RCTs enrolled 260 children and adolescents, and the OR for eradication was 1.98 (95% CI: 0.96–4.07). There was no difference in the rate of side effects between the ST and the TT (OR, 1.01; 95% CI: 0.78–1.30).**

CONCLUSIONS: **ST appears to be better than TT in the eradication of *H. pylori*. This is a promising therapy, but further trials are needed in other European countries and North America before it can be recommended as a first-line treatment.**

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INTRODUCTION

Helicobacter pylori is a major cause of morbidity and mortality worldwide. It is the principal cause of peptic ulcer disease (PUD) and gastric cancer (1). Eradication of *H. pylori* has been shown to reverse gastric atrophy, a precursor of gastric cancer. Eradication has also been shown to prevent the recurrence of PUD and to cure some localized low-grade gastric lymphomas (1). The treatment of *H. pylori* is therefore of global significance. In recent years, the success of eradication therapies has declined, in part due to the development of anti-microbial-resistant strains (1). Current guidelines still recommend proton-pump inhibitor (PPI) triple therapy (TT) as the principal treatment to be used worldwide, but it is generally accepted that new strategies are required for the treatment (2). A 10-day sequential therapy (ST) is a novel therapeutic regimen that has shown promise in controlled trials in both adults and children (3). The aim of this systematic review and meta-analysis was to determine the therapeutic efficacy of ST compared with PPI TT in adults and children. Secondary aims were to determine (i) whether differences in eradication rates

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were present in patients with PUD and nonulcer dyspepsia (NUD); (ii) the impact of the duration of TT (7 vs. 10 days) on the comparison with ST; (iii) the effect of anti-microbial resistance on the outcome of eradication; and (iv) the side-effect profile of the two treatments.

METHODS

Studies retrieval and selection

This meta-analysis follows the QUOROM (Quality of Reporting of Meta-analyses conference) statement guidelines (4). We performed an electronic search of the following databases: The Cochrane Central Register of Controlled Trials (until Issue 4, 2008), MEDLINE (1966 to 21 October 2008), EMBASE (1980 to 21 October 2008). The search strategy for MEDLINE and EMBASE had three sets of terms: (i) for the condition of interest (*Helicobacter pylori*, *H. pylori*, *H pylori*, *Campylobacter pylori*, *C. pylori*, *C pylori*, *infection*, *dyspepsia*); (ii) for the treatment evaluated (*sequential treatment or therapy, 10 day treatment or therapy, eradication treatment or therapy*, *triple therapy*); and (iii) search strategy developed by the Cochrane Collaboration (5) for the identification of randomised controlled trials and controlled clinical trials. Sets of terms (i), (ii), and (iii) were joined together with the "AND" operator to retrieve studies. The search strategy for the Cochrane Central Register of Controlled Trials was based only on the sets of terms (i) and (ii) joined together with the "AND" operator. A manual and electronic search of the abstracts from the American Gastroenterological Association (1975–2008), United European Gastroenterology Federation (1992–2008), European Helicobacter pylori Study Group (1988–2008), Asian Pacific Digestive Week (2003–2008) was also performed. We contacted individual authors to obtain the most recent data from their studies. There were no language or age restrictions in the search strategy. We did not include review articles, position papers, editorials, commentaries, and book chapters. The selection criteria for inclusion in the meta-analysis were (i) a randomized controlled trial (RCT) or a controlled clinical trial with a parallel group design comparing the ST (i.e., PPI twice daily and amoxicillin 1 g twice daily for the first 5 days followed by PPI twice daily, clarithromycin 500 mg twice daily, and tinidazole or metronidazole twice daily for the following 5 days) to a TT (i.e., PPI twice daily, clarithromycin twice daily, and amoxicillin twice daily, or metronidazole–tinidazole (instead of clarithromycin twice daily) lasting at least 7 days; (ii) patients enrolled in the trials were required not to have been treated for *H. pylori* infection before (to avoid bias caused by treatment-induced resistance); (iii) patients in the trials were required to have a follow-up test to confirm eradication performed no sooner than 4 weeks after the completion of treatment; and (iv) intention-to-treat (ITT) analysis. Quality assessment was performed using the scale described by Jadad *et al.* (6). In this scale, the scores range from 1 to 5, with a higher score indicating higher quality. Scores are based on the method of randomization, level of blinding, concealment of allocation, and complete accounting of all randomized patients. We considered RCTs with a score of 4 or 5 to be of good quality. Two investigators (L.G. and N.V.) separately and in a double-blinded manner performed the search, selected the studies, and performed data extraction using pre-defined data extraction forms. A third investigator (D.V.) arbitrated in the event of a lack of agreement.

Outcome measure and statistical methods

The pre-defined outcome measure was the proportion of patients with successful eradication after treatment. The odds ratios (ORs) of eradicating *H. pylori* infection after ST compared with TT were pooled using the fixed effects model (7). Where significant heterogeneity was detected, a random effect model was also assessed. Subgroup analyses were performed evaluating the age of patients (patients older than 18 are reported as adults, younger patients are presented as children) and duration of TT. As it has been suggested that a poorer eradication rate is seen in patients with NUD, a comparison of eradication rates in patients with a diagnosis of NUD and PUD was also performed (8). Where the data permitted, evaluation of the impact of pre-treatment anti-microbial resistance on the outcome was calculated. We also developed pooled OR for side effects of the treatments. All the outcomes were evaluated according to ITT analysis with the exception of the analyses regarding success of eradication with resistant strains. They were based on pre-treatment anti-microbial sensitivity testing. Heterogeneity between trials was assessed by χ^2 test for heterogeneity at a significance level of $P<10$. I^2 statistic was also performed and low, moderate, and high degree of heterogeneity was considered for I^2 values of 25%, 50%, and 75%, respectively (9).

