Peer Review File

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Review Comments

Comment 1: (Minor) Abstract (first impression). The following information should be included in the Methods: type of patient selection (consecutive or other), main inclusion criteria (especially: did you include patients with a prior history of PC).

Reply 1: Thank you for your valuable feedback. We have revised our abstract to include the type of patient selection and main inclusion criteria. We used a consecutive patient selection and included patients with no prior history of PCa. We believe that this will provide a more comprehensive understanding of our study and improve the clarity of our methods section.

Changes in the text: We have added this information in the text (see Page 4, line 58-60).

Comment 2: (Minor) Methods, patient selection. Please provide information in regard to type of patient selection. Did you offer participation in the study to every, consecutive patient? Most probably yes, but this information should be expressed. Also, did you expect the patients to meet one, two, or three inclusion criteria in order to be included? How many exclusion criteria must have been met to be excluded?

Reply 2: Thank you for your comment. We apologize for the lack of clarity in the patient selection process. We included patients consecutively who met at least one of the following inclusion criteria: (1) serum PSA level > 4 ng/mL; or (2) palpable nodules, induration or asymmetry on digital rectal examination (DRE); or (3) abnormal prostate structure on imaging. Our study excludes patients who: (1) have already been diagnosed with prostate cancer; (2) have received treatment that may affect PSA levels or imaging; (3) have MRI contraindications; (4) are suspected to have advanced prostate cancer (the clinical stage is greater than cT3a.); (5) refuse to undergo biopsy. Patients who meet any of these criteria will be excluded from the study.

Changes in the text: We have updated the Methods section to reflect this information (see Page 8, line 128-133).

Comment 3: (Minor) Methods, prostate biopsy. Did you use any software add-on to perform a PET-CT/ultrasound fusion biopsy? I never saw such a feature in my BK device.

Reply 3: Thank you for your comment and for taking the time to review our paper. Radiologists labeled a maximum of 2 highest scoring lesions per patients by using the BK Fusion software (MIM 6.9, MIM Software Inc.) before conducting the prostate biopsies.

Changes in the text: We have added this information in the text (see Page 9, line 159-161).

Comment 4: (Major) Methods, external validation cohort. You do discuss the limitations in regard to the validation cohort. Please, not one more thing. Validation cohort might have also differed significantly from the prospective cohort, as those patients underwent systematic biopsy only. I assume that relatively small size of the prospective sample disallowed for dividing it randomly into training and validation sets, so this was why the validation cohort was retrospective and using this data was a must. However, this issue should be adequately discussed as a risk of bias.

Reply 4: Thank you for your comment. We agree that the validation cohort may have differed significantly from the prospective cohort, not only because the patients underwent systematic biopsy only, but also because the biopsy was performed by a different urologist.

Furthermore, as you noted, the limited sample size of the prospective study precluded random allocation into training and validation sets. Therefore, we enrolled an additional 42 patients who underwent PET-CT after biopsy to serve as the training cohort, as the number of patients meeting the criteria for the training cohort was relatively small.

We appreciate your feedback and we discussed the issues in our revised manuscript to provide a more comprehensive and transparent discussion of the limitations of our study.

Changes in the text: We have added ample discussion in the text (see Page 20, line 396-414).

Comment 5: (Minor) Methods, definition of terms. You are defining things precisely. Well, how did you manage PIN HG and ASAP?

Reply 5: Thank you very much for your reminder. In our study, we defined HG-PIN and ASAP as non-neoplastic lesions based on pathological examination of biopsy specimens. We followed established guidelines for management and treatment based on the severity and progression of these conditions. We appreciate your feedback and will make sure to clarify this point in our revised manuscript to ensure that our readers have a clear understanding of how we classified these lesions.

Changes in the text: We have added this information in the text (see Page 10, line 183-185).

Comment 6: Methods, statistical analyses. You expressed continuous variables as means with standard deviations, also you used the Student t-test for comparing them. Was the data normally distributed? Did you check for normal distribution? Please, verify. In case of skewed distribution, you should provide medians with interquartile ranges and use the Mann-Whithney U test. Also, Chi-square is most commonly used for non-small samples, but Fisher's exact test is acceptable.

Reply 6: I'm sorry that my pen error has caused you confusion. We appreciate your attention to detail and your suggestions for improving our statistical analyses. To answer your question, we did check for normal distribution of our continuous variables using the Shapiro-Wilk test. The data was not normally distributed, we reported the medians with interquartile ranges and used the

Mann-Whitney U test for comparisons, as you suggested. Regarding the Chi-square test, we did use this test for categorical data in our study. However, if the sample size was small or if any expected cell count was less than 5, we used Fisher's exact test, as recommended. We appreciate your insightful feedback and will make sure to address the issues you raised in our revised manuscript.

Changes in the text: We have updated the statistical analyses section to reflect this information (see Page 10, line 188-191).

Comment 7: (Minor) Results, predictive model construction. Line 216: the word “independent” is inappropriate.

Reply 7: Thank you for your feedback on our manuscript. We appreciate your comment on line 216 where the word "independent" was used inappropriately. We have revised the sentence and deleted the word "independent". We hope that this revision addresses your concern and improves the clarity of our manuscript.

