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: see over
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's report:**

**Major Compulsory Revisions**

I have pointed out that the authors didn’t describe which part of the mucosa they analyzed. Then they answered they examined whole mucosa. They used biopsy specimens for analysis, and used whole mucosa. Usual biopsy specimens from
stomach of many patients are never uniform, and various composition of many cell
groups (foveal epithelium, foveolar neck, proprial glands, fibroblasts, endothelium,
and so on). So I have considered that they should describe the composition of the
specimens. I can’t understand why they use heterogeneous whole specimens and two
person can. They commented that they divided all slides into 5 regions automatically
by the software, which ensured the whole tissue was measured. But they don’t show
the definitions of 5 regions.
Unfortunately, I can’t accept their FISH data by the described method, in a point of
the reliability.

**Answer:** Thank you for your question.

Firstly, we have answered that we agreed with you that telomere lengths were
different in different cells which composing the gastric mucosa. However the Stomach
Age is the biological age of the stomach, therefore we were not discussing whether
the epithelial cells were aging more than the endothelial cells or not, but were
analyzing the mean telomere length of the various cells which comprise one organ
together. Just like the Southern blotting using the whole DNA from the tissue to
estimate the mean telomere length, we tried to estimate the mean telomere length of
the mucosa we got from the biopsies. We have revised paper by describe the
definition of the estimated regions (Reference 1 is showed below) on Page 9.

Secondly, we admitted that in most tissue samples, where cellular heterogeneity is the
rule, different proportions of cell subtypes having different telomere lengths may
skew the results. But we tried to minimize the skewness by the DAPI signal
normalization. We revised and described the methods on Page 9-10. Telomere signals
were quantified by a method validated recently in which the sum of pixel intensities in
the Cy3/FITC channel for a given cell nucleus is normalized to the DAPI signal.
DAPI staining provides a robust measure of DNA content, being largely insensitive to
cell type, proliferation status, and degree of chromatin condensation. The normalized
Telomere signals are linearly proportional to the mean telomere length as assessed independently by Southern blotting which may minimize the skewness (References 1-5 are showed below).

Last but not least, we will keep doing the research of the Stomach Age, and will try to understand the relation between the atrophic and aging in specific cell subtypes in further study.

Reference: