Chapter 5

The influence of *in vitro* nitroimidazole resistance on the efficacy of nitroimidazole containing anti-*Helicobacter pylori* regimens: a meta-analysis.

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Abstract.

Objective: To study the influence of nitroimidazole resistance (NIR) on the efficacy of treatment for *H. pylori* infections by meta-analysis of the world literature.

Methods: A Medline search, a hand search of all major gastroenterological journals of 1993-1997, and abstracts of gastroenterological and *H. pylori* meetings of 1993-1997 was performed. All treatment studies using a nitroimidazole, providing data about the used medication, dose frequency, total daily dose, duration of treatment, and eradication results in relation to NIR were included. Eradication had to be assessed by two biopsy based tests or a urea breath test ≥4 weeks after treatment. Individual studies were pooled into groups according to the used medication and the duration of the treatment. The pooled estimate of the odds ratio (OR) of NIR for treatment failure and its 95% confidence interval (95%CI) was calculated for each group using the logit method. To detect any possible bias, funnel plots (plots of effect estimates against sample size) were constructed.

Results: 91 treatment arms including a total of 4823 patients were evaluated. The pooled OR's of NIR for treatment failure (95% CI) of protonpump inhibitor, bismuth, and quadruple regimens were 5.2 (3.8-7.1), 5.9 (4.1-8.3), and 7.0 (3.1-16.0), respectively. Eradication rates were 90% in susceptible strains but below 75% in resistant strains. In susceptible strains neither treatment duration nor the choice of the second antibiotic influenced efficacy. In resistant strains tetracycline was more effective than amoxicillin (bismuth regimens) and the longer the duration of regimens the more effective they were (bismuth-amoxicillin regimens). Only quadruple regimens given for 1-week or more were effective in resistant strains.

Conclusions: NIR decreases treatment efficacy. Treatment duration and the choice of other drugs influence the impact of NIR on efficacy. If NIR is present a nitroimidazole-containing regimen should be avoided or a quadruple regimen should be given for more than 1-week.
Introduction.
Nitroimidazoles such as metronidazole and tinidazole are frequently used in treatment regimens for Helicobacter pylori (H. pylori) infection (1). Nitroimidazole resistance (NIR) of H. pylori, however, is very common in developing countries (2-6) and is increasingly encountered in the Western world (7-11). To what extent in vitro NIR influences the efficacy of treatment regimens containing one of these drugs is still debated. Some authors have suggested that in vitro NIR is but a laboratory phenomenon with limited or no influence on the efficacy of eradication therapy (12-15). Most investigators, however, consider the in vitro susceptibility of the infecting H. pylori strain to nitroimidazoles as one of the major factors influencing the efficacy of regimens containing one of these drugs (3,16-20). The impact of NIR on treatment efficacy may be dependent on several aspects of the treatment regimen, such as duration, the nature of the concurrently prescribed other antibiotic (usually amoxicillin, clarithromycin or tetracycline), and the other component of the regimen (either a bismuth compound or a protonpump inhibitor (PPI)). In order to clarify the significance of in vitro NIR for the outcome of the currently used treatment regimens, the world literature on this issue was evaluated by meta-analysis.

Methods.
A Medline search was performed for all clinical trials and reviews on the treatment of H. pylori infection. Furthermore, all abstracts submitted to the Digestive Disease Week, United European Gastroenterology Week, meetings of the British Society of Gastroenterology, and major H. pylori meetings of 1993-1997 were reviewed. Finally, a hand search of all the 1993-1997 issues of the main gastroenterological journals was performed and the references of all articles found were reviewed. Only studies using a nitroimidazole containing treatment regimen and providing adequate data about the used medication, dose frequency, total daily dose, duration of treatment, and eradication results in relation to nitroimidazole susceptibility were included. Eradication had to be assessed by adequate means (either two or more biopsy-based tests or a $^{13}$C or $^{14}$C-urea breath test) at least four weeks after the end of treatment. Care was taken to avoid the inclusion of covert duplicate data. As only the effect of in vitro NIR on treatment efficacy was studied, the data of the per protocol analysis (PP) were used. Individual studies were pooled into groups according to the medication used and in subgroups according to the duration of the treatment. Any
possible differences between the various bismuth compounds (bismuth subnitrate (BSN), colloidal bismuth subcitrate (CBS), and bismuth subsalicylate (BSS)), between the various PPI's (lansoprazole, omeprazole, and pantoprazole), and between the different nitroimidazoles (metronidazole, tinidazole, and ornidazole) were ignored. Furthermore, to avoid too small groups, differences in total daily dose and dosing frequency were not taken into account.

