Peer Review File

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Reviewer A

Comment 1: Title: Please modify the Title enhancing the aim in a more consistent manner.

Reply 1: Magnetic resonance imaging-based radiomics in predicting the expression of Ki-67, P53, and epidermal growth factor receptor in rectal cancer

Changes in the text: we have modified our text as advised (see Page 1, line1-2)

Keywords

Comment 2: Keywords: Please modify by specifying the predictive models.

Reply 2: Radiomics signature; Ki-67; P53; epidermal growth factor receptor

Changes in the text: we have modified our text as advised (see Page 4, line51)

Abstract

Comment 3: Please be more precise on purpose, in this section you should describe only the aim of the study in a concise manner. The background should be more synthetic in this section.

Reply 3: We have modified as "The preoperative evaluation of the expression levels of Ki-67, P53, epidermal growth factor receptor (EGFR) based on magnetic resonance imaging (MRI) of rectal cancer is necessary to facilitate individualized therapy. This study aimed to develop and validate radiomics models for the evaluation of the expression levels of Ki-67, P53, and EGFR of rectal cancer from preoperative MRI."

Changes in the text: we have modified our text as advised (see Page 3, line25-29)

Comment 4: Please briefly describe inclusion and exclusion criteria, protocol, and model building.

Reply 4: Inclusion and exclusion criteria: patients with rectal cancer who underwent preoperative MRI and postoperative Ki-67, P53and EGFR assay. Protocol, and model

building: 796 radiomics features were acquired from both diffusion-weighted imaging (DWI) and T2-weighted imaging (T2WI). Least absolute shrinkage and selection operator (LASSO) and the minimum redundancy maximum relevance (mRMR) were used to select the most predictive texture features, and then the radiomics score (Rad-score) models were derived to evaluate Ki-67, p53, and EGFR expression status based on radiomics signature.

Changes in the text: we have modified our text as advised (see Page 3, line30,33-37)

Comment 5: Please define the nature of the study and the time frame in which you enrolled the patients.

Reply 5: In this retrospective study, 124 patients were included in our hospital from June 2015 to October 2019.

Changes in the text: we have modified our text as advised (see Page 3, line30-33)

Comment 6: Please be more consistent in the conclusion, it seems to be too general. What about “immunohistochemical biomarkers”?

Reply 6: Ki-67, P53, and epidermal growth factor receptor (EGFR)

Changes in the text: we have modified our text as advised (see Page 4, line49-50)

Highlight box

Comment 7: Overall, these seem to be too general. In this section you should provide some take home messages with a more consistency

Reply 7: Immunohistochemical biomarkers replaced by Ki-67, P53, and EGFR

Changes in the text: we have modified our text as advised (see Page 5, Highlight box)

Introduction:

Comment 8: Please provide some literature data about some previous studies which tested radiomics approach with a specific task of predicting immunohistochemical biomarkers, highlighting their main limitations that should be overcome with your study.

Reply 8: Thanks for your advice, we have modified.

Changes in the text: we have modified our text as advised (see Page 7, line101-110)

Comment 9: What about adding value of your paper? Please define more consistently. Currently in case of specific immunohistochemical biomarkers is it suggested to avoid or not the chemotherapy?

Reply 9: Radiomic is different from surgery and biopsy, which is a non-invasive preoperative method for assessing Ki67, p53 and EGFR expression to guide the surgical or chemoradiotherapy strategy decision and monitor the tumor's progression or treatment response.

Changes in the text: we have added in our text as advised (see Page 6, line76-80)

Comment 10: In case of accurate pre-operative risk stratification, what are the main clinical workflows? And what about their strengths and limitations? Please discuss.

Reply 10: In the context of accurate pre-operative risk stratification, the main clinical workflows typically involve a series of steps aimed at assessing the patient's risk prior to undergoing a surgical procedure. These workflows leverage various sources of information and tools to ensure that decisions regarding treatment options, post-operative care, and potential complications are well-informed.

Main Clinical Workflows

Patient History and Physical Examination: This is the foundation of any pre-operative assessment. It involves a detailed review of the patient's medical history, including previous surgeries, medications, allergies, and comorbidities. A thorough physical examination is conducted to identify any potential risk factors or concerns that may impact the surgical outcome. Diagnostic Tests and Imaging: Additional diagnostic tests, such as blood work, urinalysis, and cardiac evaluations, may be ordered to further assess the patient's health status. Imaging studies, such as X-rays, CT scans, or MRIs, are often used to visualize the anatomical structures involved in the surgery and identify any abnormalities that may increase the risk of complications. Risk Assessment Tools and Models: A variety of risk assessment tools and models are available to clinicians, including both traditional and machine learning-based approaches. These tools

incorporate patient-specific data and clinical variables to generate a risk score or probability of various outcomes, such as post-operative complications or mortality. Multidisciplinary Team Discussion: In complex cases, a multidisciplinary team of specialists may convene to discuss the patient's condition, treatment options, and potential risks. This collaborative approach ensures that all relevant expertise is considered in making decisions about the patient's care. Patient Education and Informed Consent: Patients are educated about their condition, the proposed surgical procedure, and the potential risks and benefits involved. Informed consent is obtained, ensuring that patients understand the risks and are making an informed decision about their treatment.