The Galbraith plot was also used to analyze heterogeneity if present. In this graph, every trial is represented as number. The graphical presentation used here is one in which estimated ORs of each trial are plotted as numbers. Every estimate has the unit standard error (SE) in the y-axis direction. Trials with large SE(Δ) fall near the origin, whereas points with small SE(Δ), that is the more informative estimates, fall well away from the origin. The x axis is also marked with numerical values of 100/SE(Δ), which is the approximate percentage relative error of Ψ. Galbraith's plot is interpreted in terms of lines through the origin. The central line represents the pooled ORs. Its 95% confidence interval (CI) is represented by a segment of the arc parallel to the scale. The outer lines delimit an area called the "homogeneity area." Studies that fall outside the homogeneity area are those where there is heterogeneity among the results. Thus, it is quite easy to judge visually which subsets of the estimates are consistent with each other (10). The reason for heterogeneity was explored by stratification analysis. Factors that were defined prospectively were the Jadad score and duration of TT. Publication bias was assessed using the funnel plot asymmetry test (11). A two-sided *P*-value of 0.05 or less was regarded as significant for all other tests performed. Proportions, their differences, and 95% CIs were calculated using the method recommended by Newcombe and Altman (12). Numbers needed to treat

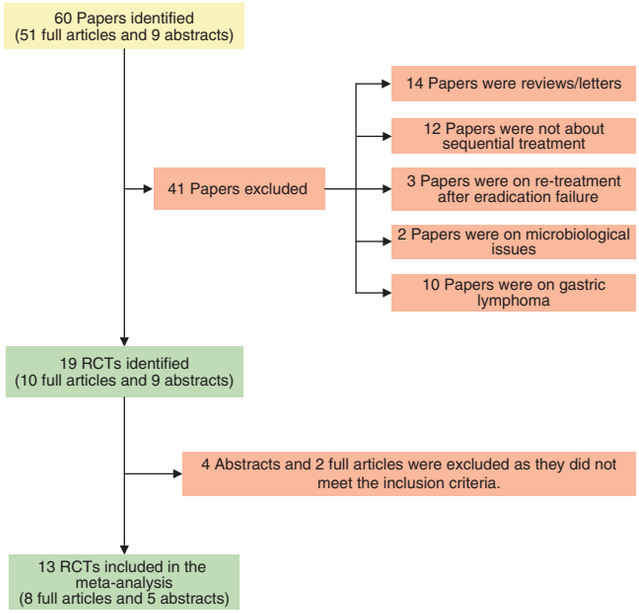

Figure 1. Meta-analysis flow. RCT, randomized controlled trial.

(NNTs) were calculated from the reciprocal of the pooled risk difference. A risk difference for each study was calculated, and then pooled using the fixed effect model as there was no evidence of heterogeneity (13). All the calculations were performed with *Meta-analysis* software (BMJ Books, Blackwell Publishing, London, UK) (14).

RESULTS

Study retrieval and inclusion

A flow diagram of the meta-analysis is shown in **Figure 1**. In all, 19 RCTs (10 articles and 9 abstracts) were identified (15–33). Four abstracts and 2 articles were excluded. Of the four abstracts, the first was excluded (20) as the data were subsequently published as a full paper (21); the second after the authors of the study informed us that larger patients sample in a later abstract included all the data that were reported in the first (15) and therefore only the latter dataset was included (16); the third because eradication assessment was performed 15 days from the end of the therapy (27); the fourth as it did not compare ST with TT (28). Of the papers, the first (30) was not included as it did not compare ST with TT; the second, was a *post hoc* analysis from an earlier published RCT (17,31). Therefore, a total of 13 studies (16–19,21–26,29,32,33) were included with a total of 3,271 patients treated. Three of these studies were performed in children: 108 patients treated with the ST and 152 with the TT (22,24,26). In one study, ST was compared with both 7- and 14-day TT in the same trial (26). The remaining 10 studies (16–19,21,23,25,29,32,33) were performed in adults, with a total of 1,400 patients treated with the ST and 1,611 with TT. Six RCTs were multi-center trials (17–19,21,23,25). Eight (16–19,21,23,29,32,33) compared the ST to TT lasting 7 days, and 4 (18,19,23,25) to TT lasting 10 days. In two studies, ST was compared with both 7 and 10 day TT at the same time (19,23). The characteristics of the studies included are shown in **Tables 1** and **2**.

Studies in adults

In all, 1,400 patients treated with ST were compared with 1,611 patients treated with TT lasting 7 or 10 days. The eradication rate was 91.0% (95% CI: 89.6–92.1) for ST and 75.7% (95% CI: 73.6–77.7) for the TT lasting 7 and 10 days, with a difference in the eradication rate of 15.3% (95% CI: 13.1–17.4). The pooled OR (**Figure 2**) with the fixed model was 2.99 (95% CI: 2.47–3.62), giving an NNT of 6 (95% CI: 5–7) favoring ST. The χ^2 test was not significant ($\chi^2 = 13.5$; degree of freedom = 9; $P = 0.13$); however, the I^2 (41%) showed a moderate degree of heterogeneity. As shown in **Figure 3**, the test for funnel plot asymmetry did not show any evidence of publication bias ($\alpha = 0.48$; 95% CI: −3.03–2.07; $P = 0.71$).

Duration of TT

In all, 1,205 patients treated with the ST were compared with 1,222 patients treated with TT lasting 7 days. The eradication rate in studies comparing ST with TT administered for 7 days was 91% (95% CI: 89.6–92.3) and 74.5% (95% CI: 72.4–76.5), respectively, with a difference in the eradication rate of 16.5% (95% CI: 14.1–19) (16,17,19,21,23,29,32,33). The pooled OR (**Figure 4**) with the random effect model was 3.22 (95% CI: 2.12–4.89), yielding an NNT of 6 (95% CI: 5–7), favoring ST. Evidence of heterogeneity was found ($\chi^2 = 14.845$; degree of freedom = 7; $P = 0.038$; $I^2 = 53\%$). The Galbraith plot showed that two studies fell outside the homogeneity area and they originated in Asia (**Figure 5**) (32,33). Only when both these studies were not included in the analysis, there was no evidence of heterogeneity ($\chi^2 = 1.349$; degree of freedom = 5; $P = 0.93$; $I^2 = 0\%$), with a pooled OR with the fixed effect model of 3.81 (95% CI: 3.02–4.80), yielding an NNT of 6 (95% CI: 5–7), favoring ST.

In all, 383 patients treated with the ST were compared with 389 patients treated with TT lasting 10 days. The eradication rate in studies comparing the ST to TT administered for 10 days was 92.4% (95% CI: 90–94.4) and 79.4% (95% CI: 75.9–83.1), respectively, with a difference in the eradication rate of 13% (95% CI: 8.9–17.1) (18,19,23,25). The pooled OR (**Figure 6**) with the fixed effect model was 2.92 (95% CI: 1.95–4.38), yielding an NNT of 8 (95% CI: 6–12), favoring ST. No evidence of heterogeneity was found ($\chi^2 = 1.49$; degree of freedom = 3; $P = 0.68$; $I^2 = 0\%$).

NUD patients

In all, 652 patients with NUD were treated with ST and 642 with TT lasting 7 days. The eradication rate was 92.2% (95% CI: 89.9–94) for ST and 73.8% (95% CI: 70.3–77.1) for TT, with a difference in the eradication rate of 18.4% (95% CI: 14.4–22.3) (16,17,19,23). The pooled OR with the fixed effect model was 3.72 (95% CI: 2.78–4.98), with an NNT of 6 (95% CI: 5–7), favoring ST. No evidence of heterogeneity was found ($\chi^2 = 2.739$; degree of freedom = 3; $P = 0.434$; $I^2 = 0\%$).

Table 1. Characteristics of the RCTs included in the meta-analysis

Reference	Country	Multi-centers	Type of publication	Population	Type of patients	No. of patients	Tests used to assess *H. pylori* status	Duration of compared therapy	Follow-up	Tests used to assess eradication	Jadad score
Focareta *et al.* (16)	Italy	No	Abstract	Adults	NUD + PUD	358	RUT	7 days	6 weeks	FT + ^{13}C-UBT	2
Zullo *et al.* (17)	Italy	Yes	Paper	Adults	NUD + PUD	1,049	^{13}C-UBT + RUT + Histo	7 days	6 weeks	^{13}C-UBT + RUT + Histo	3
De Francesco *et al.* (18)	Italy	Yes	Paper	Adults	NUD + PUD	97	^{13}C-UBT + RUT + Histo	10 days	6–8 weeks	^{13}C-UBT	3
De Francesco *et al.* (19)	Italy	Yes	Paper	Adults	NUD + PUD	347	^{13}C-UBT + RUT + Histo	7 and 10 days	6–8 weeks	^{13}C-UBT + RUT + Histo	3
Scaccianoce *et al.* (23)	Italy	Yes	Paper	Adults	NUD	213	RUT + Histo	7 and 10 days	4–6 weeks	^{13}C-UBT	3
Francavilla *et al.* (22)	Italy	No	Paper	Children	NUD + PUD	75	RUT + Histo	7 days	4 weeks and 6 months	^{13}C-UBT	3
Zullo *et al.* (21)	Italy	Yes	Paper	Adults	PUD	179	RUT + Histo	7 days	4–6 weeks	RUT + Histo	3
Vaira *et al.* (25)	Italy	Yes	Paper	Adults	NUD + PUD	300	^{13}C-UBT + RUT + Histo	10 days	4 and 8 weeks	^{13}C-UBT	5
Lerro *et al.* (24)	Italy	No	Abstract	Children	NR	50	^{13}C-UBT + RUT + Histo	7 days	6 weeks	^{13}C-UBT	1
Hurduc *et al.* (26)	Romania	No	Abstract	Children	NR	135	RUT + Histo	7 and 14 days	4–6 weeks	RUT + Histo	1
Paoluzzi *et al.* (29)	Italy	No	Abstract	Adults	NR	180	^{13}C-UBT, RUT, Histo FT*	7 days	>4 weeks	^{13}C-UBT	2
Choi *et al.* (32)	Korea	No	Paper	Adults	NR	158	Histo	7 days	8 weeks	^{13}C-UBT or ^{13}C-UBT + Histo	2
Wu *et al.* (33)	China	No	Abstract	Adults	NR	130	Histo	7 days	4–6 weeks	^{13}C-UBT	2

FAT, fecal antigen test; FT, faecal test; ^{13}C-UBT, urea breath test; Histo, histology; NR, not reported; NUD, nonulcer dyspepsia; PUD, peptic ulcer disease; RCT, randomized controlled trial.
*2 Out of the 4 tests mentioned in the table.

