Dear Editor,

Thank you for considering our manuscript, Manuscript NO.: 77387, Observational Study), for publication in the *World Journal of Gastroenterology*. We have now revised the manuscript according to your suggestions. We have not marked the ince we understand as though we should not. However, we have given exact localization of the corrected sections in the paper. We have also had it language-corrected, see certificate. We think that the manuscript has been improved after this revision, and hope that it can now be accepted for publication.

On behalf of all authors

Bodil Ohlsson, professor

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Rejection
Specific Comments to Authors: The authors set out to visualize the ENS in full thickness biopsies of the human ileum using "x-ray phase-contrast nanotomography." They examine tissue from six patients with GI dysmotility and three controls, and compare their findings to standard H&E staining. They identify several pathologic changes not seen by light microscopy and suggest that this method offers potential advantages for ENS analysis and histologic diagnosis of enteric neuropathies.

1. The image clarity and detail of the myenteric plexus provided by phase-contrast nanotomography and virtual sectioning is excellent and perhaps, as the others suggest, could help with histopathologic diagnosis of neuroenteric diseases. The study includes only a small number of patients, all with CIPO, but the clinical phenotype is not fully described. Did these patients have a known CIPO-associated mutation (e.g. ACTG2)? Was the onset in infancy or later in life? Did the histologic findings correlate in any way with their symptoms or with results of their GI workup?

Reply: You are correct that the study only included a small number of patients. This is due to that CIPO is a rare disease, and full-thickness biopsies have not been obtained from all patients. The onset of the disease was not in infancy but later in life. We did not perform any genetic analyses, so we do not know anything about their mutations. We could not find any correlations between symptoms and histological findings. Sometimes patients with severe GI symptoms have sparse histological findings, and those with milder symptoms have severe histological changes. This is discussed in the literature and may depend on that destruction of sensory nerves along with motor nerves may lead to less pain experience. Comments on this is added on page 7, the 3 last sentences of section two.

2. Many other studies have included histopathology findings of CIPO and have identified vacuolar changes, desmosis, nuclear palisading, disordered smooth muscle, intracellular inclusion bodies, and cytoplasmic aggregates. Some references include Lehtonen et al, Gastroenterology, 143:14892, 2012; Hahn et al, J Neurogastroenterol Motil, 28:104, 2022; Moreno et al, Am J Med Genet A, 170:2965, 2016. How do the findings of the current study relate to the results of those published studies?

Reply: The most published and complete publications on CIPO is summarized by the Gastro 2009 International Working Group demonstrating many cytopathological changes in the neurons apart from the muscle cells (Knowles CH et al Gut 59:882-7, 2010). The named changes are only the very few alterations among many which can be seen in the light microscope after relevant stainings.

One of the aims of the present study was to examine whether some cytopathological changes can be discovered and which ones. In this sense the current study could confirm some of the most important changes damaging the neurons such as vacuolization, autophagic vacuoles/lipofuscin, chromatolysis, and apoptosis and the presence of hyaline-body within the ganglion like those seen in the light microscope by the golden standard of various staining techniques [8]. Furthermore, p62+ sequestosome could also be observed in accordance with the findings of Alafuzoff et al [19], page 12 last sentences and page13, first sentences.

Our aim was not to analyze the myopathies or the absence of intermuscular connective tissue plate and the connective septa within the circular muscle, just to study the ganglion.

Regarding Moreon et al., that publication is considering genetic variants of CIPO. The publication briefly discussed contractile filament, α and γ actin, myosin heavy chain etc. Contractile filaments of the smooth muscle cells can be identified by immunohistochemistry and the myopathies were NOT the aim of this study. We have not performed genetic analyses because the aims were to see whether this method can be applied to the study of the neuropathology within the ENS.

Regarding Hahn et al., the description of thinner muscle layers and vacuolar changes. These findings can be observed also by the present technique. However, the aim of this publication was to describe myenteric ganglia stated in the introduction, not to describe muscular layers. As you can see in Table 1, our patients were mainly suffering from neuronal degeneration, or ganglionitis, and only secondary myopathy was present.

Regarding Lehtonen et al., a wide range of different specific staining is performed, and the reference is added to the ref list as No 21. The advantages with x-ray tomography to study large volumes of tissue may be of more importance than to stain multiple structures. Small sample areas may miss information due to discontinuous changes. Furthermore, in the future, there may be possibilities to combine x-ray scanning with immunohistochemistry or by combining x-ray scanning with FIB-SEM, page 14, last section.

3. The first sentence of the abstract is not correct since there is no submucosal plexus in the esophagus or stomach.

Reply: You are right, and this is now corrected in the first sentence of the abstract.

4. The legend in Fig. 2B states “cellular nuclei of two telocytes are seen (two dark dots).” Arrows need to be added to point out these nuclei; but more importantly, how do the authors know these are telocyte nuclei?

Reply: We suppose you mean Fig 1B. As we have explained in the method section, page 9, line 14-16, we call it telocytes when we have tissue samples of similar regions with immunostaining, ref No 17, see below. Two empty arrowheads are added according to the reviewer ś suggestion.

5. In the last paragraph of the Methods section, the authors state that telocytes, ICC, and fibroblasts cannot be differentiated without immunohistochemistry. If that's the case, why do the Results repeatedly refer to telocytes and telopodes? Could those cells instead represent ICCs or fibroblasts? Validation of the nanotomography findings with immunohistochemistry would strengthen the study.

