January 22, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: LEVOFLOXA WJG REVISED.doc) "*Low efficacy of levofloxacin-doxycycline based third-line triple therapy in an italian population*" by Omero Alessandro Paoluzi (Number ID: 01714224), Giovanna Del Vecchio Blanco, Emanuela Visconti, Manuela Coppola, Carla Fontana, Marco Favaro, Francesco Pallone.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15949

The manuscript has been improved according to the suggestions of reviewers as follows.

Reviewer 1

1. "In conclusion of abstract, authors suggested that an antibiotic susceptibility test is mandatory to tailor a third-line therapy overcoming antibiotic resistance. However, authors did not provide this efficacy":

   R: The sentence was eliminated from the abstract

2. "Eradication rate was higher in patients assuming than in those not assuming probiotics (per-protocol analysis: 55% versus 43%, intention to treat analysis: 54% versus 40%), but not significant. This result may suggest that this study has loss of sample power. ":

   R: In agreement with reviewer suggestion, the lack of significance of eradication rates according to the probiotics intake, possibly due to a small sample size, has been highlighted in Discussion (page 13, lines 3 to 5). Indeed, the study was performed to evaluate the efficacy of a levofloxacin-doxycycline triple therapy in a group of patients not responding to ≥2 *H. pylori* eradication attempts and therefore sample size was not specifically calculated to evaluate whether probiotics addiction may be effective or not in the eradication of *H. pylori*. The finding was reported only as a suggestion to reinforce evidences already present in the literature about a possible role of probiotics in the eradication of H. pylori.

3. “In this study, resistance to clarithromycin was 31%, whereas clarithromycin was used in previous eradication therapy. Prevalence of strains with resistance to clarithromycin may be low. “:

   R: Unfortunately, only one third of our patients accepted to undergo antibiotic susceptibility culture test at baseline, and in 25% of them the test did not give reliable results. On the basis of so limited data, it was chosen to omit any speculation in the paper about the prevalence of clarithromycin resistance in our patients’ sample.

4. “Please add 95%CI in eradication rate”:

   R: 95% CI have been added in eradication rates comparison (Results, page 9 line 19 to 20).

5. Authors should demonstrate association with eradication success and infection of strain with resistance to levofloxacin.”:

   R: A comment has been added in Discussion, page 12 lines 11 to 14.

6. “As authors suggested, culture test-based eradication therapy showing to cure H. pylori infection in at least 90% of non-responders to 1 or more previous attempts. Why didn’t authors set protocol to show efficacy of tailored treatment in this study?”

   R: The original aim of the study was to perform an antibiotic susceptibility assessment by culture test prior to prescribe a third line eradication therapy with levofloxacin and doxycyline. Unfortunately, majority of patients wished to eradicate *H. pylori* infection but refused to undergo upper gastrointestinal endoscopy to perform an antibiotic susceptibility culture test. This is a paradoxical but real scenario of what happens in the clinical practice. On the other hand, Maastricht IV stated that a new eradication therapy including antibiotics not already assumed is a possible approach alternative to culture test in non-responders to two eradication therapies.

7. “It is hard to understand Table 1 to 3. Authors should re-make to be clear.”

   R: Tables have been modified according to Reviewer suggestion.

Reviewer 2

1. “This study was performed on a small number of subjects at a single center. Further studies with larger series are needed to investigate the efficacy of levofloxacin-containing regimens.” :

   R: A phrase has been added in conclusions of Abstract and in Discussion (page 13 lines 18-19).

2. “Another limitation is that the treatment failure rate of levofloxacin-containing therapy regimens was not assessed with cultures to detect antibiotic resistance or susceptibility in all treated patients. Susceptibility test failed to give reliable results in 7 cultures for contamination (four patients) or absence of bacterial growth (three patients) which is more than expected for an experienced lab. Thus, we have no full data regarding levofloxacin and/or tetracycline resistance.”:

   R: In agreement to Reviewer observation, the availability of low data on antibiotic resistance has been stressed in Discussion (page 12, lines 8-10).