Table 2. Dosage and scheme of medications used in the RCTs included in the meta-analysis

Reference	Sequential treatment		Compared therapy
	First 5 days	Second 5 days	
Focareta *et al.* (16)	Esomeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Esomeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Esomeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Zullo *et al.* (17)	Rabeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
De Francesco *et al.* (18)	Rabeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
De Francesco *et al.* (19)	Rabeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Scaccianoce *et al.* (23)	Esomeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Esomeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Esomeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Francavilla *et al.* (22)	Omeprazole 1 mg/kg/day + amoxicillin 50 mg/kg/day	Omeprazole 1 mg/kg/day + clarithromycin 15 mg/kg/day + tinidazole 20 mg/kg/day	Omeprazole 1 mg/kg/day + metronidazole 15 mg/kg/day + amoxicillin 50 mg/kg/day
Zullo *et al.* (21)	Rabeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Vaira *et al.* (25)	Pantoprazole 20 mg twice daily + amoxicillin 1 g twice daily	Pantoprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg twice daily	Pantoprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Lerro *et al.* (24)	Omeprazole 1 mg/kg/day + amoxicillin 50 mg/kg/day	Omeprazole 1 mg/kg/day + clarithromycin 15 mg/kg/day + tinidazole 20 mg/kg/day	Omeprazole 1 mg/kg/day + tinidazole 20 mg/kg/day + amoxicillin 50 mg/kg/day
Hurduc *et al.* (26)	Omeprazole + amoxicillin (dosages not reported)	Omeprazole + clarithromycin + tinidazole (dosages not reported)	Proton pump inhibitor plus 2 antibiotics (dosages and type of medication not reported)
Paoluzzi *et al.* (29)	Omeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Omeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + tinidazole 500 mg	Omeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Choi *et al.* (32)	Omeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Omeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + metronidazole 500 mg	Omeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily
Wu *et al.* (33)	Rabeprazole 20 mg twice daily + amoxicillin 1 g twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + metronidazole 400 mg twice daily	Rabeprazole 20 mg twice daily + clarithromycin 500 mg twice daily + amoxicillin 1 g twice daily

RCT, randomized controlled trial.

In all, 320 patients with NUD were treated with the ST and 323 with TT lasting 10 days. The eradication rate was 92.8% (95% CI: 90.1–94.9) for ST and 76.2% (95% CI: 72–79.8) for TT, with a difference in the eradication rate of 16.6% (95% CI: 12–21.2) (18,19,23,25). The pooled OR with the fixed effect model was 3.53 (95% CI: 2.30–5.41), giving an NNT of 6 (95% CI: 5–9), favoring ST. No evidence of heterogeneity was found ($\chi^2 = 1.754$; degree of freedom = 3; $P = 0.625$; $I^2 = 0\%$).

PUD patients

In all, 321 patients with PUD were treated with the ST and 344 with the TT lasting 7 days. The eradication rate was 96% (95% CI: 93.7–97.4) for ST and 78.8% (95% CI: 74.9–82.2) for TT, with a difference in the eradication rate of 17.2% (95% CI: 13.1–21.3) (16,17,19,21). The pooled with the fixed effect model was 4.06 (95% CI: 2.29–7.23), giving an NNT of 6 (95% CI: 5–8), favoring ST. No evidence of heterogeneity was found ($\chi^2 = 0.962$; degree of freedom = 3; $P = 0.81$; $I^2 = 0\%$).

In all, 63 patients with PUD were treated with the ST and 66 with TT lasting 10 days. The eradication rate was 90.5% (95% CI: 82.6–95) for ST and 95.5% (95% CI: 87.5–98.4) for TT, with a difference in the eradication rate of −5% (95% CI: −13.3–2.8); the pooled OR with the fixed effect model was 0.50 (95% CI: 0.13–1.90), indicating no significant difference between the two treatments (18,19,25). There was no significant heterogeneity ($\chi^2 = 2.32$; degree of freedom = 2; $P = 0.314$; $I^2 = 13.8\%$).

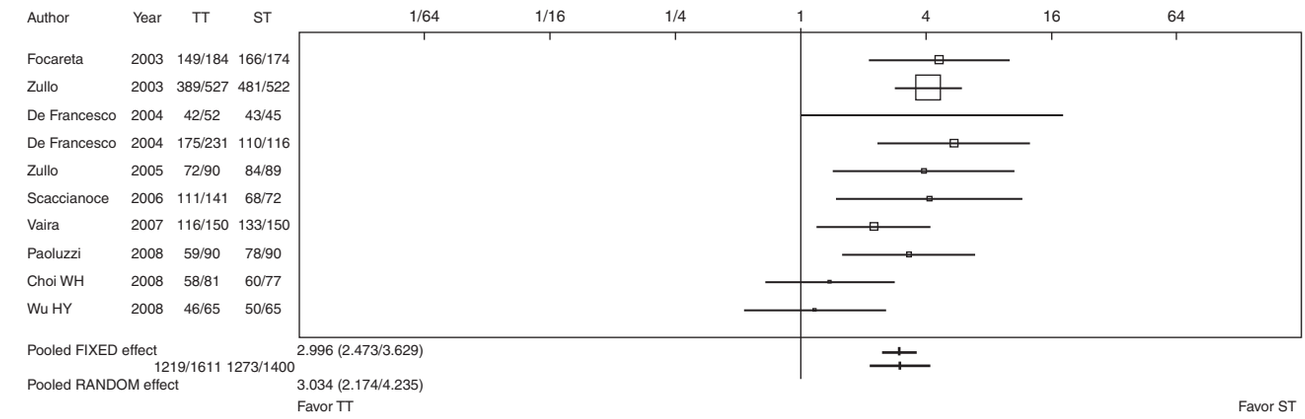

Figure 2. Forest plot of adults treated with sequential or triple therapy. Estimates of odds ratio obtained with the fixed effect model for eradicating *H. pylori* infection with their 95% confidence interval according to the intention-to-treat analysis. ST, sequential therapy; TT, triple therapy.

