Author's response to reviews

Title: Evaluation and clinical significance of the Stomach Age model for evaluating aging of the stomach----A multicenter study in China

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Author's response to reviews:

Editorial Office of BMC Clinical Pathology
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Dear Sir,

We appreciate your consideration of this manuscript (MS: 1761542674840832 - Evaluation and clinical significance of the Stomach Age model for evaluating aging of the stomach----A multicenter study in China#) and the reviewers’ helpful suggestions.

We have revised the paper in accordance with the reviewers’ comments. The comments are addressed point by point below. We hope to have the opportunity to publish our study in BMC Clinical Pathology.

Sincerely,

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Reviewer: 1
Comments to the Author
Reviewer: Kazuhiko Inoue
Reviewer's report:
I read this article about `Stomach Age' determined by FISH examination of telomere length with interest. The evaluation of chronic atrophic gastritis and the intestinal metaplasia is important in gastric cancer screening. The authors clarified the relationship between the telomere length and chronic atrophic gastritis, and they set `real age' and 'Stomach Age'. If we can measure `Stomach Age' more easily, we may use it for gastric cancer surveillance.

The wrong use of a term: page 8; `13C urease breath testing' #13C urea breath test

Answer: Thank you for your careful checking. We have revised the paper and the error has been corrected (Page 8).

Reviewer: Junko Aida
Reviewer's report:
Major Compulsory Revisions
1. Problems of the FISH analysis
The authors analyzed at 5 regions of the biopsied gastric mucosa, however, they didn't describe which part of the mucosa they analyzed. There are differences in telomere lengths among the each cell composing gastric mucosa, i.e. foveolar epithelium, fundic or pyloric gland cells, fibroblasts etc.(Aida J, Hum Pathol 2007). The specimens of active gastritis, there should be many inflammatory cells, that have telomerase and long telomeres. So the intensity of the 'region' is considered to depend on the amount of inflammatory cells. Therefore, the reliability of the telomere length data they show, as far as they test them by a described method.

Answer: Thank you for your question. We agree with you that telomere lengths were different among the different cells which composing gastric mucosa. Pervious results indicated that telomere lengths were reduced in turn from fibroblasts to fundic gland cells, to glandular neck cells, and then to surface foveolar cells (Aida J, et al. Hum Pathol. 2007 Aug;38(8):1192-200). Although fluorescence in situ hybridization (FISH) is regard as a quantitative method, we could not examine telomere labeling for each kind of cell. The intensity of telomere labeling was the reflection of the whole mucosa. For this reason, we chose the professional image analysis software (Image-Pro Plus) to analyze the whole tissue. All the slides were divided into 5 regions automatically by the software which ensured the whole tissue was measured. The intensity of the fluorescence was utilized to reflect this. Each region of the tissue section was
examined for telomere labeling independently by two experienced technicians at different time. The results were analyzed independently, but the correlation coefficients were over 0.95. In order to obtain reproducible results, all the image acquisition were captured in the same conditions (ie, exposure times). However the repeatable results proved the reliability of the method. We have revised our paper to describe the method in detail (Page 9).

Furthermore the participants we included in our study did not have acute gastritis. So there were not a lot of inflammatory cells which may confuse the results.

2. Problems of the flow cytometry

The authors described the biopsy specimen were prepared as single cell suspensions. The biopsy specimens are not simple tissue, including gastric epithelium (foveolar epithelium, proprial gland, or goblet cells and so on), fibroblasts, vascular endothelia, inflammatory cells, and so on. Those samples are not examined histologically, so they might include tissue include inflammatory cells or fibroblasts. The method is for cultured cells, originally. Therefore, the reliability of the flow cytometric data they show, as far as they test them by a described method.

Answer: Thank you for your question. The kit we used to prepare as single cell suspensions was KeyGen tissue dissociation Kit (Cat number#KGA829 http://www.keygentec.com.cn/productdetails/163.html). According to the manufacturer’s protocol, the concentration of working solution, the time and temperature of digestion were different in different types of cell. With the use of different sizes of cell mesh, we could get the specially cell which we need. So the final suspensions were only contained epithelium cell, but no fibroblasts or inflammatory cells.

Minor Essential Revisions

1. Figure 1 and Figure 2 are not consistent with the text of the description. They should reverse them.

Answer: Thank you for your careful checking. We have revised the paper and the error has been corrected (Page 28).

2. Figure 3 legend include abbreviated word CSG. This word is the first appearance, but there isn't description the abbreviation of what words.

Answer: Thank you for your question. We have revised the paper accordingly (Page 28).

CSG stands for chronic superficial gastritis. In order to be consistent, we have changed it into CNAG (chronic non-atrophic gastritis), which was defined as any grade of inflammation with no atrophy in both the corpus and the antrum.

3. In the Figure 3, the differences are shown, but it is not clear whether it is the differences between which bar and which bar.

Answer: Thank you for your question. We have revised the Figure accordingly in order to make it clearly (Fig 3).
The m decreased in individuals whose Stomach Age was greater than their real age in younger adult group and the older adult group. But the shift in ratio in the older group was not as great as in the younger group (P < 0.05).

4. In the Figure 5, the numbers of group A, B, C, so the ratios should be described in the y-axis, not number.
   Answer: Thank you for your suggestion. We have revised the Figure accordingly by using ratios instead of number in the Y-axis (Fig 5).

5. In the Figure 6, the name of y-axis is 'Percent survival', but the percent data are not shown.
   Answer: Thank you for your question. We have revised the Figure in order to make it easy to understand (Fig 6). The Y-axis means the percent of subjects who remains in good prognosis.

6. The name of the author of the reference number #9 is error.
   Answer: Thank you for your careful checking. We have revised the paper and the error has been corrected (Page 23).

Discretionary Revisions
Discussion is too long and not so clear. What is the basis that the authors consider that CAG in young adults is quite different to the CAG in older people.
At least, the third paragraph in page 18 is too long to read and grasp the authors' meaning clearly.
Answer: Thank you for your suggestion. We have revised our paper by simplify the discussion, especially the paragraph in Page 18.