Strengths

Personalized Approach: Risk stratification allows for a more personalized approach to surgical care, tailoring treatment plans to the individual patient's needs and risks.

Improved Outcomes: Accurate risk stratification can help clinicians identify high-risk patients who may benefit from additional monitoring, interventions, or alternative treatment options, ultimately leading to improved patient outcomes. Cost-Effectiveness: By identifying patients who are at low risk for complications, clinicians can avoid unnecessary interventions and resources, leading to cost savings.

Limitations

Data Limitations: The accuracy of risk stratification tools depends heavily on the quality and completeness of the data available. Incomplete or inaccurate data can lead to biased risk estimates. Model Limitations: Risk assessment models have inherent limitations, including the potential for overfitting, underfitting, and a lack of generalizability to all patient populations. Patient Variability: Patients can have unique responses to surgical procedures and interventions, making it difficult to accurately predict outcomes in all cases. Ethical Considerations: Risk stratification can raise ethical concerns, such as the potential for discrimination based on risk scores or the use of limited resources for patients with lower risk scores.

Overall, accurate pre-operative risk stratification is a critical component of surgical care, enabling clinicians to make informed decisions about treatment options and potential

risks. While it has numerous strengths, it is important to acknowledge its limitations and to continuously improve risk assessment tools and models to ensure that patients receive the best possible care.

Changes in the text: we have modified our text as advised (see)

Comment 11: Concerning radiomics limitations, you should add some information literature based.

Reply 11: We have added some literature

Changes in the text: we have modified our text as advised (see Page 7, line95-98, References, 11, PMID:34072366)

Materials and methods:

Comment 12: Please describe in a detailed manner how do you select the genetic mutations and all clinical data

Reply 12: We have described in “Immunohistochemical biomarkers tests”

Changes in the text: we have modified our text as advised (see Page 9, line141-146)

Comment 13: Did you use the TNM staging or not? Please clarify.

Reply 13: Yes. We used pathological TNM stage

Changes in the text: we have modified our text as advised (see Page 8, line126)

Comment 14: What about the expertise of the radiologists? Please define.

Reply 14: 10 years’ experience in diagnosis of rectal cancer

Changes in the text: we have modified our text as advised (see Page9, line153-154)

Comment 15: How did you select only T2WI and DWI for the tumor segmentation? Please clarify.

Reply 15: The selection of T2-weighted (T2WI and Diffusion-Weighted Imaging (DWI) sequences for tumor segmentation is typically based on their unique abilities to highlight different aspects of tumor tissue. This choice is informed by several

considerations. 1.Tissue Contrast: T2WI: T2-weighted imaging provides excellent contrast between normal tissue and lesions. This makes T2WI useful for identifying and outlining the overall extent of the tumor, including its peripheral edema. DWI: Diffusion-Weighted Imaging is sensitive to the microstructural characteristics of tissue, particularly the ability of water molecules to diffuse freely. In tumors, regions with restricted diffusion can be highlighted. DWI is therefore valuable for identifying the core region of the tumor, which often has a higher cellular density and may not be as clearly visible on T2WI due to its overlap with edema. 2. Complementarity: By combining T2WI and DWI, researchers and clinicians can take advantage of the complementary information provided by each sequence. T2WI provide a broad overview of the tumor's spatial extent, while DWI focuses on the tumor's more cellular core. This combined approach often leads to more accurate and precise tumor segmentation. 3.Availability and Practicality: T2WI and DWI sequences are routinely acquired as part of a standard MRI protocol for patients with suspected rectal cancer. Therefore, selecting these sequences for tumor segmentation is practical and does not require additional scanning time or resources.4. Diagnostic Accuracy: Numerous studies have demonstrated the diagnostic accuracy and value of T2WI and DWI in tumor segmentation, particularly for identifying tumor boundaries and distinguishing between tumor tissue and edema. This evidence supports their use in clinical practice and research. In summary, the selection of T2WI and DWI for tumor segmentation is based on their ability to provide complementary information about tumor extent and tissue characteristics, their wide availability as part of standard MRI protocols, and their established diagnostic accuracy in this context.

Changes in the text: we have modified our text as advised (see Page 14, line279-281)

Comment 16: Did you evaluate the inter-reader variability among radiomic features?

Reply16: Yes.

Changes in the text: we have modified our text as advised (see Page 9, line157-158)

Comment 17: Please be more consistent in the description of “excessive artifacts

Reply 17: It means “Poor image quality”

Changes in the text: we have modified our text as advised (see Page 8, line124, and our figure 1)

Comment 18: Was it a single center study?

Reply 18: Yes

Changes in the text: we have modified our text as advised

Comment 19: Data about survival were available. This aspect could be relevant

Reply 19: Thank you for your valuable advice, which we will expand on in the next step of our research

Changes in the text: we have modified our text as advised

Comment 20: What about mucinous adenocarcinoma? These were included or not? Please clarify

Reply 20: Thank you for your question, you are an expert in rectal cancer research. Mucinous adenocarcinoma is a more dangerous type, prone to recurrence, and the prognosis is relatively poor, but unfortunately there are only three of the 124 cases in our study, so we did not separate them for separate analysis, which will be a good research direction in the future.