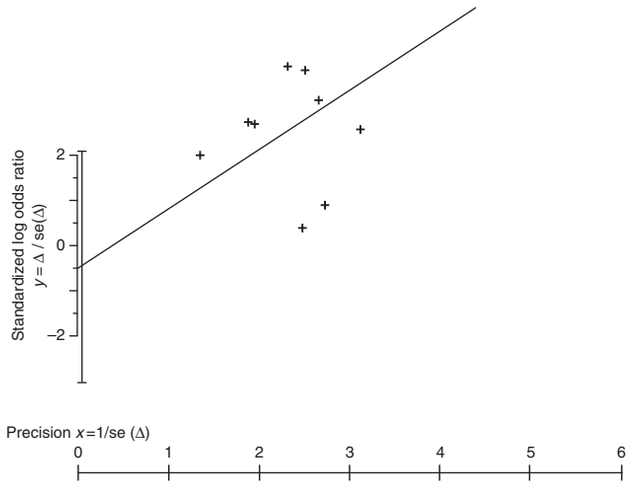

Figure 3. Test for funnel plot asymmetry in papers with an adult population ($\alpha = 0.48$; 95% CI: −3.03–2.07; $P = 0.71$).

Anti-microbial resistance

Only two RCTs (performed in adults) provided data on eradication according to pre-treatment anti-microbial susceptibility testing (17,25). In all, 18 patients harboring strains resistant to clarithromycin were treated with ST and 27 with TT. The eradication rate was 83.3% (95% CI: 60.8–94.2) for ST and 25.9% (95% CI: 13.2–44.7) for TT, with a difference in the eradication rate of 57.4% (95% CI: 37.5–83.7). The pooled OR with the fixed effect model was 10.21 (95% CI: 3.01–34.58), yielding an NNT of 2 (95% CI: 1–3) favoring ST ($\chi^2 = 0.002$; degree of freedom = 1; $P = 0.968$; $I^2 = 0\%$). In all, 71 patients harboring strains resistant to metronidazole were treated with ST and 59 the TT. The eradication rate was 95.8% (95% CI: 88.3–98.6) for sequential and 78% (95% CI: 65.9–86.6) for TT, with a difference in the eradication rate of 17.8% (95% CI: 6.4–30.2). The pooled OR with the fixed effect model was 4.70 (95% CI: 1.62–13.62) giving an NNT of 8 (95% CI: 4–34) ($\chi^2 = 0.088$; degree of freedom = 1; $P = 0.767$; $I^2 = 0\%$). Finally, 14 patients harboring strains resistant to both clarithromycin and metronidazole were treated with ST and 12 with TT. The eradication rate was 57.1% (95% CI: 32.6–78.6) for ST and 33% (95% CI: 13.8–60.9) for TT, with a difference in the eradication rate of 24.1% (95% CI: −13.1–52.8), indicating no significant difference; the pooled OR with the fixed effect model was 1.65 (95% CI: 0.278–9.80; $\chi^2 = 2.46$; degree of freedom = 1; $P = 0.117$; $I^2 = 59.3\%$). Because of the small numbers of patients with anti-microbial susceptibility testing, caution is necessary in interpreting these results.

Side effects

The rate of side effects was 13.6% (95% CI: 11.7–15.8) for ST and 12.9% (95% CI: 11.1–14.8) for TT, with a difference of 0.7% (95% CI: −2.0–3.5) (17,19,21,23,25,32,33). The pooled OR for developing side effects with the fixed effect model was 1.01 (95% CI: 0.78–1.30), indicating no significant difference. The heterogeneity test was not significant ($\chi^2 = 3.864$; degree of freedom = 6; $P = 0.7$; $I^2 = 0\%$).

Test for funnel plot asymmetry did not show any evidence of publication bias ($\alpha = 1.60$; 95% CI: −0.82–4.02; $P = 0.19$).

Children

In all, 108 children were treated with ST and 152 with TT. The eradication rate in children was 90.7% (95% CI: 83.8–94.9) for ST and 82.9% (95% CI: 76.1–88.1) for TT, with a difference in the eradication rate of 7.8% (95% CI: −0.8–15.8); the pooled OR, obtained with the fixed effect model was 1.984 (95% CI: 0.96–4.07) (22,24,26) The χ^2 test was not significant ($\chi^2 = 3.359$; degree of freedom = 2; $P = 0.186$); however, the I^2 (40%) showed a moderate degree of heterogeneity.

The test for funnel plot asymmetry did not show any evidence of publication bias ($\alpha = 4.57$; 95% CI: −0.35–9.48; $P = 0.07$).

When studies using TT for 7 days were compared with ST, the eradication rate was 90.7% (95% CI: 83.8–94.9) for ST and 78.5% (95% CI: 69.8–85.2) for TT, with a difference in the eradication rate of 12.2% (95% CI: 3.1–22.0) (22,24,26). The pooled OR (**Figure 7**), obtained with the fixed effect model was 2.53 (95% CI: 1.21–5.29), giving an NNT of 8 (95% CI: 5–32), favoring the ST. There was no significant heterogeneity