The pooled estimate of the odds ratio (OR) of NIR for treatment failure (95% confidence interval) was calculated for each group using the logit method (21,22). By this method each individual study is weighed according to its inverted variance. Thereby it is ensured that larger studies with comparable numbers of patients infected with susceptible and resistant strains have more impact on the pooled OR than smaller studies with a disproportionate ratio of patients infected with susceptible and resistant strains.

To detect any possible bias, funnel plots (plots of effect estimates against sample size) as described by Egger et al. were constructed (23). This method is based on the mathematical fact that precision in estimating the OR will increase as sample size increases. In our study the inverted variance was used instead of the actual sample size. Results of studies with a small variance will form the top of the funnel. Results of studies including only a small number of patients, with a disproportionate fraction of subjects infected with either susceptible and resistant strains will scatter at the bottom of the graph. In the absence of bias the plot will be a symmetrical inverted funnel. In the presence of bias the funnel will be skewed. Funnel plot asymmetry was analyzed by a linear regression approach, so that deviation of the intercept from zero represents bias (23). Evidence for funnel plot asymmetry was based on P < 0,1 and intercepts are presented with 90% confidence intervals (24,25).

Pooled PP eradication results were estimated in a similar way as described for the OR and are presented as percentage (95% confidence interval) (26). Differences in eradication rates were tested at a level of significance of p = 0,05.

**Results.**

**Bismuth-based triple therapy.**

Twenty studies using a classical bismuth based regimen and fulfilling the inclusion criteria were identified (27-46). These studies comprised 27 separate treatment arms, including a total number of 1691 patients. Treatment regimens consisted of a bismuth
compound (CBS in 22 treatment arms and BSN in 5 treatment arms), a nitroimidazole (metronidazole in all but three treatment arms, ornidazole in one, and tinidazole in two), and either tetracycline (18 treatment arms) or amoxicillin (9 treatment arms). Treatment duration ranged from 4 to 28 days; in 8 treatment arms the regimen was given for 7 days and in 14 it was given for 14 days or more.

Nitroimidazole susceptibility was mostly tested with a disk diffusion method; a few studies used the E-test or agar dilution.

NIR was significantly associated with treatment failure (OR: 5.9; 4.1-8.3), with an eradication rate of 89% (80-97%) in case of susceptible strains vs. 64% (52-77%) in case of resistant strains (p < 0.01). The funnel plot, however, was significantly skewed (intercept: -35 (-62 to -7.5)). This suggests a bias, by which the smaller studies are overestimating the effect of NIR as compared to the larger studies.

When the treatment arms were pooled into four subgroups (one subgroup using amoxicillin and with a treatment duration of 7 days (AMO7, n=5), one group using
tetracycline and with a treatment duration of 7 days (TET7, n=3), one group using amoxicillin and with a treatment duration of 14 days or more (AMO14, n=2), and one group using tetracycline and with a treatment duration of 14 days or more (TET14, n=12)), NIR was significantly associated with treatment failure in all subgroups (OR: 15.8 (6.9-36.2), 13.4 (5.1-35.6), 5.3 (2.3-14.8), and 3.2 (2.0-5.3) respectively). In only two subgroups (AMO7 and TET14) numbers were sufficient for the construction of funnel plots. In AMO7 the funnel plot did not suggest any bias (intercept -21 (-115 to 75)), but in TET14 the funnel plot was skewed (intercept -36 (-62 to -7.5)). Pooled eradication rates and 95% confidence intervals are summarized in figure 1. Amoxicillin and tetracycline containing regimens were equally effective in susceptible strains (eradication rate 85% (70-100%) and 90% (80-100%), p = 0.60). In resistant strains, however, tetracycline containing regimens were more effective than amoxicillin containing regimens (eradication rates 75% (60-90%) and 40% (17-63%), respectively, p < 0.01). In the same way, treatment duration had no influence on treatment efficacy in susceptible strains (AMO7: 81% vs. AMO14: 92% (p = 0.56) and TET7: 94% vs. TET14: 92% (p = 0.90), figure 1). In resistant strains, however, one-week treatment with amoxicillin was less effective than treatment given for two weeks or more (AMO7: 21% vs. AMO14: 66%, p = 0.04). Such an effect was not significant for tetracycline (TET7: 50% vs. TET14 79%, p = 0.21, figure 1).