Changes in the text: we have modified our text as advised

Results

Comment 21: Please expand data about sensitivity, specificity, and accuracy of radiomics signature also in in the main text. These are relevant data that should be reported in the main text

Reply 21: We have reported relevant data

Changes in the text: we have modified our text as advised (see Page 12, line217-223)

Comment 22: Please expand the Clinical usefulness, several data seem to be missing.

Reply 22: Decision curve analysis (DCA) was adopted to evaluate the clinical utilities. We can see the Figure 6. The horizontal line represents the assumption of no positive patients. The blue curve represents the standardized net benefit and was classified as positive (high risk) by the radiomics model at each threshold probability. The green curve represents the standardized net benefit of true positives at each threshold probability.

Changes in the text: we have modified our text as advised (see Page 12, line217-223)

Discussion and Conclusion

Comment 23: In the first paragraph you should sum up the main results achieved in a more consistent manner, by proving some relevant information.

Reply 23: We have modified our text as advised

Changes in the text: we have modified our text as advised (see Page 12-13, line239-244)

Comment 24: Please discuss more consistently your results in comparison with the previous studies, the listing and the description seems to be reductive.

Reply 24: we have modified our text as advised

Changes in the text: we have modified our text as advised (see Page 14, line286-289; Page 14-15, line290-315);

Comment 25: What about the added value of your paper in clinical setting, especially in case of you patients who could avoid or benefit of the aggressive treatment? Please expand upon it.

Reply 25: Radiomic is a more accurate prediction of tumor cells and Ki-67, p53 and EGFR expression, which may help prognosticate the heterogeneous clinical behavior, and reduce time-consuming and costly procedure. In clinical setting, radiomics is potential to inform and guide clinical decision-making, particularly for patients who may be able to avoid or benefit from aggressive treatment options. Patient Stratification: Our research may help identify specific patient subgroups that are more likely to

respond favorably or adversely to aggressive treatments. By understanding these differences, clinicians can tailor treatment plans to individual patients, potentially sparing some from unnecessary harsh therapies and directing others towards more effective interventions. Risk-Benefit Assessment: The findings of our study can contribute to a more nuanced risk-benefit analysis for patients facing aggressive treatment decisions. By providing insights into the likelihood of success or complications associated with different treatment options, our work can empower patients and clinical physician to make more informed choices. Improving Treatment Outcomes: By highlighting factors that predict better or worse outcomes with aggressive treatments, our paper can help optimize treatment strategies. This may lead to improved overall survival rates, reduced treatment-related morbidity, and enhanced quality of life for patients. Cost-Effectiveness: In addition to clinical benefits, our research may also have implications for clinical physician cost-effectiveness. By identifying patients who are unlikely to benefit from aggressive treatments, resources can be redirected towards more effective interventions for others, potentially reducing overall healthcare costs. Our work can pave the way for future research and the development of new, more targeted therapies. In summary, the value of our paper in a clinical setting lies in its potential to improve patient outcomes, enhance decision-making, in a way that ultimately benefits patients who may be considering aggressive treatment options.

Changes in the text: we have modified our text as advised (see Page15, line 315-320)

Comment 26: Concerning the limitations, you might rewrite in a more synthetic and clearer manner.

Reply 26: we have modified our text as advised

Changes in the text: we have modified our text as advised (see Page 15, line310-318)

References:

Comment 27: Please add to the references PMID:34072366

Reply 27: Tanks your advice. We find another article with PMID:34063937. Radiomics

in Oncology, Part 1: Technical Principles and Gastrointestinal Application in CT and MRI; and the article with PMID:34072366 is Part 2: Thoracic, Genito-Urinary, Breast, Neurological, Hematologic and Musculoskeletal Applications. The two articles have been cited.

Changes in the text: we have modified our text as advised (see References 11, PMID:34072366; see References 40, PMID: 34063937)

Tables:

Comment 28: Please add some dedicated table concerning MRI protocol.

Reply 28: we have added some dedicated table concerning MRI protocol

Changes in the text: we have added table as advised (see table1)

Figures:

Comment 29: Please improve the layout of Figure 1.

Reply 29: we have improved as advised

Changes in the text: we have modified our text as advised (see Figure 1)

Reviewer B

Comment35: Line 34: “radiomics” (with “s”, please check across the whole article)

Reply 35: We have check

Changes in the text: we have modified our text as advised (see Page 4, line49)

Comment 36: Line 40: “ranks third highest” => “ranks as the third highest”

Reply 36: we have modified “ranks as the third highest”

Changes in the text: we have modified our text as advised (see Page5, line55)

Comment 37: Line 70: please first define “AUC” in the main text (abbreviations have to be defined in both the Abstract and the Main Text.)

Reply 37: we have defined as advised

Changes in the text: we have modified our text as advised (see Page7, line103)

Comment 38: Line 198: please review “order s”???

Reply 38: It means orders

Changes in the text: we have modified our text as advised (see Page 13, line256)