Changes in the text: We have reviewed the entire manuscript and made revisions to similar expressions (see Page 12, line 223-224).

Comment 8: (Major) Results, predictive model construction. To my opinion, including an independent variable with no significant association into a model only based on the “importance in detecting prostate cancer (no reference given)” is inappropriate. If you have a clinical basis for doing so, you should first run the AUC ROC for two models, one including PSAD and the other one not including PSAD and demonstrate whether addition of PSAD resulted in any increment in AUC.

Reply 8: Thank you for your feedback on our manuscript. We appreciate your comments on the inclusion of an independent variable with no significant association into our predictive model. We agree that adding variables to a model based solely on their importance in detecting prostate cancer, without a clinical basis, is inappropriate.

Therefore, we have performed additional analysis as suggested by you. We compared the AUC of two models, one including PSAd and the other one not including PSAd. We found that there was no significant difference in their ability to diagnose prostate cancer. We have added the result in the revised manuscript. We hope that this revision addresses your concern and strengthens the clinical basis of our study. Once again, thank you for your valuable feedback.

Changes in the text: see Page 13, line 244-254, Page 17, line 329-350.

Comment 9: Discussion, Lines 297-300. You refer to a meta-analysis. However, the paper you refer to provides different values, with the upper boundary of the 95%-CI being much closer to your results. Please double-check.

Reply 9: Thank you for bringing this to our attention. We apologize for any confusion caused by our oversight and have rechecked the references, correcting any numerical errors in the text. We hope that this revision clarifies any confusion regarding our reference to the meta-analysis.

Changes in the text: We have revised the text in lines 358-360 accordingly to reflect this correction.

Comment 10: There is plenty of data in the literature in regard to the high predictive value of pre-biopsy PSMA-PET imaging. Your results are unique, as you are the first to combine PSMA-PET results with other parameters to form a predictive model. However, the comparison of ROC AUC of the nomogram and the ROC AUC of SUVmax demonstrates that the increment in AUC was, in fact, only minor. The absolute difference between the AUCs was 0.01, which is still better than none, but the clinical significance is not obvious. To me, this rather serves as evidence that the predictive value of SUVmax itself is so good that it might independently discriminate csPC from PC in clinical practice. Please, discuss it.

Reply 10: Thank you for your valuable feedback. We appreciate your acknowledgment of the uniqueness of our study and the significance of combining PSMA-PET imaging with other parameters to form a predictive model.

Although there is small difference in AUC between SUVmax and our predictive model, it still has some clinical significance. In our analysis, ^{68}Ga-PSMA PET-CT yielded high sensitivity (0.9, 95% CI, 0.90-0.99) but relatively moderate specificity (0.66, 95% CI, 0.52-0.78). And the positive predictive value of SUVmax was also relatively moderate (0.660; 95% CI 0.507-0.791), which may lead to a higher false positive rate of csPCa and unnecessary prostate biopsies. Compared with SUVmax alone, the novel predictive model demonstrated excellent diagnostic performance with significantly improved specificity (0.910 95%; CI 0.824-0.963) and positive predictive value (0.811 95%; CI 0.648-0.920) at a slight sacrifice of sensitivity.

Thank you for your valuable input. We address the issues you raised in an extra paragraph in result section of revised manuscript.

Changes in the text: We also have added ample discussion in the text (see Page 16, line 303-328, Page 18, line 351-361).

Comment 11: You did discuss that PSMA-PET is not an easily accessible nor affordable imaging study. In fact, in my practice, I would not offer PSMA-PET to every patient suspected of harboring csPCa. Based on your data, are you able to identify which patients would be more likely to benefit from a pre-biopsy PSMA-PET? Yes, you did compare the nomogram to SUVmax, PSAD and PSA

in selected populations. However, enhancing your manuscript with additional analyses, aimed at identifying risk factors of a patient being false-negative at MRI and true-positive at PSMA-PET (i.e., risk factors of being likely to benefit from additional PSMA-PET in order not to miss csPC) would add even more value to your conclusions. This is not a call for a revision. However, I would appreciate adding, or at least discussing the above considerations.

Reply 11: Thank you for your insightful comment. We agree that identifying risk factors for patients who are more likely to benefit from pre-biopsy PSMA-PET imaging would be of great clinical relevance.

While we did not specifically analyze risk factors for false-negative MRI and true-positive PSMA-PET in our current study, we acknowledge that this is an important area for future research. We agree that further research is needed to identify additional risk factors for false-negative MRI and true-positive PSMA-PET. This could potentially involve larger studies with more extensive patient data, as well as machine learning algorithms that can identify patterns and predictors of imaging results.

We add a detailed discussion that specifically addresses the issue of identifying which patients may benefit most from pre-biopsy PSMA-PET imaging. We appreciate your feedback and suggestions for improving our study. **Changes in the text:** We have added ample discussion in the text (see Page 19, line 375-395).

Many grammatical or typographical errors have been revised.

All the lines and pages indicated above are in the revised manuscript.