Protonpump inhibitor-based triple therapy.

Thirty-eight studies using a PPI-based triple therapy regimen and fulfilling the inclusion criteria were found (27,47-80). In these studies 48 different treatment arms were investigated including a total of 2454 patients. The regimens consisted of a PPI, a nitroimidazole (metronidazole in 39 treatment arms, and tinidazole in 9), and either amoxicillin (28 treatment arms) or clarithromycin (20 treatment arms). In most studies the E-test was used for susceptibility testing. A minority of the studies used disk diffusion or agar dilution.

When all PPI based regimens were considered together, NIR was significantly associated with treatment failure (OR: 5.2; 3.8-7.1). Overall efficacy dropped from 93% (86-99%) in nitroimidazole susceptible strains to 69% (60%-79%) in resistant strains (p < 0.00001). The funnel plot was symmetrical (intercept -2 (-15 to 12)) suggesting no significant bias in the studies.
Figure 2. Pooled eradication rates and 95% CI in relation to nitroimidazole susceptibility in patients treated with PPI-triple therapy. AMO = amoxicillin; CLA = clarithromycin; 7, 10, and 14 = treatment duration of 7, 10, and 14 days, respectively.

Susceptible vs. resistant: AMO7: p = 0.01, AMO10: p = 0.18, AMO14: p = 0.01, CLA7: p = 0.06, CLA10: p = 0.93, CLA14: p = 0.57.

The treatment arms could be divided into six different subgroups: either amoxicillin or clarithromycin containing regimens given for 7, 10, or 14 days (AMO7, AMO10, AMO14, CLA7, CLA10, and CLA14). These subgroups contained 10, 5, 13, 16, 2, and 2 treatment arms, respectively. In all but the last two subgroups nitroimidazole resistance was significantly associated with treatment failure: OR’s were 9.6 (5.2-17.8), 4.2 (2.1-8.3), 8.7 (4.0-18.8), 3.5 (1.9-6.3), 1.7 (0.5-16.6) and 2.8 (0.5-16.6), respectively. Funnel plots in the 4 larger subgroups were symmetrical (intercept -33 (-88 to 22), -1 (-3 to 0.1), 10.2 (-27 to 48), and -6 (-29 to 17.8)).

Pooled eradication rates in the 6 subgroups are summarized in figure 2. In nitroimidazole susceptible strains neither treatment duration (AMO7 vs. AMO10: p = 0.64, AMO7 vs. AMO14: p = 0.85, AMO10 vs. AMO 14: p = 0.52, CLA7 vs CLA10: p = 0.90, CLA7 vs. CLA14: 0.81, CLA10 vs. CLA 14: p = 0.91, figure 2) nor the choice
of the second antibiotic (93% (84-100%) for all amoxicillin containing regimens vs. 92% (82-100%) for all clarithromycin containing regimens, p = 0.89) influenced efficacy. Also, in resistant strains, treatment duration had no significant influence on efficacy (AMO7 vs. AMO10: p = 0.81, AMO7 vs. AMO14: p = 0.34, AMO10 vs. AMO14: p = 0.43). The data suggest that amoxicillin is less effective than clarithromycin in resistant strains (pooled eradication rate of all amoxicillin containing regimens was 64% (50-77%) and for clarithromycin containing regimens 76% (62-90%)), but the difference failed to reach statistical significance (p = 0.18).

Only a few studies used an H2-receptor antagonist instead of a PPI and these were not included in this analysis (81-84). A reduction in efficacy in resistant strains, however, was also suggested in these studies.

Quadruple therapy.

Eight of the 15 studies using quadruple therapy and fulfilling the inclusion criteria were performed by de Boer et al (28,85-98). Sixteen different treatment arms were evaluated including a total of 678 patients.

All but 3 regimens consisted of a PPI, CBS, tetracycline, and metronidazole. One regimen used BSS instead of CBS (88), one used amoxicillin instead of tetracycline (91), and one used amoxicillin instead of tetracycline and tinidazole instead of metronidazole (97). Treatment duration varied from one to twelve days. In all but one study tetracycline and CBS were given at least four times daily. In one study, medication was given bid and this was less effective (88). This study is considered separately.

NIR was significantly associated with treatment failure (OR 7.0 (3.1-16.0)), and the funnel plot was symmetrical, be it with a large confidence interval (intercept -36 (-91 to 19)). Overall eradication rates were 91% (80-100%) in susceptible and 77% (53-100%) in resistant strains (p = 0.19). Treatment arms were pooled into two different subgroups according to treatment duration. (≤4-days (quad 4; n=8) and ≥7-days (quad 7; n=7)). OR’s were 6.4 (1.9-22.1), 4.2 (0.9-21), respectively. Funnel plots could not be constructed in the two subgroups due to the small number of studies and the small number of patients with resistant strains in some of these studies.

Efficacy increased with treatment duration in both susceptible and resistant strains, but the difference failed to reach statistical significance (quadr 7 vs. quadr 4 in susceptible
Figure 3. Pooled eradication rates and 95% CI in relation to nitroimidazole susceptibility in patients treated with quadruple therapy. Susceptible vs. resistant: 1-4 days: p = 0.23, 7-12 days: p = 0.81.

strains: p = 0.22, and in resistant strains: p = 0.17) When quadruple regimens are given for seven days or more NIR had no significant influence on efficacy (eradication rate of 92% (63-100%), figure 3). Although the bid regimen was given for ten days, metronidazole resistance decreased its efficacy from 90 to 41% (P = 0.02, Fisher's exact test) (88).

Ranitidine bismuth citrate based triple therapy.
At the time of completion of this meta-analysis there were hardly any data on the efficacy of ranitidine bismuth citrate (RBC)-based nitroimidazole-containing regimes in relation to nitroimidazole susceptibility (99). Any meta-analysis is, therefore, futile. The one available study evaluated a 1-week regimen consisting of RBC, tetracycline, and metronidazole. Efficacy was 100% in susceptible strains (31 patients successfully treated) and 57% in resistant strains (4 of 7 patients successfully treated) (p = 0.0005; Fisher's exact test).
Discussion.

Meta-analyses of clinical trials have become popular in clinical medicine (100). These meta-analyses, however, are hazardous and in recent years some of them have been contradicted by large randomized studies performed after they were published (101-103). In general, the access of data, publication bias (studies which show effect are more likely to be published than those showing no effect), multiple publication bias, poor methodological design of clinical studies, inadequate analysis of the data of these studies by the investigators, and even fraud may bias the results of a meta-analysis (23,103).

The construction of a funnel plot, as described by Egger et al. (23) is a crude method to detect any kind of such bias. In our study, significant bias could not be demonstrated, as nearly all plots were symmetrical. Therefore, although there were major variations between the individual studies, our estimation of the effect of NIR on the efficacy of the different regimens, as represented by the OR, seems reasonable. Only in the classical bismuth group the funnelplot suggested some form of bias.