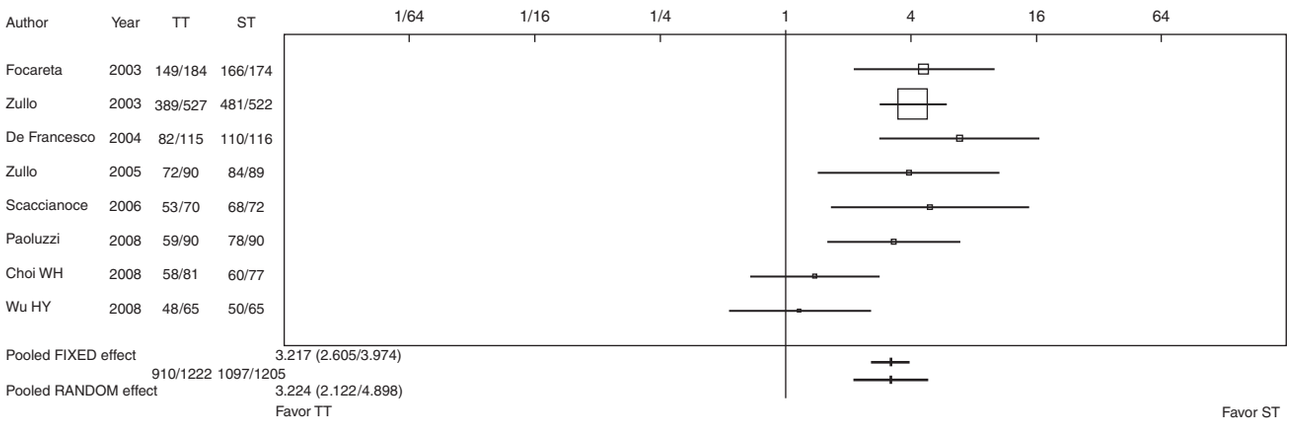

Figure 4. Forest plot of adults treated with sequential or triple therapy lasting 7 days. Estimates of odds ratio obtained with the fixed effect model for eradicating *H. pylori* infection with their 95% confidence interval according to the intention-to-treat analysis. ST, sequential therapy; TT, triple therapy.

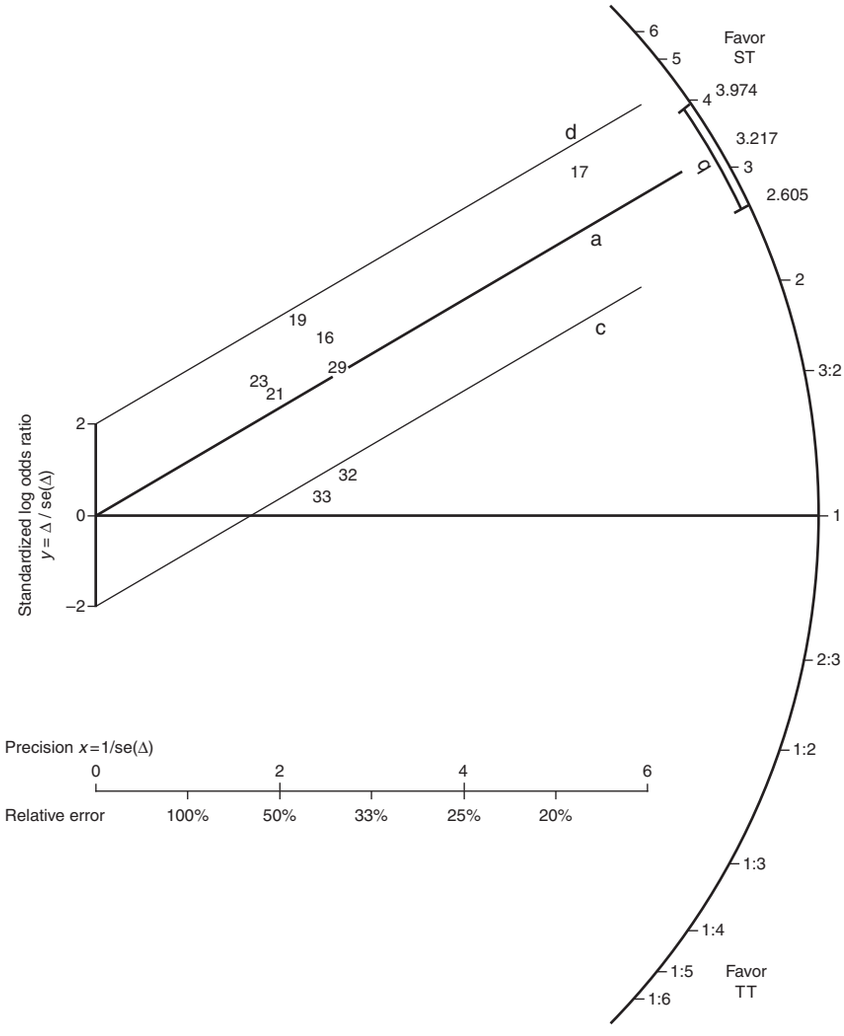

Figure 5. Meta-analysis of studies enrolling adults treated with sequential or triple therapy lasting 7 days shown as Galbraith's radial plot. Galbraith's plot is interpreted in terms of lines through the origin. The middle line represents the pooled odds ratio (*line a*). Its 95% CI is represented by a segment of the arc (*b*) parallel to the scale. The lines *c* and *d* delimit the homogeneity area. Studies shown as numbers (studies are numbered according to the References) that fall outside the homogeneity area are ones in which there is heterogeneity among the results. ST, sequential therapy; TT, triple therapy.

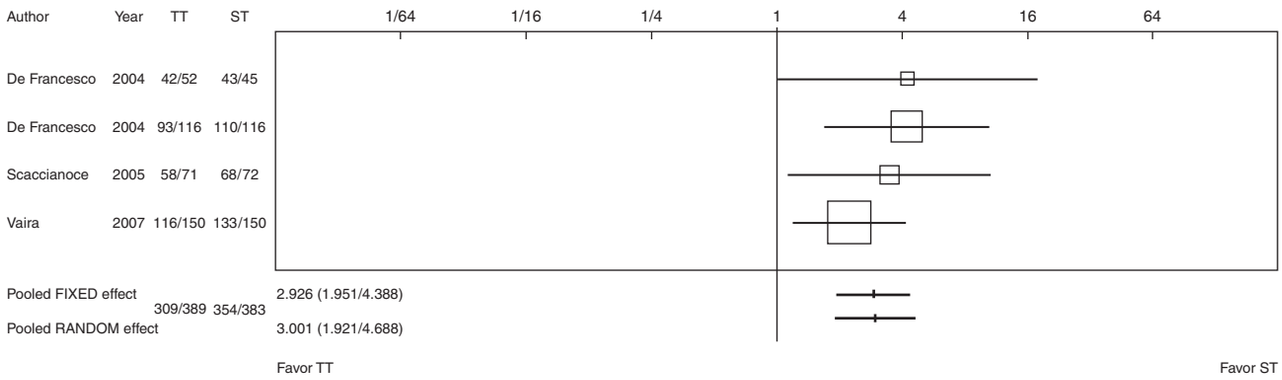

Figure 6. Forest plot of adults treated with sequential or triple therapy lasting 10 days. Estimates of odds ratio obtained with the fixed effect model for eradicating *H. pylori* infection with their 95% confidence interval according to the intention-to-treat analysis. ST, sequential therapy; TT, triple therapy.

