Peer Review File

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Reviewer A

The authors determined factors which predict seminal vesicle invasion (SVI) in 262 patients who underwent radical prostatectomy and had a preoperative MRI which was negative for SVI. In a multivariable model they found that SVI at surgery was associated with biopsy Gleason grade group and PI-RADS score.

Comments as follows:

Comment 1. The title is inappropriate and does not comply with the TRIPOD guidelines the authors stated they followed. Furthermore, I cannot find any data in the results section which supports the statement in the manuscript title.

Reply 1. Thank you for the title suggestion. The precedent version of the title has been replaced, becoming “Predictive model containing PI-RADS v2 score for postoperative seminal vesicle invasion among patients with PSA <10 mg/mL”.

Changes in the text: See page 1 line 1-2

Comment 2. PI-RADS was used but then grouped into a 3 point “Likert” scale in the model. Why was this done? PI-RADS 1-5 should have been used in the models – this makes me believe they did not rigorously apply the PI-RADSv2 criteria to the pre-operative MRIs.

Reply 2. According to PI-RADS v2 assessment categories, clinically significant cancer

is highly unlikely or unlikely to be present in lesions of PI-RADS 1 or 2.(1,2). What's more, lesions with PI-RADS >2 were defined as MRI-visible lesion, which could be considered for targeted biopsy. For patients with PI-RADS 3, it may be beneficial to perform follow-up rather than immediate biopsy, as most lesions can be reclassified after a manageable period of time (3). PI-RAD 4 or 5 means highly or very highly likely existence of clinically significant cancer, which biopsy should be considered (2). Targeted MR biopsy should be considered for PI-RADS assessment category 4 or 5 lesions but not for PI-RADS 1 or 2 (4). The 3 point "Likert" scale was associated directly with clinical decisions, which was the reason for grouping PI-RADS score into a 3 point "Likert" scale.

Comment 3. The models have not been adjusted for fundamental variables such as clinical stage. Ideally their models should have also been adjusted for a well validated risk stratification score such as CAPRA.

Reply 3. We have calculated CAPRA score for each patient, and the relevant information has been added in article. Univariate and multivariate binary logistic regression analyses were conducted to identify weather clinical stage or CAPRA score were independent predictors of SVI. We found that patients with SVI seemed to have higher CAPRA score (OR 1.388; 95% CI1.063-1.814; p= 0.016). But in the multivariate analysis, CAPRA score was insignificantly associated with SVI, suggesting that it wasn't an independent risk predictor for the diagnosis of SVI (table S1).

In the final predictive model, the crude OR of PI-RADS and GGG was 3.359 and 1.535 respectively. After containing the clinical stage as a confounding factor, the adjust OR

of PI-RADS and GGG was 3.678 and 1.555 respectively, in which no significant change occurring. Thus, the clinical stage didn't impact GGG and PI-RADS as independent risk predictors for the diagnosis of SVI.

Change in the text: page 9 line 1-5; page 12 line 8-16; page 20 line 9-15

Comment 4. In the cohort, 11.5% of men were found to have SVI after surgery. This is a large percentage and suggests the cohort was biased.

Reply 4. Thank you for pointing out this. We have retrieved recent literature that containing SVI percentage. The incidence of SVI seemed to be heterogeneous, ranging from 3% to 17.6% (5-8), and in the literatures published in 2020 which the median PSA level of the patients (5.9-7.8 ng/ml) is similar with ours(7.51 ng/ml), the prevalence of SVI is 11%-17.6% (6-8). In our cohort, the rate of SVI was 11.5%, which is consistent with the rate reported in recent literatures. The average incidence of SVI of the whole population and the heterogeneity of it in different literatures require further research and analysis.

Change in the text: See page 6 line 16; page 14 line 10-18

Comment 5. What percentage of men were upgraded at surgery compared to biopsy? Was this accounted for in the models?

Reply 5.

Compare to biopsy, there are 52 patients (19.8%) upgraded form GG < 2 to GG ≥2 at surgery. And among 52 patients who upgraded, there are 4 patients with SVI on radical prostatectomy specimens. There is no significant connection between GG upgrading and SVI (p=0.342).

