

Response letter

I. Response to 1st Reviewer

Reviewer's code: 00028194

Reviewer's country: United States

Date sent for review: 2015-12-22 14:19

Date reviewed: 2016-01-07 02:23

Comment: The authors do an excellent job of assessing AFI endoscopy for its utility in the evaluation of NERD versus FH. It appears to be a more detailed follow-up from their previous manuscript published in the Journal of Gastroenterology and Hepatology (Volume 29, Issue 7, pages 1442-1448, July 2014). The flow diagram explaining patient recruitment and exclusions is extremely helpful. The color images are exceptionally well done, and very instructive. The manuscript is well written. The authors did a nice job with the English language. The authors are encouraged to continue this line of work, which is of great potential importance, especially given the dramatic rise in GERD as the rate of obesity rises.

Answer: Thanks for the reviewers' favorable remarks. Our group does have further studies focusing on GERD

II. Response to 2nd Reviewer

Reviewer's code: 02567645

Reviewer's country: Italy

Date sent for review: 2015-12-22 14:19

Date reviewed: 2015-12-23 04:33

1. **Comment:** Introduction section. Please, replace "complicated diagnostic methods" with "invasive diagnostic tests".

Answer: "Complicated diagnostic methods" was replaced with "invasive diagnostic tests" as suggested.

2. **Comment:** Also, a reference here should be more than appropriated (Savarino E et al. NERD: an umbrella term including heterogeneous subpopulations Nat Rev Gastroenterol Hepatol. 2013 Jun;10(6):371-80. doi: 10.1038/nrgastro.2013.50. Epub 2013 Mar 26. and Savarino E, et al. The added value of impedance-pH monitoring to Rome III criteria in distinguishing functional heartburn from non-erosive reflux disease. Dig Liver Dis. 2011 Jul;43(7):542-7. doi: 10.1016/j.dld.2011.01.016. Epub 2011 Mar 3)

Answer: Reference 1 and 2 were added as suggested.

3. **Comment:** Introduction section. The sentence "As is known to all, NERD is characterized etiologically of acid reflux, whereas FH is not." Is somehow misleading. It is well known now that NERD may be secondary to acid and/or weakly acidic reflux as suggested by several papers

(Savarino E, et al. The role of nonacid reflux in NERD: lessons learned from impedance-pH monitoring in 150 patients off therapy. Am J Gastroenterol. 2008 Nov;103(11):2685-93. doi: 10.1111/j.1572-0241.2008.02119.x. Epub 2008 Sep 4)

Answer: We agree that not only acid reflux but also weakly acid and non-acid reflux may contribute to GERD. Impedance can help to find other kinds of reflux besides acid reflux. So we have changed “acid reflux” to be “pathologic reflux”.

4. **Comment:** Introduction section. Please, replace “classic reflux symptoms” with “typical reflux symptoms”.

Answer: “Classic reflux symptoms” was replaced with “typical reflux symptoms” as suggested.

5. **Question:** Methods section. Which rescue therapy was allowed during the wash-out period? Alginate? Magaldrate? I suppose that they undertook some kind of rescue medication (Savarino E, et al. Alginate controls heartburn in patients with erosive and non-erosive reflux disease. World J Gastroenterol. 2012 Aug 28;18(32):4371-8. doi: 10.3748/wjg.v18.i32.4371)

Answer: Thanks for the reviewer’s question. According to the protocol of ambulatory 24h pH/impedance monitoring in our hospital, patients are asked to stop PPIs during wash-out period before examination and oral antacid (Hydrotalcite Tablets) may be provided as rescue medication. In the present study, oral antacid was also allowed as rescue medication during one-month withdrawal of PPIs before endoscopy. We added one sentence to Method section “oral antacid was allowed as rescue medication during wash-out period”

6. **Question:** Do you have any data about impedance baseline levels between NERD and FH? - If yes, did the authors correlate the AFI results with baseline impedance levels? This could be an easy and very good study to perform

Answer: So far a limited number of researches have been reported regarding the application of baseline impedance analysis for the diagnosis of GERD. In China, no software regarding how to analyze baseline impedance was available on market. We asked the Sierra Scientific Instruments Inc., our supplier of AccuTrac pH-Z System, for the protocol of baseline impedance analysis but they could not provide it. Hence, no baseline impedance analysis was involved in the present study. We realize it will be an interesting work if we may have data of baseline impedance analysis. And our group will focus on it in the future study.

III. Response to 3rd Reviewer

Reviewer’s code: 00214251

Reviewer’s country: Romania

Date sent for review: 2015-12-22 14:19

Date reviewed: 2016-01-03 16:44

1. **Comment:** Authors should estimate the sample size and the risk of alpha and beta errors for this study.

Answer: As a new attempt in differentiating NERD and FH, we estimated the sample size with references to previous studies in this field at first. According to reference 19 and 20, 52-84 cases were enrolled in their studies. In another two narrow band imaging (NBI) studies concerning its application in GERD reported by Fock KM and Sharma P, 107 cases were screened(Clin Gastroenterol Hepatol. 2009 Jan;7(1):54-9; Gastroenterology. 2007 Aug;133(2):454-64).

At present, based on our data, the sample size was estimated to be 79 if $\alpha=0.05$ and $\beta=0.10$. Finally in this study 127 patients were screened and 84 patients were included. Therefore, the sample size of our study is reasonable.

2. **Comment:** Being the first research on this topic, given the limited number of subjects; the manuscript should bear the subtitle "preliminary data" or "pilot study".

Answer: Thanks for the reviewer's proposition. We added the subtitle "a pilot study" to the title. Therefore, the new title is "Autofluorescence imaging endoscopy can distinguish non-erosive reflux disease from functional heartburn: A pilot study".

3. **Comment:** The philosophy of the study is based on a single reference (3), thus it is necessary to elaborate more on the premises of this study and on the pitfalls of the method.

Answer: We agree with the reviewer's comments. Most of the studies regarding the application of AFI in the gut are targeted at cancerous or precancerous conditions such as Barrett's esophagus [10-12]. So far no similar studies to ours were reported. We realize it is important to elaborate more on the premises of this study and on the pitfalls of the method. The readers can find the premises of the study in the Discussion section, Paragraph 2. The mechanism how AFI endoscopy works rely on that changes in tissue components, regardless of whether they are caused by neoplasia or inflammation, can alter the density of autofluorescence emitted from lesions[13-15]. These findings suggest that AFI may also be useful to evaluate diseases caused by inflammation, such as esophagitis [5].

Of course, it also brings about the pitfalls of the method that AFI itself cannot differentiate inflammation from neoplasia. But it does not undermine the application of AFI in the diagnosis of NERD and FH in the study. Because tri-modal endoscopy, which combines AFI with white light imaging (WLI) and narrow band imaging is helpful to exclude gastrointestinal neoplasia, and patients with any esophageal abnormality on WLI would be excluded in this study according to our exclusion criteria. Hence, we need not worry about this pitfall.