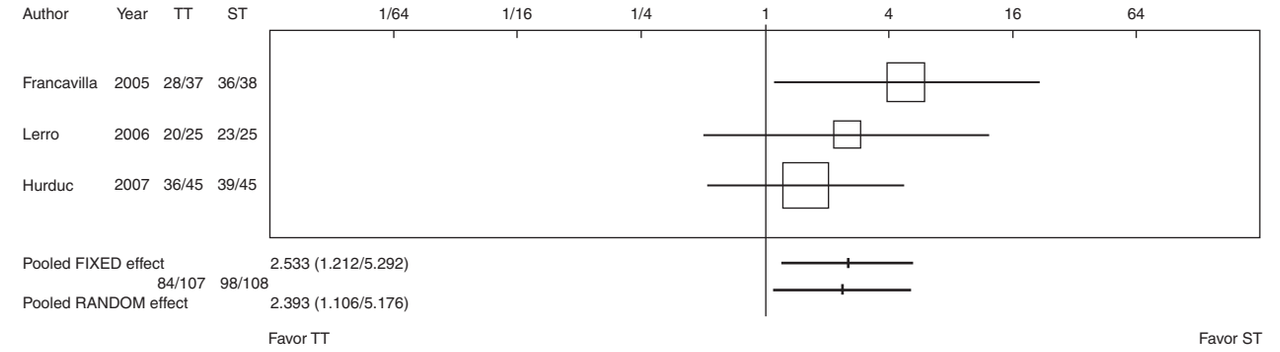

Figure 7. Forest plot of children treated with sequential or triple therapy lasting 7 days. Estimates of odds ratio obtained with the fixed effect model for eradicating *H. pylori* infection with their 95% confidence interval using intention-to-treat analysis. ST, sequential therapy; TT, triple therapy.

($\chi^2 = 1.64$; degree of freedom = 2; $P = 0.441$; $I^2 = 0\%$). In the study comparing the ST to the TT for 14 days (26), the eradication rate was 86.7% (95% CI: 73.8–93.7) for ST and 93.3% (95% CI: 82.1–97.7) for TT, with a difference in the eradication rate of −6.6% (95% CI: −20.2–6.6), indicating no significant difference. Only one study (22) reported the rate of side effects: 13.5% (95% CI: 5.9–28) with ST and 10.8% (95% CI: 4.3–24.7) with TT, with a difference of 2.7% (95% CI: −13.1–18.6), indicating no significant difference.

DISCUSSION

The success of eradication therapy for *H. pylori* has been declining and recent studies performed in the United States and Europe have shown that the eradication rate has dropped below the 85% threshold that most consensus guidelines recommend (1,2,34). Resistance rates for the commonly used anti-microbial agents in current treatment regimens have been rising and this is an important cause of treatment failure (31). A recent review has summarized the resistance rates for commonly used anti-microbial agents in different parts of the world and shows that clarithromycin resistance is a major problem in most developed countries with a few notable exceptions such as Scandinavia (1). Anti-microbial resistance is not the sole explanation for falling eradication rates. In one US trial, resistance was present in only one-third of patients who failed therapy (35). Nonadherence with treatment is a well-recognized factor in failed eradication but is difficult to measure in clinical trials. Side effects may influence adherence with treatment regimens and therefore an evaluation of side effects is important with any new therapy. In our analysis, the side-effect profile of ST was similar to that of TT. Although more anti-microbial agents are used with ST, the duration of exposure to each agent is short and this may limit the development of side effects.

The underlying disease state may also have a function in failure. Some studies have suggested that lower eradication rates are obtained when patients with NUD are treated compared with patient with PUD but other trials have not shown this effect (1,8). Several strategies have been proposed to improve eradication rates with existing therapies. It has been suggested that lengthening the duration of TT may increase the success but a recent meta-analysis failed to show any advantage for lengthening the duration of TT beyond 7 days (34). Quadruple therapy administered for 7–10 days has been proposed

as a solution for the declining eradication rates seen with TT. A large RCT compared a 7-day quadruple therapy with a 7-day TT showing similar eradication rates (82% and 78%, respectively) (36). A meta-analysis that evaluated quadruple therapy found that there was no significant difference between TT and quadruple therapy when clarithromycin resistance rates were <15%, as is currently the case in much of North America (37). Levofloxacin-based TT is another possible alternative, but the high-prevalence rates of levofloxacin-resistant strains is a cause for concern in many countries. In Italy, fluroquinolone-resistant *H. pylori* are found in 19.5% of subjects and in Germany in 22% (38,39). Few other alternatives exist. Rifabutin-based TT is used as a salvage regimen when other treatments have failed but side effects including bone-marrow toxicity limit its widespread use (40). Recent expert consensus statements and society guidelines recommend TT for the eradication in many regions of the world (1,41,42). The results of this meta-analysis, however, suggest that ST is superior to TT in the eradication in adults and that it should be given more consideration as a first-line therapy. Our analysis also suggests that the superior results with ST cannot be explained by publication bias or duration of TT against which ST was compared. However, in interpreting these positive results, it has to be remembered that most of the studies scored 3 on the Jadad scale, with only 1 good quality trial (Jadad = 5) with low risk of bias (25).