Table a. GS shift

	Total cohort (n=262)	SVI (+) (n=30)	SVI (-) (n=232)	p
Any upgrade	90/262 (34.4%)	13/30 (43.3%)	77/232 (33.2%)	0.271
From GG < 2 to GG ≥2	52/262 (19.8%)	4/30 (13.3%)	48/232 (19.0%)	0.342
Any upgrade from GG≥2	38/262 (14.5%)	9/30 (30%)	29/232 (12.5%)	0.023

Reviewer B

Wang and colleagues have performed a retrospective analysis of 262 patients who underwent RP to identify pre-operative predictors of pathological invasion of the seminal vesicles identified final whole mount histology.

Unfortunately, the cohort included only a minority of men with SVI (n= 31/11%). However, the authors were able to generate a model and the findings of a GG <8 and PI-RADS score <4 leading to a NPV of 98% for SVI are interesting and have some clinical utility in pre-operative planning and counselling patient on the need for multi-modal therapy.

Generally, the manuscript is well written.

Title: Appropriate

Comment 1. Abstract: Use PIRADS abbreviation to improve read.

Reply 1. Thank you for your kind suggestion for this item. For easy reading, we

modified the abstract with abbreviation, including PI-RADS, mpMRI and SVI.

Change in the text: See page 4-5

Comment 2. Do you have a 95% CI for NPV available, suggest inclusion here.

Reply 2. We have calculated the 95% CI for sensitivity, specificity, positive predictive value and negative predictive value. The relevant information has been supplemented in the article.

Change in the text: See page 5 line 11-12; page 13 line 9-11

Comment 3.-Was there ethical approval? is there an institutional number?

Reply 3. During to no influence on therapeutic strategy or need for patients' follow-up, the Institutional Review Board approved this study without an ID of ethical approval replied that there was no need for ethical approval. Related illustration was supplemented.

Change in the text: See page 11 line 9-13; page 22 line 12-16;

Comment 4.-Statistical approach is valid although event rate is low here so the MVA must be interpreted with caution.

Reply 4. Thank you for pointing out this. We have to admit the shortage of sample size and the number of patients with SVI due to low incidence of SVI. The baseline clinical and pathological characteristics of the total 262 patients were analyzed objectively by the appreciate statistic method. In the multivariate analysis, GGG and PI-RADS remained significantly ($p<0.01$) associated with SVI. And the predictive model for postoperative SVI was constructed and has shown a high negative predictive value of 96.5% (95% CI, 91.5%-98.7%) at the optimal cutoff predictive value, though the

accuracy of this model requires internal and external validation to assess its wider applicability. These limitations have been explained in the discussion (page 20 line 12-15).

Results:

- Appropriately presented

Comment 5. - Higher PI-RADS score (OR, 4.095; 95% CI, 1.688–9.938; p = 0.002). The 95% CI for PIRADS is wide and may suggest an overfitted model. Have the authors confirmed this is not the case?

Reply 5. The reason for the wide range of 95% CI for PI-RADS can be partly explained by the relatively small sample size. Though the range of 95% CI is wide, the lower limit (1.688) is much higher than 1, suggesting PI-RADS v2 score was an independent risk predictor for the diagnosis of SVI.

Discussion:

Comment 6. "On the contrary, in the case of high likelihood of SVI, additional therapies should be discussed. In terms of prognosis and therapy strategy, preoperative prediction of SVI is important"

- Could you go further how does this fit within the knowledge from ARTISTIC which included the RADICALS study that delayed radiotherapy has the same outcomes as early salvage radiotherapy?

reply 6. The RADICALS trail has showed no statistically significant difference with regards to biochemical progression-free survival and freedom from subsequent

hormonal therapy between adjuvant radiotherapy and early salvage radiotherapy. It's worth pointing out that patients of this clinical trial must have at least one of the following risk factors: a) positive margins, b) extraprostatic extension (EPE) with or without seminal vesicle involvement (pT3a or pT3b). The RADICALS study focused on postoperative therapeutic schedules for patients underwent RP. However, the aim of this study is to create a model that includes PI-RADS v2 score to predict postoperative SVI in patients without SVI on mpMRI. According to NCCN guideline version 2.2020, patients with SVI are defined as very high-risk group, which treatment should think carefully for them. Duo to poor prognosis of SVI, for patients with SVI, RP requires carefully consideration, which RP is only recommended for patients with >5 years life expectancy or with symptomatic. Asymptomatic patients with < 5 years life expectancy are only considered for androgen deprivation therapy (ADT), external beam radiotherapy or observation. Advanced knowledge of SVI may influence the preoperative treatment and surgical options.

