Whole Lesion Histogram Analysis of Apparent Diffusion Coefficient (ADC) Predicts Therapy Response in Locally Advanced Rectal Cancer (LARC).

Jiménez de los Santos ME *et al*. **Histogram analysis of ADC predicts therapy response.**

We thank the reviewers and editor for their valuable comments, which helped strengthen our manuscript. All comments were accepted, and the appropriate changes were made in the manuscript using "track changes." Thank you for your time and consideration of our manuscript.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: ADC histogram has the potential ability to show tumor heterogeneity. This study analyzes and evaluates its prediction ability in rectal cancer, which has certain clinical significance.

I think the subject is relatively relevant and appropriate.

Thank you very much for the positive feedback. We appreciate it.

The study have shown that ADC histogram analysis is a valuable method to distinguish the treatment response of LARC. Further research is needed to determine its clinical application.

Thank you very much for the positive feedback. We appreciate it.

1. Patient inclusion criteria "B) greater than stage T2 on pre NCRT MR

Thank you for the feedback. The paragraph was modified as follow:

…b) greater than T2 on pre-nCRT MR imaging; with or without regional lymph node metastases and no distant metastases.

2. Table 1 shows that the MR parameters of all cases are fixed values. This study retrospectively analyzed the eligible cases for 5 years and 8 months. This is a relatively long time period. It is necessary to confirm whether the MRI parameters used can be consistent or within a certain range.

Thank you very much. We really appreciate your comment. All patients are part of an internal MRI accreditation program that had been running during the last six years. Therefore, standardized and reproducible imaging protocols have been applied to obtain the best images in the evaluation of rectal cancer, but also reproducible interpretations which facilitate the widespread use of this technique. Patients included in the present work are part of a unique protocol that has proven to be the best so far.

3. "In brief, we obtained standard T2W spin-echo sequences (FSE) in axial, coronal, and sagittal directions. All of these sequences were planned perpendicular to the longitudinal axis of the tumor." Axial images can be planned perpendicular to the longitudinal axis of the tumor. How can the sagittal and coronal images be perpendicular to the longitudinal axis of the tumor?

We really appreciate your comment. To clarify our image acquisition protocol, we have added the next paragraph in the new version of the manuscript:

…In brief, we obtained standard T2W spin-echo sequences (FSE) in axial, coronal, and sagittal directions. To improve tumor tissue visualization (including the

delineation of muscular layer), these planes were planed perpendicular to the main
axis of the tumor.

4. "Eight four patients" should be "forty eight patients".

   Thank you for the comment. We are submitting the corrected
   manuscript with the suggestion incorporated.

5. "TRG Ryan classification" please provide specific classification indicators.

   In this work, we used the tumor regression grading system according to the
   Ryan score, which classified patients according to four-point grades based
   on residual tumor and fibrosis. TRG Ryan classification is described as
   follow in the new version of the manuscript:

   The pathological response of primary tumor was estimated using the modified
   Ryan's as follows: TRG 0, complete response with no viable cancer cells; TRG1
   moderate response with single cancer cells or small groups of cancer cells; TRG2,
   minimal response with residual cancer outgrown by fibrosis, and TRG3, poor
   response with minimal or no tumor killing and extensive residual cancer. Only
   patients with TRG0 were considered as complete responders (R)

6. "2.4 Statistical analysis"should be"**2.5** Statistical analysis"。

   Thank you for bringing this point to our attention.

7. "Post CRT" and "post NCRT" appear many times in the text, which should
   be consistent.

   Thank you for the comment. We have scrupulously revised the new version
   of the manuscript to avoiding errors related with abbreviations.

Thank you for bringing this point to our attention. As we stated in figure 2, ROIs were drawn manually slice by slice on DWI images along the edge of the lesion to cover as much tumor area as possible without excluding cystic, fibrotic or necrotic areas.

We really appreciate your comment. To clarify our sample size, we have included the next paragraph in the new version of the manuscript:
Among 58 patients originally included in the present study, 10 had severe imaging artifacts. Thus, our final sample included 48 patients whose clinical and pathological characteristics are described in table 2.

Thank you for the comment. Data was recalculated and reconfirmed. Really sorry for the mistakes we made due to frequent modification of manuscript before submission.

Thank you for bringing this point to our attention. This is not the case of a radiomic study. We apologize for any confusion being caused by the "title" of the first version of our manuscript. However, we sustain that the results extracted from the present study can be further used to develop computational models using advanced machine learning algorithms.

2. It is necessary to simplify the results.

We appreciated the comment. Results have been re-organized and simplified in the new version of the manuscript.

3.I couldn't understand why the word in the title "Radiomics" was only used twice in the text.

Histograms is a basic concept from mathematics, which has been amply used in more complex mathematical models to predict treatment response in several types of cancer, such as radiomics. However, strictly, this is not a work of radiomic. Sorry for the confusion, but on literature search for histogram analysis on radiology related publications, parameters such a kurtosis, skewness, entropy, etc., are well accepted as high-order statistical radiomics features

4. I don't think A2 and B2 in Figure 2 are really demonstrative. Why was the hypointense area between 5-8 o'clock included in figure A2? Figure B2 includes hyperintense focus for a reason.

