Sequential therapy for *Helicobacter pylori* eradication: the time is now!

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Helicobacter pylori treatment still remains a challenge for physicians, and no current first-line therapies are able to cure the infection in all treated patients. Two very large meta-analysis studies showed that standard 7–14 days triple therapies fail to eradicate *H. pylori* infection in up to 20–25% of patients [Fuccio *et al.* 2007; Zullo *et al.* 2007]. In addition, the efficacy of these regimens is even decreasing worldwide. Indeed, during the last few years, different studies have found that the success rate following such regimens is disappointingly low, with values less than 45–60% in some countries [Gumurdulu *et al.* 2004; Vakil *et al.* 2004]. This phenomenon most likely depends on an increased bacterial resistance to antibiotics, particularly against clarithromycin – the key antibiotic in *H. pylori* treatment [Megraud 2004]. In detail, clarithromycin resistance reduces success rate of standard triple therapies to mean values as low as 18–44%. Consequently, several patients deserve two or more therapeutic attempts to cure *H. pylori* infection in clinical practice. Therefore, as well as first-line regimens, both second-line and 'rescue' therapies have been advised in the updated international guidelines for *H. pylori* management [Caselli *et al.* 2007; Malfertheiner *et al.* 2007]. However, to cure the infection following a failed initial triple therapy is particularly difficult and costly, due to the need for further therapies and diagnostic tests. In a recent study, the cumulative eradication rate was 89.6% by using three consecutive standard therapies in patients treated in clinical practice, being only 70.3%, 69.1% and 70% following first-, second-, and third-line regimens, respectively [Rokkas *et al.* 2009]. All these observations clearly suggest that more efficacious therapy regimens are required as an initial therapy for clinical practice, the best first-line treatment being still regarded as the best 'rescue' therapy [Huang and Hunt 1999].

In 2000, we conceived a novel therapeutic approach to cure *H. pylori* infection, namely a 10-day sequential therapy, which achieved a very high eradication rate [Zullo *et al.* 2000]. The sequential therapy is a simple dual therapy including a proton pump inhibitor (PPI) plus amoxicillin 1 g (both twice daily) given for the first 5 days followed by a triple therapy including a PPI, clarithromycin 500 mg, and tinidazole (all twice daily) for the remaining 5 days [Zullo *et al.* 2007]. To date, the efficacy of such a therapy regimen has been investigated in several trials overall enrolling more than 2000 patients. Based on these results, sequential therapy is now recognized as first-line therapy in the current Italian guidelines [Caselli *et al.* 2007].

How effective is sequential therapy?

To date, 22 trials on sequential therapy have been published. Following this therapy regimen, *H. pylori* eradication was achieved in 2181 out of 2388 treated patients, corresponding to an eradication rate of 91.3% [95% confidence interval (CI = 90.2–92.5) and 93.7% (95% CI = 92.7–94.7)] at 'intention to treat' (ITT) and 'per protocol' (PP) analysis, respectively (Table 1). The PPI administered did not significantly affect the eradication rate [Zullo *et al.* 2007]. On the contrary, therapy

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Table 1. Overall eradication rate at intention to treat (ITT) and per protocol (PP) analysis.

Country	Setting	Patients	Cured	ITT (%)	PP (%)	Reference
Italy	Adult	52	51	98.1	98.1	Zullo *et al.* [2000]
Italy	Adult	65	61	93.8	96.8	De Francesco *et al.* [2001]
Italy	Adult	94	90	95.7	95.7	Focareta *et al.* [2003]
Italy	Adult	522	481	92.1	95.1	Zullo *et al.* [2003]
Italy	Adult	152	142	93.4	96.6	Hassan *et al.* [2003]
Italy	Adult	174	166	95.4	95.4	Focareta *et al.* [2003]
Italy	Adult	162	151	93.2	94.4	De Francesco *et al.* [2004a]
Italy	Adult	45	43	95.6	97.7	De Francesco *et al.* [2004b]
Italy	Adult	116	110	94.8	95.6	De Francesco *et al.* [2004c]
Italy	Adult	40	38	95	97.4	Zullo *et al.* [2004]
Italy	Geriatric	89	84	94.4	96.6	Zullo *et al.* [2005]
Italy	Peadiatric	38	36	94.7	94.7	Francavilla *et al.* [2005]
Italy	Adult	72	68	94.4	97.1	Scaccianoce *et al.* [2006]
Italy	Paediatric	40	33	82.5	82.5	Lionetti *et al.* [2006]
Italy	Paediatric	25	23	92.0	92.0	Lerro *et al.* [2006]
Romania	Paediatric	45	39	86.6	86.6	Hurduc *et al.* [2007]
Italy	Adult	146	133	91.1	93.0	Vaira *et al.* [2007]
Italy	Paediatric	108	92	85.2	85.2	Francavilla *et al.* [2008]
Italy	Adult	90	78	86.7	88.6	Paoluzi *et al.* [2008]
Spain	Adult	139	117	84.2	90.7	Sanchez-Delgado *et al.* [2008]
Taiwan	Adult	66	59	89.4	98.3	Wu *et al.* [2008]
Korea	Adult	77	60	77.9	85.7	Choi *et al.* [2008]
Panama	Adult	76	65	85.5	85.5	Ruiz-Obaldia *et al.* [2008]
Total		2.433	2.220	91.2	93.7	

