**Peer Review File**

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**Reviewer A**

This is a retrospective analysis of patients from a single institution. While bias is inherent to retrospect studies, this a nice exploration of PDA density and it's role on prostate cancer.
While the idea and findings of the study are not new and ground breaking, this study elucidates more light on the PSA and it's relationship with prostate size in terms of the risk of prostate cancer

**Reviewer B**

Introduction

L56 You should present the reference.

The utility and effectiveness of TRUS in the diagnosis of prostate cancer patients with the level of PSA>2.5ng/ml should be described in the introduction. If you answered that content, please mention the reference.

We wholly agree with the reviewer's opinion. Thank you for your kind reminders

We presented the reference for the sentence (page3, line58)

We added some sentence as follows (Page 3, line 62-65)

Transrectal ultrasound (TRUS) is used worldwide because it can be easily performed in consulting rooms, is cost-effective, and takes only 10 minutes. However, since PCa often appears hypoechoic in TRUS and it does not increase PCa detection compared to biopsy of isoechoic lesions, TRUS alone has limitations in diagnosing PCa (7).

Methods

L70 "PSA levels of 2.5-20.0 ng/mL who have a low-to- intermediate risk of PCa ~" It should be mentioned the references for this sentence.

L80 If it is approved by the IRB, the approval number should be noted.

The present study included DRE and TRUS. These are individual findings and are biased. Therefore, these should be excluded. Please provide the reasons for including them.

Thank you for your kind reminders.

We mentioned the reference for the sentence. (page4, line 75)

We added the approval number, (NO. 2021-10-001) (page 5, line 88)

We added this as follows. (page 11-12, line 223-237)

Abnormal DRE and TRUS are individual findings and are biased, and not all physicians performing DRE or TRUS to the one patient give the same results. According to a recent study, Chang et al. reported positive or ambiguous DRE findings lead to only 42.1% sensitivity for detection of csPCa (27). And about 30–40% of PCa are not hypoechoic (28,29), TRUS has disadvantage of low specificity (30) for PCa. In our study, One physician reported abnormal DRE or TRUS findings in 76 of 141 patients (53.9%), and another submitted only 5 of 52 patients(0.09%), which we expected to be somewhat less reliable. Also, there was no significant difference between the PCa group and Non-PCa group for abnormal TRUS findings (p= 0.953). In case of DRE, 6 patients (3.7%) in the Non-PCa group and 11 patients (9.9%) in the PCa group were abnormal, showing a little difference (p=0.035). However, only one physician reported 14 patients (82.4%) out of all abnormal DRE findings, with confirmation bias, and as a result of analyzing these findings separately, there was no difference between the two groups (p=0.261). Therefore, in this study, both subjective visual and tactile information were judged

to be less accurate, and both were included in the study at PSA 2.5-20.0 ng/mL.

Results

L131 TRUS should be excluded.

L137 -138 “TZ-PSAD was the next~” this sentence seems unnecessary.

L139 “a PSA level of 4.0-20.0ng/mL” this sentence should be corrected “a PSA level of 2.5-20.0ng/mL”.

In the Table 2 and Table 3, the best cut-off of PSA was described. The best cut-off of PSA was lower in the predicting csPCa than in the predicting PCa. Although the PSA value is likely to be a higher for csPCa, the reason should be mentioned in the discussion.

Thank you for pointing this out.

We exclude the word “TRUS” (page8, line 143)

We deleted the sentence, TZ-PSAD was the next best predictor, followed by PZ-PSAD, total PSA, age, TPV, and PZV (page8,line 149)

We revised the word as follows : a PSA level of 2.5–20.0 ng/mL (page8, line 151)

As you advised, we mentioned as follows (Page12, line 237-245)

In the Table 3 and Table 4, the best cut-off of PSA was lower in the predicting csPCa than in the predicting PCa (p= 0.680, 0.659 respectively). Yusim et al. reported that 167 of 338 overall PCa had csPCa (49.4%) (13), while our study suggested 88 of 111 overall PCa had csPCa (79.3%), showing larger proportion in our study. This seems to be because the total number of study participants was small and there was not much difference between the number of csPCa and non-csPCa. Also PCa. is the result of diagnosis by TRUS-Bx. and is not the final Bx. after radical prostatectomy, so the possibility of downgrade cannot be ruled out (31). However, this study included real world data and proves that PSA alone cannot predict csPCa in patients with a PSA level of 2.5–20.0 ng/mL.

Though univariate analysis has been performed, multivariate analysis should be performed. It should be prepared as a new Table. Furthermore, the categories of PPV, NPV and accuracy should be added.

We think this is an excellent suggestion.

Table 2,3 and 4 has been updated to table 3,4, and 5.

We added this as follows (page 2, line 32-37)

In univariate and multivariate analysis, all categorical variables were divided based on the cut-off value and used to predict PCa. Those with a PSAD of ≥0.218 ng/mL$^2$ were found to be at an increased risk of PCa than those with a PSAD of <0.218 ng/mL$^2$ (odds ratio [OR] 3.51, 95% confidence interval [CI] 1.306-9.415), which was the best result, followed by TZ-PSAD with a cut-off value of 0.353.

We added this as follows (page 9, line 165-169)

In the multivariate analysis, patients with age ≥ 68.5 (OR 3.51, 95% CI 1.920-6.417), TZ-PSAD ≥0.353 (OR 2.26, 95% CI 1.019-4.993) and PSAD ≥0.218 ng/mL2 (OR 3.51, 95% CI 1.306-9.415) were found to be at the predictors of PCa, whereas PSA (p=0.656), PZ-PSAD (p=0.370) were not (Table 5).