Thank you very much for the feedback. The case presented in figure 2 was change to a more representative one. The new figure is presents as follow.
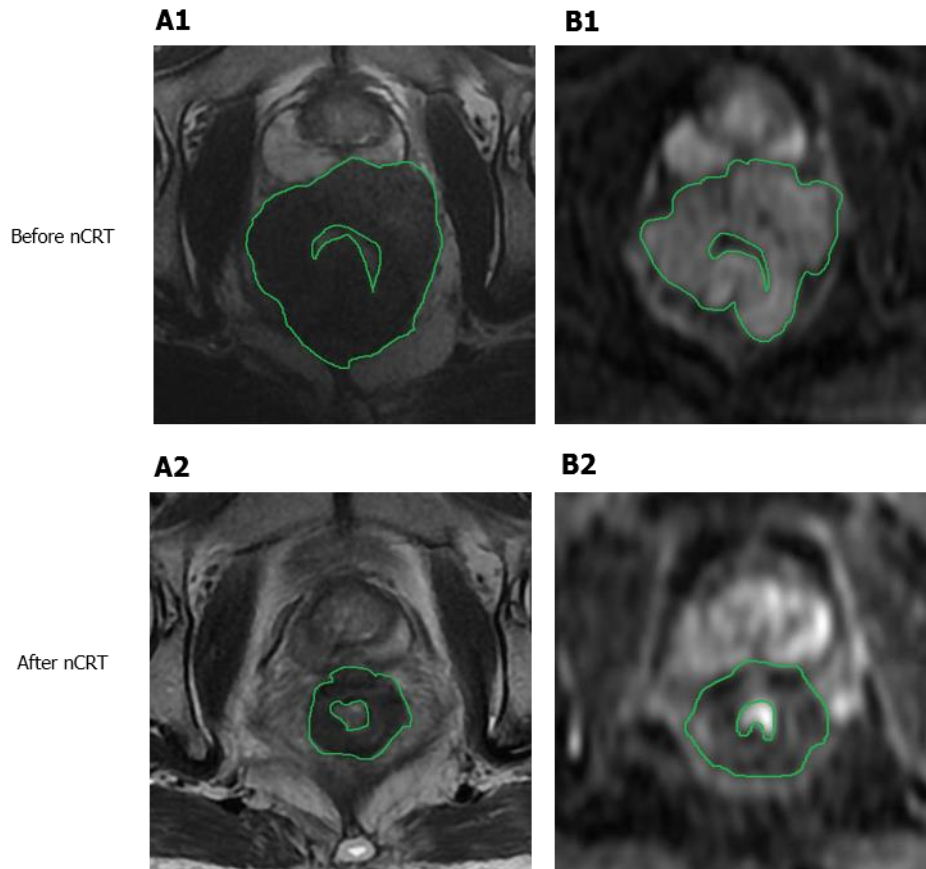
Figure 2. Images of rectal tumor before (A1-B1) and after (A2-B2) nCRT. A) T2-weighted MR images (T2WI) obtained in 67-year-old man with rectal tumor (histopathologic response Ryan 1) to evaluate tumor volume. B) Diffusion-weighted images (DWI) that were obtained from the same patient. As we can see in the present case, ROIs were drawn manually slice by slice on DWI images along the edge of the lesion to cover as much tumor area as possible without excluding cystic, fibrotic or necrotic areas.

Title: The main idea of using volumetric ADC and Histogram analysis is not clearly stated on the title

Abstract: The words exceeds of ==250 words==. please add the conclusion

We appreciate your comment. The conclusion has been added in the new version of the manuscript. Moreover, the abstract was adapted accordingly with the author's instructions.

Introduction: The main idea is clearly described

We appreciate your comment. Thankyou very much.

Methods: Detail but some unnecessary information mentioned resulted in too long paragraphs to methods section Results/

We appreciated the comment. The methods have been simplified in the new version of the manuscript.

DIscussion : Too many and complicated tables , make the results is difficult to be followed.

We appreciated the comment. Both results and discussion have been simplified in the new version of the manuscript.

The result is weak seen only few patients (18 patients / 36%) achieved a histopathologic complete response. It's still questionable for the larger percentage of pts who did not have the comparable histopathologic proven result. The variety of pts tumor staging might interfere the results of the nonresponden group

We agree that our sample of responders are small; however, these patients have a complete pathological response. Several authors have groups partial and complete responders to analyze their data, expanding their sample, but also the standard deviations of the mean values. The power and sample size were always taken into

account in this study in order to select statistical analysis to control the family wise error. We consider that the main advantage of our analysis was de avoidance of multiple or "family" comparisons between the data set by using two group comparison tests, which enhance the p values.

Science editor:

The authors evaluate whether ADC histogram profiling can assist distinguish therapy response in patients with locally advanced rectal cancer who underwent neoadiuvant radiochemotherapy. They concluded that kurtosis might be a useful indicator in the evaluation of pathological complete response, however they recognized a high interobserver reliability for measurements of all of the histogram-derived parameters analyzed and therefore underlined the challenges to replicate the model. The manuscript is interesting and well written, however it is too long: some of the results could simplified and the discussion could be shortened.
Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade C (Good)

All requested revisions have been made. Thank you for your review. Accordingly, methods, as well as results and discussion sections, have been simplified in the new version of the manuscript.

Company editor-in-chief:

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.

Sorry that we should have followed the submission guidelines more carefully. Now we have rewritten the manuscript according to the technical comments and editorial policies.

Sincerely,

Isabel Sollozo-Dupont, PhD.