success changed according to the nitroimidazole type used, i.e. a therapy regimen with metronidazole giving poorer results as compared to tinidazole. Indeed, at ITT analysis, *H. pylori* infection was cured in 301 (84.1%; 95% CI = 80.3–87.9) out of 358 patients receiving a metronidazole-based regimen enrolled in four trials [Choi *et al.* 2008; Ruiz-Obaldia *et al.* 2008; Sanchez-Delgado *et al.* 2008; Wu *et al.* 2008] as compared to 1880 (97.4%; 95% CI = 91.5–93.7) out of 1930 treated with a tinidazole-based therapy ($p < 0.0001$). Most likely, such a phenomenon depends on a markedly higher half-life of tinidazole as compared with metronidazole [Lamp *et al.* 1999]. Similarly, we computed that the eradication rate achieved in those studies performed in clinical practice was significantly lower than in clinical trials (84.6% *vs* 90.9%; $p < 0.0001$). The difference rate (−6.3%) between clinical practice studies and therapeutic trials on sequential therapy would appear lower than 10% considered acceptable for standard triple therapies. Of note, at multivariate analysis, the success rate following sequential regimen was not significantly affected by those factors responsible for standard triple therapies failure, such as CagA-negative status of bacterial strains, presence of nonulcer dyspepsia, and smoking habit [De Francesco *et al.* 2004b]. These findings have been confirmed in a recent trial [Sanchez-Delgado *et al.* 2008], although a trend towards a lower eradication rate in smokers has been observed in this study.

Is sequential therapy more effective than triple therapies?

The efficacy of sequential therapy has been compared to that of standard 7–10 days triple therapies in several randomized trials. The success rate of the sequential regimen was distinctly higher than that achieved by either 7-day and 10-day standard triple therapies [Marshall 2008; Moayyedi 2007]. A comprehensive pooled-data analysis found that the sequential regimen was significantly ($p = 0.001$) superior to either 7-day and 10-day standard triple therapies in all studies were a 'head-to-head' comparison was performed, with an overall eradication rate of 93.7%, 75.9% and 79.6%, respectively [Zullo *et al.* 2007]. These results were substantially confirmed in a meta-analysis which also excluded the presence of a publication bias [Jafri *et al.* 2008]. In another recent meta-analysis, overall including 3271 patients, it was calculated that a number needed to treat (NNT) of only 6 and 8 patients favouring the sequential therapy as compared with the standard 7- or 10-day triple therapy [Gatta *et al.* 2009]. Of note was that the sequential regimen and standard triple therapies are characterised by a similar patient compliance, the incidence of side effects and therapy

interruption rate. Indeed, a good compliance (consumption of $>90\%$ of prescribed drugs) was observed in 92.6% of patients receiving the sequential regimen and in 94% of patients treated with a triple therapy. The incidence of side effects was 9.9% and 9.8%, requiring the interruption of therapy in only 0.003% and 0.007% of patients, respectively [Zullo *et al.* 2007].

Is sequential therapy effective in either children or geriatric patients?

H. pylori eradication is indicated in nonulcer dyspepsia and peptic ulcer in children [Bourke *et al.* 2005]. Moreover, younger age at acquisition has been suggested to increase the risk of developing cancer later in life [Blaser *et al.* 1995], and some trials found that *H. pylori* eradication fails to prevent gastric cancer in those adults already harbouring precancerous conditions, such as intestinal metaplasia in the stomach [Wong *et al.* 2004]. Thus, strategies to limit the burden of *H. pylori* infection and its complications are needed, and prevention by treatment of childhood *H. pylori* infection has been suggested [Francavilla *et al.* 2005]. A review of data in children reported variable eradication rates according to the different therapeutic regimens, with success rates as low as 68–75% following standard triple therapies, and prolonging the duration of these schedules does not significantly increase the efficacy [Oderda *et al.* 2007]. To date, five trials performed in children evaluated the efficacy of sequential therapy with weight-adjusted drug dosage. These studies found an eradication rate at ITT of 87.1% on 256 enrolled cases (Table 1).

As far as the efficacy of sequential therapy in geriatric patients is concerned, a trial enrolling elderly (age >65 years) patients with peptic ulcer found that the *H. pylori* eradication rate following sequential therapy was significantly higher when compared with the standard 7-day triple therapy (94.4% *vs* 80.0%; $p=0.008$) [Zullo *et al.* 2005].