Chang in the text: See page 15 line 4 – page 17 line 5

Comment 7.- Have you employed this model in your clinical practice locally i.e. you counsel your patients for risks of ADT and salvage radiotherapy pre-operatively?

reply 7. We have not employed this model in clinical practice due to lack of internal and external validation, however, further studies were ongoing for validation.

Comment 8. Limitations appropriate including the need to valid this on an external data-set

Reply8. Further studies would be performed in the future and these limitations have

been explained in the discussion. See page 20 line 17- page 18 line 2

Reviewer C

This retrospective study analyses imaging, clinical and histopathological factors associated with SVI and creates a model for SVI-prediction. These are my comments:

Comment 1. Title: The title is very specific. I would propose to formulate a more general title.

Reply 1. Thank you for the title suggestion. The precedent version of the title has been replaced, becoming "Predictive model containing PI-RADS v2 score for postoperative seminal vesicle invasion among patients with PSA <10 mg/mL".

Changes in the text: See page 1 line 1-2

Comment 2. - PI-RADS v2.1 is the current version since 03/2019. Why was v2 the version used in this analysis? This aggravates transferability for clinical usage.

Reply 2. We totally understand the reviewer's concern. PI-RADS v2.1 released in 2019 aims to improve inter-reader variability and simplify PI-RADS assessment of prostate MRI (9). In PI-RADS v2.1, there are there are technical revisions in the three sequences to be routinely acquired, as follows: (1) T2WI: obtain axial and at least one additional orthogonal plane, (2) DWI: clarification of b-values used in DWI acquisition and apparent diffusion coefficient (ADC) map calculation, and (3) DCE: recommendation to decrease temporal resolution to 15 s or less during DCE acquisition and preferably using three-dimensional (3D) T1-weighted imaging (10).

As for DWI acquisition, PI-RADS v2.1 recommends that the highest b value used to

calculate ADC is ≤1000 s/mm^2, to avoid the impact of calculation of ADC values. Unfortunately, the MRI performed previously in our institution could not meet the PI-RADS v2.1 technical stander. However, PI-RADS v2 has showed satisfactory inter-reader variability in previous study (11). This limitation has been explained in the discussion.

Changes in the text: page 21 line 2-8

Abbreviations:

Comment 3. - Needs some corrections: PI-RADS = Prostate Imaging Reporting and Data System; TRUS = transrectal ultrasound

Reply 3. We gratefully appreciate for your valuable suggestion. We have corrected the abbreviations carefully.

Changes in the text: See page 6 line 4-11

Introduction: Appropriate.

Methods:

Comment 4.- Only patients with a PSA level < 10ng/ml were included. Why did you exclude patients with higher PSA levels?

Reply 4. Patients with a PSA level < 10ng/ml make up most of the population of PCa. In recent study, there are 78.6% patients (213/271) with PSA level <10ng/ml (12). The possibility of SVI in people with a PSA level < 10ng/ml is low and those patients with SVI were easily missed. In addition, PSA level ≤10 ng/mL was one of the critical inclusion criteria for low risk group and patients who underwent nerve-sparing RP (13,14) . Thus, we mainly focus on patients with PSA level <10ng/ml.

Comment 5.- mpMRI interpretation: Were the images re-read for this study by a second radiologist after the first radiologist performed analysis in clinical routine? If yes, was he blinded to the histopathological and clinical results?

Reply 5. In this study, the images were re-read and interpreted retrospectively by one of the two experienced radiologists with > 5 years' experience in reading prostate MRIs. Although the images were not routinely re-read by both of radiologists, any questions in the process of interpretation was resolved by the senior adjudicating radiologist.

And they are blinded to histopathological results, but relevant clinical characters before biopsy, including age, family history, PSA, and digital rectal examination results were provided.

Comment 6. - Statistical analysis: "The endpoint of the study was the identification of the presence of SVI on the final biopsy" - I thought that the endpoint was SVI on prostatectomy specimen? Please explain.

Reply 6. Thank you for your kind suggestion for this item. The endpoint has been modified to SVI on radical prostatectomy specimens.