We also showed that ST may be superior in eradication in patients with NUD and in individuals with clarithromycin-resistant strains but the results may not be better in patients with PUD. As most patients treated in the Western world do not have PUD, these results apply to many Western countries (43).

The exact mechanism by which ST works is uncertain. Several possibilities exist but all remain unproven at this time. One possibility is that decreasing the bacterial density in the stomach with a drug such as amoxicillin (to which resistance is rare) with the initial 5 days of therapy, improves the efficacy of the subsequently administered combination of clarithromycin and tinidazole. It is known that bacteria can develop efflux channels for clarithromycin, which rapidly transfer the drug out of the bacterial cell, preventing binding of the antibiotic to the ribosome (31). Therefore, another possibility is that amoxicillin acts on the bacterial cell wall and weakens it in the initial phase of treatment thereby preventing the development of efflux channels by weakening the cell wall of the bacterium. A final possibility is that the sequential administration of medication has no specific benefit but that the advantage of this regimen is the number of anti-microbials to which the organism is exposed (three instead of two with the TT). The main limitation with the available data on ST is that most of the RCTs are from Italy and there are no North American studies at the present time. However, a recent study from Spain that is not a RCT (and therefore not eligible for our analysis) found comparable eradication rates as in the trials we reported as well as did another unblinded study from Taiwan that also did not meet criteria for inclusion (28,44).

We found heterogeneity when ST was compared with TT lasting 7 days. The Galbraith plot showed that Asian studies fell outside the homogeneity area (32,33). Only when both these studies were excluded from the analysis, there was no evidence of heterogeneity. We were not able to explain this by planned analysis, also because the number of the studies was small ($n = 2$). Future Asian trials may help to clarify these differences.

If in adults there are numerous reviews and several meta-analyses describing the efficacy of anti-*H. pylori* treatments, only few studies exist regarding treatment consideration in children (45,46). No meta-analysis was published earlier on the efficacy of ST in children. The only meta-analysis published in children looked at the efficacy of the triple therapies. For the TT with PPI, clarithromycin and amoxicillin lasting 1 or 2 weeks, the eradication rate ranged from 29.2% to 92%, and the incidence of side effects was 15% or higher; for the TT with PPI, nitromidazole, and amoxicillin lasting 1 or 2 weeks, the eradication rate ranged from 64.3% to 92%, but no data on side effects were reported (46).

Our results show that the ST in children eradicated the 90.7% of patients according to ITT analysis, and it was 8% more effective of triple therapies lasting 7 or 14 days. This difference failed to reach the statistical significance, but considering the CIs (95% CI: −0.8–15.8) it is reasonable to argue that a large sample size might have reached the statistical significance. When compared with triple therapies lasting only 7 days, the ST in children was the 12% (95% CI: 3.1–22.0; $P = 0.014$) significant more effective, with an NNT of 8. Side effects were reported from one study only, and there was no significant evidence of incidence between the two treatments (22). All these data seem to indicate that also in children ST might be an effective and safe first-line treatment. However, only 1 trial scored 3 on the Jadad scale (22), and more well-designed and high-quality RCTs are necessary.

Other attempts have been made to summarize data with ST (43,47). However, our analysis has several unique features compared with an earlier meta-analysis (47).

We contacted authors of the studies and were able to identify two abstracts containing overlapping data (15,16). After consultation with the authors of the abstracts, we included only one set of data (16), thereby avoiding counting the same patients twice (47). The study of De Francesco *et al.* (30) was not included in the review as it compared ST with a 5-day therapy with ranitidine bismuth citrate that cannot be considered a TT (2). Data on PUD and NUD not published earlier are included in this analysis (16,25), and also estimates of eradication rates of the ST compared with the TT lasting 7 and 10 days are reported for patients with NUD and PUD. All our analysis are based on ITT analysis, and NNT are provided to make easier to intercept the results. For the first time, results of two novel Asian RCTs are reported, and this might account for the absence of publication bias in studies enrolling adults in our results (47).

Our interpretation of data also differs from the study of Jafri *et al.* (47). For example, in two papers (19,23) patients were randomized to three arms: sequential treatment, TT lasting 7 days, and TT lasting 10 days. For these studies, we considered patients randomized both to 7 and 10 days triple therapy. Other differences concern the Jadad score: we scored 2 instead of 1 the study of Focareta *et al.* (16) as they described their study as a randomized trial, and having contacted the authors, we became aware of the method used to generate the sequence of randomization (computer-generated). Two studies of Zullo *et al.* (17,21) were scored 3 instead of 2 as they were described as a randomized trials, reported the method used to generate the sequence of randomization (computer-generated), and provided a description for withdrawals and dropouts. Finally, our review provides also data on the performance of ST in RCTs enrolling children.

In conclusion, ST appears to be better than TT in the eradication of *H. pylori*. This is a promising therapy but needs further high-quality RCTs in other European countries and in North America before it can be recommended as first line.

CONFLICT OF INTEREST

Guarantor of the article: Dino Vaira, MD.

Specific author contributions: Participation in the study conception, study design, article retrieval, data abstraction, analysis of results, and preparation and editing of the final report: Luigi Gatta and and Nimish Vakil; participation in the study conception, study design, analysis of results, and preparation and editing of the final report: Dino Vaira; participation in the statistical analysis, analysis of results, and editing of the final report: Gioacchino Leandro; participation in the preparation and editing of the final report: Francesco Di Mario.

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Potential competing interests: Nimish Vakil is a consultant for Astra-Zeneca and Axcan. He and Dino Vaira have stock ownership or options (other than mutual funds) in Meridian Bioscience.

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