

Jan. 11th, 2017

Dear Damian Garcia-Olmo, MD, PhD, Stephen Storm, PhD, Andrzej Tarnawski, DSc, MD, PhD:

**RE: "Second-line bismuth-containing quadruple therapy
for *Helicobacter pylori* eradication and impact of diabetes"**

Thank you very much for giving us an opportunity for revision.

Accurate and kind comments by the reviewer have been addressed in the Material and Methods, and Discussion. We also believe that these comments improved our manuscript. Highlight the changes have been made by changing the font to [Blue](#) in the main manuscript body in the revised manuscript to avoid any confusion.

I anticipate good response.

Thank you!

Sincerely yours,

Corresponding author

Reply to Reviewer's comments

Reviewer: 02941672

Comments to the author:

The efficacy and safety of the second-line bismuth-containing quadruple therapy for *Helicobacter pylori* eradication is interesting and the conclusions are reasonable, but I have some questions as follows.

1. The author confirmed *H. pylori* eradication by a ¹³C-urea breath test or a rapid urease test. The rapid urease test is known to have higher false negative rate comparing ¹³C-urea breath test. Is there any difference of eradication rate among these two methods?

Answer: Thank you for the accurate comment. When we analyzed the eradication rates according to the methods, there was no significant difference between two groups [93.4% (141/151, rapid urease test) vs 95.1% (327/344, ¹³C-urea breath test), *P* = 0.519]. We added these results in Discussion (page 11, line 24-27):

“We found that eradication rates based on the ¹³C-urea breath test and the rapid urease test were 95.1% (327/344) and 93.4% (141/151), respectively (*P* = 0.519). Therefore, there was no significant difference between the two methods.”

2. The ¹³C-urea breath test or rapid urease test needs to avoid taking PPI at least two weeks to prevent false negative. This matter should be mentioned in manuscript.

Answer: Thank you for your kind comment. We added the sentence as you mentioned (page 7, line 23-25):

“Afterwards, a ¹³C-urea breath test or a rapid urease test was conducted to assess *H. pylori* eradication at least 4 weeks after the treatment completion, and at least 2 weeks after cessation of PPIs or histamine (H₂) receptor antagonists.”

3. There are difference between kinds of PPI in eradication rates. The kinds of PPI and those eradication rate needs to be described.

Answer: Thank you for your accurate comment. All enrolled patients received rabeprazole 20 mg with standard-dose PPI. Therefore, we modified the sentence as you mentioned in Method (page 7, line 21-23):

“The latter was comprised of 20 mg [rabeprazole](#) twice daily, 500 mg metronidazole three times daily, 300 mg tripotassium dicitrato bismuthate, and 500 mg tetracycline four times daily for 7 days.”

Reviewer: 02520359

Comments to the author:

Interesting study in a large cohort of patients using bismuth containing therapy as second line therapy, in patients failed triple, clarithromycin containing regimen. The limitations of this study are the absence of any sensitivity data to antibiotics, the use of rapid urease test to evaluate eradication and its retrospective design. In the introduction the authors have to state that the use of the triple, clarithromycin containing regimen is given only in areas with low (<20%) resistance to clarithromycin, and that other first line regimens have emerged. They have also to give data on how many patients have tested with rapid urease test, to evaluate eradication success as this might influenced the results. Minor language polishing is needed

Answer: Thank you for the accurate comment. We added the sentence in Introduction and these limitations in Discussion as you mentioned (page 6, line 18-21; page 11, line 14-18; page 11, line 24-27):

“In general, [clarithromycin-containing therapy is recommended for first-line eradication treatment in low \(<20%\) clarithromycin resistance areas^{\[4\]}. However, the eradication rates for clarithromycin-containing triple therapy have been decreasing significantly in Korea in recent years due to increased *H. pylori* antibiotic resistance^{\[6\]}.](#)”

71.”

“In addition, antibiotic susceptibility tests were not conducted in this study. Culturing *H. pylori* is difficult, and the response rates for antibiotic susceptibility tests are relatively low. Therefore, this was hard to inspect in all enrolled patients, and there were no standard criteria for identifying antibiotic resistance^[9].”

“We found that eradication rates based on the ¹³C-urea breath test and the rapid urease test were 95.1% (327/344) and 93.4% (141/151), respectively ($P = 0.519$). Therefore, there was no significant difference between the two methods.”

Reviewer: 02954663

Comments to the author:

This is a well-designed, although retrospective study including a high number of patients. The methods used are appropriate, the statistics is sound. The difference between ITT and PP eradication rates reflects the real life, while a proportion of patients lost to follow up is high.

Please comment on followings:

- Proportion of patients with Type 1 and Type 2 diabetes and eradication results in these 2 types.

Answer: Thank you for the accurate comment. There was no type 1 diabetes in enrolled patients. A recent study in Korea, the prevalence of type 1 diabetes was 0.017% to 0.021% of the entire population in Korea. (Epidemiology of Type 1 Diabetes Mellitus in Korea through an Investigation of the National Registration Project of Type 1 Diabetes for the Reimbursement of Glucometer Strips with Additional Analyses Using Claims Data. Song SO, et al. Diabetes Metab J. 2016;40:35-45) Therefore, patients with type 1 diabetes may not be included in the current study.

- Proportion of patients loss in diabetics and non-diabetics

Answer: Thank you for the accurate comment. Among the 141 patients [Follow-up loss (n = 138) + poor compliance (n = 3)], we could analysis 126 patients (Missing

values were 15), and 9 patients (7.1%) had diabetes. The proportion of patients with diabetes is slightly low, however, we think that it doesn't affect the results of the current study. And, we'll actively consider your accurate comments in further study.

- Were antimicrobial resistance studies performed before 2nd line treatments?

Answer: Thank you for the accurate comment. Unfortunately, we didn't perform the antimicrobial resistance before 2nd line treatment, however we will try to evaluate the antimicrobial resistance in the next study. We added these limitations in Discussion as you mentioned (page 11, line 14-18):

*"In addition, the antibiotic susceptibility tests were not conducted in this study. The culture for *H. pylori* is difficult and the response rates of antibiotic susceptibility tests are relatively low, therefore, it is hard to inspect in all enrolled patients and there are no criteria for identifying antibiotic resistance⁹."*

Reviewer: 00503623

Comments to the author:

The manuscript reports on the efficacy of the seven days second-line bismuth-containing quadruple therapy for Hp eradication and the impact of diabetes. The studies were conducted with 636 patients who failed first-line therapy, and the eradication rate of Hp was assessed 4 week following the completion of the second-line quadruple therapy by the 13Curea breath test or urease assay. The results revealed that 7 days bismuth-containing quadruple therapy resulted in an 80% eradication rate, with less than 15% adverse effects frequency. Interestingly, the rate of Hp eradication failure was not affected by such factors as gender, smoking, alcohol consumption or even aspirin. However, the eradication rate was affected by diabetes mellitus, probably, as suggested, due to microcirculatory complications and resulting gastric mucosal impaired absorption of antibiotics. This study offers only lateral extension of our knowledge on Hp eradication.

The authors really appreciate the reviewer's kind and accurate comments. The revision based on these comments made this manuscript more accurate and the quality improved. Thank you again.