Changes in the text: See page 5 line 4; page 10 line 8; page 12 line 1;

Comment 7. "...categorical variables were compared using Pearson's test and Chi-square test, as appropriate" - What do you mean with Pearson`s test? (Pearson`s) Chi-square test or Fisher`s exact test are used for categorical variables.

Reply 7. We used Pearson's chi-square test (also known as a chi-square test) or Fisher's exact text for categorical variables. When the sample size is small, we used Fisher's exact test. We are sorry for confusing the basic concepts of statistics. The relevant

concept has been modified.

Change in the text: See page 10 line 15-16

Results:

Comment 8.- p. 11, l. 3: Please see above: analysis was not performed on biopsy but on radical prostatectomy specimen

Reply 8. The endpoint has been modified to SVI on radical prostatectomy specimens.

Changes in the text: See page 5 line 4; page 10 line 8; page 12 line 1;

Discussion:

Comment 9.- p. 13, l. 8-9: In fact, the rate of SVI in this cohort is higher than in the series described for comparison. Please try to explain.

Reply 9. Thank you for pointing out this. We have retrieved recent literature that containing SVI percentage. The incidence of SVI seemed to be heterogeneous, ranging from 3% to 17.6% (5) (6-8), and in the literatures published in 2020 which the median PSA level of the patients (5.9-7.8 ng/ml) is similar with ours(7.51 ng/ml), the prevalence of SVI is 11%-17.6% (6-8). In our cohort, the rate of SVI was 11.5%, which is consistent with the rate reported in recent literatures. The average incidence of SVI of the whole population and the heterogeneity of it in different literatures require further research and analysis.

Change in the text: See page 6 line 16; page 14 line 10-18

Comment 10. - p. 14: I propose to remove the part of SV-sparing surgery because this is no current treatment standard. Preoperative knowledge of a potential SVI will not change the surgical way of prostatovesiculectomy itself, but might influence the choice

of nerve-sparing. Thus, this should be highlighted here.

Reply 10.

We are extremely grateful for pointing out this problem. According to EAU-ESTRO-SIOG Guidelines on Prostate Cancer, high risk of extracapsular disease is contraindication for nerve-sparing RP, and the EPE predictive model and mpMRI can help make the decision whether nerve-sparing RP can be performed (15). In our cohort, as discussed previously, EPE and SVI coexisted in 46.7% (14/30) of patients, and the other 16 patients without EPE. Epstein et al. reported 11.7% patients (7/60) with SVI but without EPE (16), and Billis et al. found 11% patients (3/28) with similar phenomenon (17). Thus, it's important to predict SVI before surgery. The relevant discussion has been added in the article and the part of SV-sparing surgery has been removed as you advised.

Changes in the text: See page 15 line 4- page 17 line 5;

Comment 11.- p. 15: The existing models of imaging, clinical or combined parameters show better performances compared to this new model here. This raises the question why to introduce your model, if other models better predict SVI.

Reply 11. Thank you for this valuable feedback. Our research is the first study to show that PI-RADS v2 score has a great value in predicting SVI. Other models for predicting SVI containing mpMRI findings mainly rely on the negative or positive SVI results of mpMRI. Our model focus on SVI (-) patients on preoperative mpMRI. In this study, the area under the curve of the model was 0.746 ($p < 0.001$). The PI-RADS v2 score < 4 and Gleason grade < 8 yielded only a 1.8% incidence of SVI with a high negative predictive value of 96.5% (95% CI, 91.5%-98.7%). Our research may provide a new

predictive model for postoperative SVI.

References

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14. Asimakopoulos AD, Topazio L, De Angelis M, et al. Retzius-sparing versus standard robot-assisted radical prostatectomy: a prospective randomized comparison on immediate continence rates. Surg Endosc 2019;33:2187-96.

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Reviewer D

I have read with interest the manuscript entitled: Predictive model containing PI-RADS v2 score for postoperative seminal vesicle invasion among patients with PSA <10 mg/mL

In general it is a well written article, with a coherent development.

I believe that the development of algorithms and predictive models is the future in the process of making decisions to perform biopsies, planning surgery and predicting recurrence

I only suggest some minor considerations and changes.