Why sequential therapy is more effective than standard triple therapies

Sequential therapy was conceived based on a previous observation that the eradication rate achieved with a therapeutic strategy of initially administering 14-day dual therapy (PPI plus amoxicillin) followed by 7-day triple therapy in individuals who failed the original therapy was significantly better than the reverse sequence (7-day triple therapy as an initial strategy with 14-day dual therapy for failures) [Rinaldi *et al.* 1997]. Therefore, we hypothesised that a dual therapy followed by a triple therapy was able to eradicate the infection in a very large number of patients. We chose to reduce each treatment schedule to 5 days. Indeed, it was known that a dual therapy (PPI plus amoxicillin) administered for less than 7 days was able to achieve a cure rate of up to 50%, and that the efficacy of a triple therapy (PPI, clarithromycin and tinidazole) was inversely related to the bacterial load with higher eradication rates being achieved in those with a low bacterial density in the stomach. The use of amoxicillin – to which resistance is rare – in the initial therapeutic phase offers further advantages. 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. Because amoxicillin acts on the bacterial cell wall and damages it, the initial phase of treatment may prevent the development of efflux channels by weakening the cell wall of the bacterium. Moreover, it has been found that regimens containing amoxicillin prevent the selection of secondary clarithromycin resistance [Murakami *et al.* 2002]. Different studies have found that sequential therapy is significantly more effective than standard triple therapies in those patients infected with *H. pylori* strains harbouring primary clarithromycin resistance. In these patients, the eradication rate following sequential and triple therapies were 83.9% and 35.1%, respectively [Gatta *et al.* 2008]. In detail, the sequential therapy is successful even against those clarithromycin-resistant strains harbouring the A2143G point mutation which markedly reduces the efficacy of standard triple therapy [De Francesco *et al.* 2006]. Moreover, the cure rate following sequential therapy is not affected by the presence of isolated metronidazole resistance, whilst it seems unsuccessful in the presence of double (clarithromycin and metronidazole) resistance [Vaira *et al.* 2007].

How to treat those patients who fail sequential therapy

Despite the high efficacy of sequential therapy, some patients do fail to eradicate *H. pylori*. Data on second-line treatment in these patients have been reported in a recent study [Zullo *et al.* 2006]. In detail, a triple therapy including PPI, levofloxacin (250 mg) and amoxicillin (1 g), all administered twice for 10 days, was administered to 35 patients enrolled in two centres, achieving an 85.7% and 88.2% eradication rate at ITT and

PP, respectively. Therefore, a valid second-line therapy is available for those patients who failed the sequential therapy. Indeed, the 10-day sequential regimen as first-line and the 10-day levofloxacin-based triple therapy as second-line treatment are recognised in the updated Italian guidelines as a 'therapeutic package' for *H. pylori* management in clinical practice.

Possible limitations

The main disadvantage of the sequential regimen is that it includes amoxicillin and therefore is not suitable for patients with a penicillin allergy. However, such a limitation also applies for one of the standard triple therapies suggested in the European guidelines, which are the most currently used first-line therapy regimens in clinical practice both in Europe and the US.

Another limitation is that data regarding the efficacy of the sequential regimen mainly came from Italian studies. However, different studies have been recently performed in other geographic areas suggesting an overall good efficacy, although the use of metronidazole instead of tinidazole could reduce the eradication rate. Consequently, the introduction of tinidazole in other countries could be advisable.

Conclusions

H. pylori infection is a worldwide disease causing significant morbidity and mortality. Current international guidelines advise its eradication for several clinical conditions, including patients with nonulcer dyspepsia, peptic ulcer, gastric MALT-lymphoma, those with gastric remnant for gastric cancer and first-degree relatives of these patients, and in those patients with idiopathic thrombocytopenia. The ideal treatment – a single drug able to cure all infected patients – is not currently available and, most likely, it remains a chimera. The efficacy of currently advised 7–14 days triple therapy is decreasing worldwide with disappointing low eradication rate in several countries. Sequential therapy seems to be the most effective first-line therapy available at the moment, being distinctly superior to standard triple therapy as pointed out on more than 2300 treated patients. Indeed, the cumulative eradication rate was > 90% at ITT analysis. Some evidence demonstrates that sequential therapy is less affected by primary clarithromycin resistance as compared with triple therapies. However, as happened for the standard triple therapies for which a clear decline in efficacy has been observed in the last 10 years, it is likely that a similar phenomenon will occur for sequential therapy. A further increase in primary clarithromycin resistance – which is increasing even in children [Koletzko *et al.* 2006] – or a change in the point mutations involved in this mechanism could play a role. Therefore, the time for sequential therapy is now!

Conflict of interest statement

None declared

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