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August 9, 2013

Dear Editor,
Please find enclosed the edited manuscript in Word format (file name: 4084-edited.doc)

Title: Validation of methods to assess potential biomarkers in pediatric patients with esophageal eosinophilia

Author: Jennifer M. Colombo, Nancy A. Neilan, Jennifer Verrill Schurman, Craig A. Friesen

Name of Journal: *World Journal of Gastrointestinal Pharmacology and Therapeutics*

ESPS Manuscript NO: 4084

The manuscript has been improved according to the suggestions of reviewer:

1. Core Tip has been added
2. Comment Section has been completed
3. Revision has been made according to suggestions of the reviewer

(1) a) We have supported our decision to limit analysis to subjects of eosinophil counts of >20 or <5 for purposes of this initial validation of methods study in the Discussion, page 11, paragraph 1, line 9. Future work will include varying degrees of eosinophil counts.

b) We have clarified the location of the biopsies in Materials and Methods section, page 5, paragraph 2, line 1.

c) We did not review any of the patients' medical records for this initial validation study as we were interested in validation of methods and not clinical correlation at this time. This is stated in the Materials and Methods section, page 5, paragraph 2, line 3. This is a valid point and a great idea for a future study.

(2) a) Legends were added to all figures.

b) In response to the reviewer's question on the clinical implication of this research, there is no immediate clinical impact of the validation study. The methodology of immunohistochemical staining to differentiate varying degrees of eosinophilia is validated in this study. Applying this method to future studies with less extreme degrees of eosinophilia is warranted. If this method can accurately distinguish eosinophilic esophagitis from reflux esophagitis on the initial biopsies, then follow-up biopsies needed to clarify the diagnosis can be eliminated. This point is emphasized in the Discussion, page 11, paragraph 2.

(3) a) We justified other equally challenging cases in children associated with esoinophilia in the Introduction, page 3, paragraph 1, line 3.

b) We discussed the challenge with identifying biomarkers in the Discussion, page 11, paragraph 1, lines 1-6.

(4) a) This reviewer has a very valid point in regards to the Gold Standard for diagnosis is biopsy and we are continuing to propose biopsying of tissue for evaluation of eosinophil granules. An ideal biomarker would be non-invasive. At this time, a non-invasive biomarker does not exist. Our study, if taken further, would eliminate the repeated biopsy that is often needed to clarify or confirm the diagnosis of eosinophilic esophagitis. This is discussed further in the Discussion, page 11, paragraph 2.

b) We do agree with this reviewer that correlation of pathology severity and disease severity needs to occur. We are planning future studies with this in mind.

c) We revised the Discussion to focus on the primary aim of the study per this reviewer's comment.

(5) a) We concluded that MBP appeared to be the most promising method for differentiating EoE and RE based on ROC curve analysis in terms of sensitivity and specificity. This was added and emphasized in the Discussion, page 13, paragraph 2, line 2.

b) We discussed the ideal biomarker and methodologies specifically using immunohistochemical staining for identifying biomarkers for eosinophilia in the Discussion, page 11, paragraph 2, line 1. We agree that this is an important topic for future studies. This initial validation study is the first step in identification of a biomarker, next we will need to correlate with disease activity and response to treatment.

4. Figures supplied are decomposable in PowerPoint format

Thank you again for considering our manuscript in the *World Journal of Gastrointestinal Pharmacology and Therapeutics*.

Sincerely yours,

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