

Dr. Ze-Mao Gong,
Science Editor, Editorial Office,
Baishideng Publishing Group Inc.

October 4, 2015

Dear Dr. Ze-Mao Gong:

Thank you very much for your e-mail of Sep 15, 2015 regarding our manuscript entitled “Recent advances in photodynamic diagnosis of gastric cancer using 5-aminolevulinic acid” by Noriaki Koizumi et al. (ESPS Manuscript No.:21841). After carefully reading your and the reviewer's comments, we have revised the manuscript by clarifying all the points suggested. We have addressed the criticisms that the reviewer raised, and the responses to the reviewer's comments are attached. We would very much appreciate it if you would consider our revised manuscript as now suitable for publication in the *World Journal of Gastroenterology*. We look forward to hearing from you at your earliest convenience.

Sincerely yours,

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RESPONSES TO REVIEWER

Comment: *This appears to be an exploratory study since there are many unresolved issues related to the unfavorable effect of tissue autofluorescence and insufficient depth penetration of excitation light. The authors need to provide detailed analysis of the usefulness of the method in many diagnostic evaluations and provide a statistical justification about the utility of the procedures. The real advantage of this technique is to detect the peritoneal dissemination and lymph node metastasis for gastric cancer patients without histological examination. More data will be necessary to validate the procedure.*

Response: Thank you very much for your helpful comments regarding our review article. We agree with your comment that we should describe the clinical usefulness of this procedure based on the statistical results of several studies in detail. We have revised our manuscript carefully in accordance with the reviewer's comment as follows.

Regarding the detection of peritoneal dissemination, we have added the following text and paragraphs to the "5-ALA-PDD FOR PERITONEAL DISSEMINATION IN GASTRIC CANCER PATIENTS" section of the manuscript:

"The authors reported that 5-ALA-PDD increased the detection rate of malignant

peritoneal nodules by 21% to 34% over white light alone." (page 9, line 23-24)

and

"Detailed analyses of animal models and clinical trials have indicated satisfactory diagnostic performance of 5-ALA-PDD in gastric cancer[15, 16, 40]. Kishi et al[40] first validated the efficacy of 5-ALA-PDD by analyzing 729 peritoneal nodules obtained from 8 mice. In that study, the detection rate with 5-ALA-PDD was 72%, which was significantly higher than that for the white light observation (39%). In addition, in a clinical trial for patients with advanced gastric cancer, Murayama et al[16] evaluated the availability of 5-ALA-PDD. The trial consisted of 13 patients, 5 of which were diagnosed with peritoneal dissemination by 5-ALA-PDD. The diagnoses were confirmed by subsequent histopathological examination. Whereas 14 peritoneal nodules with suspected dissemination were detected under white light observation, only 12 nodules showed red fluorescence by 5-ALA-PDD. Histopathological examination indicated that only those 12 nodules were disseminated. Therefore, the diagnostic accuracy of 5-ALA-PDD was concluded to be greater than that of white light imaging[16]."

"Furthermore, Kishi et al[15] demonstrated the usefulness of adding 5-ALA-PDD to conventional white light laparoscopy in another clinical trial with 52 advanced gastric cancer patients. The authors reported that, using white light observation, 24 of the 52 patients showed no macroscopic evidence of peritoneal dissemination, but when

5-ALA-PDD was used dissemination was detected in 5 of these 24 patients. Thus, the authors concluded that 5-ALA-PDD improved the sensitivity for the detection of peritoneal dissemination[15]."

"On the basis of these clinical trials, 5-ALA-PDD appears to be a useful and promising procedure for the detection of peritoneal dissemination." (page 9, line 25 - page 10, line 19)

In addition, regarding the detection of lymph node metastasis, we have added the following text to the "5-ALA-PDD FOR LYMPH NODE METASTASIS OF GASTRIC CANCER" section of the manuscript:

"... with 14 patients with advanced gastric cancer, and examined a total of 144 lymph nodes." (page 11, line 13-15)

We have also changed the following text, in order to indicate detailed results of the clinical trial:

"Through the study, we have reported its efficacy to detect metastatic lymph nodes in patients with gastric cancer with 92.4% diagnostic accuracy."

has been changed to

"Although some non-metastatic lymph nodes were also fluorescent, these nodes could be distinguished from metastasis-bearing lymph nodes by their characteristic follicular fluorescence pattern. Using a diagnostic algorithm for the fluorescence pattern, we found that 5-ALA-PDD achieved an acceptable diagnostic power, with 92.4% accuracy (133/144).

Furthermore, we performed a quantitative analysis based on the nodules' fluorescence intensities. Metastatic lymph nodes were significantly brighter than non-metastatic lymph nodes ($P < 0.0001$)[17]. Subsequent receiver operating characteristic analysis revealed 70.8% sensitivity and 94.4% specificity, with an area under the curve of 0.832. Although a sustained effort to improve the diagnostic sensitivity is required, future clinical application is strongly anticipated based on clinical results that will gradually accumulate in the near future" (page 11, line 17 - page 12, line 1)

Furthermore, we have added the following text to the " FUTURE PROSPECTS" section, in order to demonstrate recent efforts to reduce the unfavorable effect of tissue autofluorescence:

"Specifically, we demonstrated an improved diagnostic performance of 5-ALA-PDD, with 88.3% sensitivity, 92.0% specificity, and 87.4% accuracy. The receiver operating characteristic analysis revealed that the spectral unmixing method improved the area

under the curve up to 0.95, indicating that the method might be an innovative solution."

(page 12, line 22-26)