Reply: The reviewer is right. We therefore validated the method and have published those results previously, Veress & Ohlsson 2020. We performed double immunostanings for telocytes and ICC and could clearly localize these cells in the light microscope. Therefore we think that is right to call the spindle shape cells COVERING the ganglion telocytes. If there is some distance from the ganglion in the surrounding tissue we did not call the cells by their specific names. We can also refer to the "Image analyses" in the Methods section, page 9, line 14-16.

Ref No 17, Veress B, Ohlsson B. Spatial relationship between telocytes, interstitial cells of Cajal and the enteric nervous system in the human ileum and colon. *J Cell Mol Med* 2020;**24**:3399-3406 [PMID: **31983076** DOI: 10.1111/jcmm.15013]

6. The Results section refers to Fig. 5 before Fig. 4. The order of these figures may need to be changed.

Reply: Fig 4 is mentioned before fig 5 on page 9, last section and page 19, first section, where the figures are referred to for the first time.

7. How widely available is x-ray phase-contrast nanotomography? Is this a technique that hospitals can easily employ?

Reply: X-ray phase-contrast nanotomography is an emerging technique, which is already available to international scientific user communities with relevant scientific cases, based on a peer-reviewed beamtime allocation system, at several synchrotron radiation sources, in Europe, North Americal and Asia. to name two prominent examples in Europe, the P10 beamline of the PETRAIII storage ring, and the ID16a beamline of the European Synchrotron Radiation Facility are readily available, and fully equipped instruments, where users will receive expert help form beamline scientists during their beamtime.

More relevant of hospitals is the fact, laboratory µCT sources setups are also already available from some providers (see those mentioned in the manuscript), and further significant technology transfer (including a planned spin-off company at the university of Göttingen) is currently planned to provide fully automated and user friendly instruments which can easily be operated in pathology units. Altogether, current developments will lead to a signficant dissemination of the imaging technology presented here, after a phase dominated by early adaptors.

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: Re: Manuscript NO: 77387 This is an experimental study dealing with the morphology of the myenteric plexus under normal and pathological conditions examined with a new methodological approach. The authors have a great expertise

in this field, with several publications on this specific topic. Some changes are suggested to improve the paper.

1 Title. Does the title reflect the main subject/hypothesis of the manuscript? Yes.

Reply: Thank you

2 Abstract. Does the abstract summarize and reflect the work described in the manuscript? Yes.

Reply: Thank you

3 Key words. Do the key words reflect the focus of the manuscript? Yes.

Reply: Thank you

4 Background. Does the manuscript adequately describe the background, present status and significance of the study? Yes.

Reply: Thank you

5 Methods. Does the manuscript describe methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail? Yes.

Reply: Thank you

6 Results. Are the research objectives achieved by the experiments used in this study? What are the contributions that the study has made for research progress in this field? The results fulfil the proposal of the study and provide new data on the morphology of the human myenteric plexus under normal and pathological conditions.

Reply: Thank you

7 Discussion. Does the manuscript interpret the findings adequately and appropriately, highlighting the key points concisely, clearly and logically? Are the findings and their applicability/relevance to the literature stated in a clear and definite manner? Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently? Yes.

Reply: Thank you

8 Illustrations and tables. Are the figures, diagrams and tables sufficient, good quality and appropriately illustrative of the paper contents? Do figures require labeling with arrows, asterisks etc., better legends? Yes.

Reply: Thank you

9 Biostatistics. Does the manuscript meet the requirements of biostatistics? Not applicable.

Reply: Thank you

10 Units. Does the manuscript meet the requirements of use of SI units? Yes.

Reply: Thank you

11 References. Does the manuscript cite appropriately the latest, important and authoritative references in the introduction and discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? The authors have a large experience in this topic and are appropriately self-cited.

Reply: Thank you

12 Quality of manuscript organization and presentation. Is the manuscript well, concisely and coherently organized and presented? Is the style, language and grammar accurate and appropriate? Minor style and language corrections are suggested.

Reply: We have performed language corrections, see certificate

13 Research methods and reporting. Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study. Did the author prepare the manuscript according to the appropriate research methods and reporting? Yes.

Reply: Thank you

14 Ethics statements. For all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics documents that were reviewed and approved by their local ethical review committee. Did the manuscript meet the requirements of ethics? Yes.

Reply: Thank you

Specific comments I congratulate the authors on this new morphological approach. In the discussion section I suggest a deeper comment about the comparison of classic histology with the new method. Replace “resistent” with “resistant”. Style and language improvement is suggested.

Reply: We have changed to resistant. The language has been corrected. We also have tried to further discuss the comparison with classic histology, page13, last section and page 14, first section.

1) Science editor:

1)The theme of the manuscript fall within the scope of the journal,2)no academic misconduct was found, 3) the manuscript is an experimental study dealing with the morphology of the myenteric plexus under normal and pathological conditions examined with a new methodological approach, 4) Author supplies Non-Native Speakers of English Editing Certificate, 5) Major revision.

Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade C (Good)

Reply: We have had the language corrected by a professional editing company and performed a revision of the whole manuscript.

(2) Company editor-in-chief:

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend:
https://www.wjgnet.com/bpg/gerinfo/240.

Reply: We have now separated figures and tables accordingly and have had the language corrected by a professional editing company. We have also performed a revision of the whole manuscript.