3. “In what method was the eradication confirmed and what is its specificity and sensitivity in this country and ref. To assess the post-treatment eradication response two different noninvasive tests (i.e., the urea breath test and the stool antigen test) should be used.”

   R: Eradication of *H. pylori* was assessed by urease breath test, as indicated in the section *Patients and Methods*, which, according to Maastricht IV, is considered the gold standard in the assessment of *H. pylori* infection in the clinical practice and also in the clinical research. In Europe, and Italy also, sensitivity and specificity of urea breath test is about 95-100%. Majority of our patients performed also stool antigen test which was in agreement with urea breath test. The use of stool antigen test, originally omitted in the paper as considered not essential, was introduced in Patients and Methods (page 7 line 23).

4. “This prospective trial demonstrates that levofloxacin-doxycycline triple therapy regimen have low H. pylori eradication rates and is well tolerated. The levofloxacin-containing quadruple therapy is likely the best treatment option for a second-line therapy, this should be emphasized by the authors with references.”:

   R: A sentence in Discussion (Page 13, lines 16-18) and a reference have been added.

Reviewer 3

1. The study did not keep the journal’s style.

   R: The article was revised and changed according to journal style information

2. “There are several typographical errors in page 10”:

   R: The English style was revised and typographical errors have been corrected

3. “There is no data about sample size calculation”:

   R: The paper reports on our experience, in an open prospective setting investigation, with a levofloxacin-doxycycline triple therapy in a group of patients not responding to ≥2 eradication attempts. Thus, no sample size was specifically calculated as there was not any comparison between two or more regimens. Only probiotics were randomly administered in addiction to

eradication triple therapy as several suggestions in the literature report a beneficial effect in the eradication of *H. pylori*. A sentence about randomisation of probiotica has been added in Patients and Methods (Page 7, lines 17-19).

4." I do not understand the meaning of statistical analysis of table 1 (male vs. female)."

R: Table I has been changed according to Reviewer suggestion.

5. "There should be data about randomization method, study flow, table of ITT and PP analysis. Article should be written following CONSORT checklist."

R: Changes and addictions, including a Figure showing study flow diagram and a Table (Table III) reporting *H. pylori* eradication rates according to ITT and PP analysis, have been made according to Consort Checklist, as suggested by Reviewer.

Reviewer 4

1- "The patients with periodontal disease or with lingual burning and halitosis should be excluded. There is evidence that shows the recurrence of the Helicobacter pylori when it is also present in mouth. The bibliography should be cited here."

R: In agreement with reviewer suggestion, a phrase has been added in patients and methods and a reference has been added (Patients and Methods, page 6, lines 14 to 15).

2- "The levofloxacin administration is not clear in the 142 patients, having 7 of them resistant to it."

R: Results of sensitivity culture test were acquired following administration of therapy in some patients.

3- "A table showing the results of the eradication rate in patient having probiotic and non probiotic would be necessary."
R: A table was added in the manuscript.

Reviewer 5

1. "Susceptibility testing results should be more clearly stated. What kind of media were used. E-test method? Disk-diffusion method? What kind of breakpoints were used in interpretation of susceptibility testing, according to what kind of recommendations: EUCAST or NCLS?"

R: Methodology of culture test has been more diffusely described in Patients and Methods (Page 7, lines 5-15)

2. "It is surprising why so few patients (29%) agreed to undergo EGDS with culture sensitivity test. Our experience shows that patients who did not respond to two or more previous regimens are ready to do everything to be completely cured."

R: Authors completely agree with Reviewer opinion. It was really surprising that majority of patients wished to eradicate *H. pylori* infection but refused to undergo upper gastrointestinal endoscopy to perform an antibiotic susceptibility culture test. The reluctance was probably due to the knowledge that a sensitivity culture test is not an absolute warranty of success of an *H. pylori* eradication therapy. There was also an economical reason in some instances (EGDS and culture test are not free of charge in Italy).

3 References have been corrected as suggested

Clinical trial registration number was not included as the study was not registered. Ethical approval document may be furnished in about one month, together with audio core tip, if the paper will be accepted for publication.
Thanking for you attention, waiting an your kind reply, I send my

Best regards

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