Although we performed an extensive search of the literature, were watchful about possible multiple publication, weighed every study carefully, and checked them for methodological weaknesses as far as possible, some bias cannot be completely excluded. Several studies were published as abstracts only and the methodology could not be checked extensively. Furthermore, as subgroups would get too small otherwise, differences in dose and dosing frequency had to be ignored. This could have influenced our results as recent data suggest that higher doses of a nitroimidazole can, at least partly, overcome resistance (104). Also, dosing frequency could be of influence as suggested by the one study investigating a bid quadruple regimen (88). Furthermore, different methods of susceptibility testing were used and this may have influenced our results. Although agar dilution is accepted as the gold standard only 5 studies used this method for susceptibility testing. In most studies of 'classical' bismuth based triple therapy disk diffusion was used, while in the more recent studies of PPI-based triple therapy and quadruple therapy, susceptibility testing was mainly done with the E-test. In our and other laboratories these tests yield comparable results (105-107), but this has not been confirmed by other investigators (108,109). If either one of these tests systematically under – or overestimates the prevalence of NIR, this may cause significant bias when different regimens are compared (109). Finally, one could imagine that in studies showing no effect of NIR, susceptibility data were omitted from
publication. Such publication bias could be the explanation for the skewed funnel plot in the bismuth based triple treatment group. Notwithstanding these possible drawbacks, our meta-analysis shows that NIR is associated with treatment failure. Pooled OR's of PPI, bismuth, and quadruple regimens were 5.2, 5.9, and 7.0, respectively. In almost all subgroups including more than four treatment arms NIR significantly influenced efficacy.

Our findings are relevant for clinical practice. All currently used regimens have acceptable eradication rates of around 90% in susceptible strains (110,111). Eradication rates of nearly all nitroimidazole-containing regimens, however, are far below 75% in resistant strains, an exception being quadruple regimens for one week or more. Data on RBC triple therapy are still limited, but preliminary results, not included in this meta-analysis, suggest that a 1-week regimen combining RBC with clarithromycin and a nitroimidazole is also effective in resistant strains (112-114). Further evaluation of this regimen, however, is needed.

Confirming the data of other investigators (115,116), our meta-analysis shows that in classical bismuth-based triple therapy tetracycline is more effective than amoxicillin. This is, however, only apparent in nitroimidazole resistant strains.

As far as duration of the different treatment regimens is concerned, our study shows that prolongation of amoxicillin and bismuth containing treatment regimens increases the eradication rate in resistant strains. In concurrence with the data of de Boer et al. such an effect is also suggested in the quadruple regimens (16).

As the influence of NIR on the efficacy of treatment regimens is dependent on such different factors, like the nature of the other antibiotic and the duration of treatment, it is likely, that some common mechanism is involved. Probably, both factors influence the intrinsic efficacy of the regimen and it is likely that this is critical in determining whether or not NIR diminishes its efficacy. In patients with susceptible strains the influence of these factors on efficacy can not be shown due to the very high eradication rates achieved by these regimens. In nitroimidazole resistant strains, however, where one component of the regimen is less effective, these other factors are much more important in determining its efficacy. This results in lower eradication rates, when a less effective combination is used. This is consistent with the observations that also the dosage of the nitroimidazole (104), as well as the nature of the anti-ulcer drug (28) influences the effect of NIR.
In summary, this meta-analysis shows that NIR has a statistically significant and clinically relevant influence on treatment efficacy (110,117). The impact of nitroimidazole resistance seems to be dependent on the intrinsic efficacy of the regimen. In our opinion, nitroimidazole susceptibility data in the population or in the individual patient are essential to determine the appropriate treatment regimen for clinical practice. If NIR is present, as frequently occurs after treatment failure, a nitroimidazole-containing regimen should be avoided. If this is not feasible, a regimen with high intrinsic activity, like quadruple therapy given for one week or more, should be chosen. Furthermore, studies investigating the efficacy of nitroimidazole containing regimens for *H. pylori* should provide data on nitroimidazole susceptibility.

References.


110. De Boer WA, Tytgat GNJ. 90% Cure; which anti-*Helicobacter* therapy can achieve this goal? [editorial]. Am J Gastroenterol 1995; 90:1381-2.


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