Comment 1: The discussion seems to me very long, I think you could save words

Reply 1: We tried to condense the DISCUSSION and some paragraphs have been deleted with the precondition of not affecting the descriptive integrality, succeeding in condensing it to 22% of its original length. And some paragraphs related to the main results and the emerging technologies have been added according to your kindly suggestions.

Change in the text: Page 11-13

Comment 2: In general, I like it and suggest that the first paragraph of the discussion expresses the main results of the work.

Reply 2: The main results of our work have been added in the first paragraph according to your suggestion.

In our cohort, SVI was reported on the RP specimens in 30 patients (11.5%). Using the selected risk factors, containing biopsy Gleason grade group and the PI-RADS v2 score, a predictive model for postoperative SVI was constructed, which revealed a high negative predictive value of 96.5% (95% CI, 91.5%-98.7%) at the optimal cutoff predictive value.

Change in the text: page 11, line 15-19

Comment 3: I recommend to add some paragraph dedicated to the potential of emerging technologies such as microultrasounds and predictive models based on microultrasounds + mpMRI and biomarkers.

Reply 3:

We gratefully appreciate for your valuable suggestion. We have added some paragraphs ralated to micro-ultrasound to the Discussion section.

Emerging technologies and prostate cancer biomarkers are playing a vital role in prostate cancer diagnosis and treatment (1). Micro-ultrasound is a novel high-resolution imaging technology for diagnosing prostate cancer which is complementary for mpMRI (2) (3) (4) (5). Compare to mpMRI, micro-ultrasound, which promises real-time visualization of suspicious lesions and targeting of biopsies, has shown same or superior sensitivity (3). For detecting clinically significant PCs, micro-ultrasound biopsy has shown a higher rate with fewer biopsied cores (6), and could found PCs missed by all other techniques (7). However, additional studies are needed to explore the application of micro-ultrasound for PCs staging and predicting SVI.

Change in the text. Page 17, line 3-12

Comment 4: I personally think that MRI should be offered to all patients who are going to undergo biopsy and surgery, if the local conditions of the system and reimbursement allow it.

Reply 4: We totally agree with you form this point. MpMRI and the PI-RADS has shown a great value in predicting biopsy outcome (8), biochemical recurrence (9). Our previous studies have shown similar results, confirming the value of PI-RADS in predicting prostate cancer and clinically significant prostate cancer in men undergoing repeat prostate biopsy and in predicting pelvic lymph node metastasis at RP (10,11).

Just as you said, MRI is recommended to all patients who are suspected of PCs in our institution.

Change in the text. Page 14, line 16-19

Comment 5: The way PIRADS is expressed in table 1 is a bit confusing.

Reply 5: According to PI-RADS v2 assessment categories, clinically significant cancer is highly unlikely or unlikely to be present in lesions of PI-RADS 1 or 2.(12,13). What's more, lesions with PI-RADS >2 were defined as MRI-visible lesion, which could be considered for targeted biopsy. For patients with PI-RADS 3, it may be beneficial to perform follow-up rather than immediate biopsy, as most lesions can be reclassified after a manageable period of time (14). PI-RAD 4 or 5 means highly or very highly likely existence of clinically significant cancer, which biopsy should be considered (13). Targeted MR biopsy should be considered for PI-RADS assessment category 4 or 5 lesions but not for PI-RADS 1 or 2 (15). The 3 point "Likert" scale was associated directly with clinical decisions, which was the reason for grouping PI-RADS score into a 3 point "Likert" scale. which was the reason for grouping PI-RADS score into a 3 point "Likert" scale.

Change in the text. Page 8 line 20 to page 9 line 5

Comment 6: In the title you could perhaps avoid the part that says at the end "...among patients with PSA <10 mg/mL"

Reply 6: Thank you for the title suggestion. The precedent version of the title has been replaced, becoming "Predictive model containing PI-RADS v2 score for postoperative seminal vesicle invasion among prostate cancer patients".

Change in the text. Page 1, line 1-2

1. Kohaar I, Petrovics G, Srivastava S. A Rich Array of Prostate Cancer Molecular Biomarkers: Opportunities and Challenges. Int J Mol Sci 2019;20.

2. Ghai S, Eure G, Fradet V, et al. Assessing Cancer Risk on Novel 29 MHz Micro-Ultrasound Images of the Prostate: Creation of the Micro-Ultrasound Protocol for Prostate Risk Identification. J Urol 2016;196:562-9.