We revised this as follows (page 13 line 274-275)

univariate and multivariate analyses of PCa-associated factors. Patients with a PSAD of 0.218 ng/mL$^2$ or greater had an 3.5-fold increased risk of PCa

Although it is divided by TRUS findings, since TRUS is not reproducible, it is not appropriate as a characteristic for investigation. please explain why you selected TRUS as a characteristic for investigation.

Thank you for pointing this out. We have added more details to this. (page13, line254-258)

Although many diagnostic techniques such as mpMRI are being developed, TRUS is the world's most popular, inexpensive, and has few complications. It can also provide exact size and anatomy of each zone in detail (33). Because it provide efficient information for cancer diagnosis, TRUS which is described as a urologist's finger, was selected as a characteristic for investigation. Also, TRUS is always necessary for prostate biopsy.

Discussion

The effectiveness of PSAD with a PSA level of 10-20.0 ng/mL should be mentioned. If PSAD was 0.218 or lower in patients with PSA levels of 10-20.0ng/mL, would you avoid prostate biopsy?

L190-191 “when PSA is ambiguously elevated (PSA levels of 2.5-20.0)~ ” PSA level of 20.0 seems to be unambiguously elevated.

L225-232 This paragraph discusses the efficacy of PSAD in gray zone. It is not appropriate in the present study. Hence, please omit this paragraph.

Reference

There are two No.27. Please correct them all together, including the reference number in the text.

Thank you very much for the reminder.

We added this as follows (Page14 line 277-282)

Although not listed in the table , the patient with PSAD of ≥0.218 ng/mL2 with a PSA level of 10-20.0 ng/mL also showed increased risk of PCa than those with a PSAD of <0.218 ng/mL2 (p=0.001) However, at PSA with 10-20 ng/mL, the possibility of chronic inflammation should always be borne in mind. Indeed, in this study, the number of chronic inflammation was significantly higher in PSA with 10-20 ng/mL than PSA with 0-10 ng/mL (p=0.024).

We deleted the word. “ambiguously” (page 10,line 206)

We omitted the paragraph discusses the efficacy of PSAD in gray zone. (page 14)

We corrected the wrong number of reference and cite the consecutively in the order of appearance.

**Reviewer C**

Article is written in good English lanugage. The points that should be further elucidated are:
1. MRI of the prostate +/- fusion biopsy has become mainstream in the last 5 years. It is not clear, how many patients in the study had MRI and whether findings of MRI were used to target lesions (cognitive). Furthermore discussion should compare results of the present study with growing information in the literature about MRI guided biopsies. Authors should also present their view on use of PSAD in the diagnostic workup in the ideal scenario, i.e. MRI is available and cost of MRI is not an issue.

Thank you very much for the reminder.
We wrote accordingly. (Page12, line 246-252)
In this study, patients underwent TRUS-Bx. without the aid of mpMRI. However, mpMRI for the evaluation of PCa have been covered by the National Health Insurance in Republic of Korea since 2019. Currently, MRI of the prostate +/- fusion biopsy has become mainstream. According to recent study by Zhen et al., a meta-analysis of 29 studies with 8503 patients reported the sensitivity and specificity of pre-boipsy mpMRI were 0.87 [95%CI, 0.81–0.91] and 0.68 [95%CI,0.56–0.79] respectively (32). Our view on use of PSAD in the diagnostic workup in the ideal scenario is apply PSAD prior to performing mpMRI to aid in PCa. Diagnosis.

2. In the lines 164-166 authors state that this is the first study on PSAD in patients with PSA range 2.5-20, yet numerous research articles were published on PSAD since 1994 (which are also listed in the reference). Was there no article including this PSA range in almost 30 years?
We deeply appreciate the reviewer's thoughtful comments. We revised the sentence as follows (Page9, line 178-181).
Many studies related to PSAD in men with PSA levels in the gray zone (11,15,16) as well as in PSA <20 ng/mL (9,10,13,17,18) have been conducted, we investigate PSAD as a risk factor for PCa in patients with low-to-intermediate risk (PSA levels 2.5–20.0 ng/mL), and found that the risk of PCa increased in this patient cohort when PSAD was 0.218 or higher.

3. What was the template for systematic biopsies - was it the same in all patients (disregarding the difference in number of cores)
Thank you very much for pointing this out.
We wrote accordingly (Page6, line 104-108)
Between 12-15 cores were obtained from each patient. Systematic 12 core biopsy were

performed, including 6 sextant combined with 6 laterally directed cores at the apex, mid and base, bilaterally. Two or three biopsies of the transition zone at the mid gland were added to the routine biopsy scheme depending on the physician's preference.

4. Authors should clarify the role of TRUS alone, since on several places in the article term "ambigous finding of TRUS" is found. It is known that standard 12 MHz TRUS cannot reliably show suspicious PCa areas, since they are hipoehogenic in only 50%, while the rest are isoehogenic.

Thank you very much for the reminder. We added as follows (Page3,line62-65).

Transrectal ultrasound (TRUS) is used worldwide because it can be easily performed in consulting rooms, is cost-effective, and takes only 10 minutes. However, since PCa often appears hypoechoic in TRUS and it does not increase PCa detection compared to biopsy of isoechoic lesions, TRUS alone has limitations in diagnosing PCa (7).

5. Authors should stratify positive biopsy findings according to Gleason score and compare these strata according to PSAD and other variables studied in the article.

We appreciated the feedback.

We stratified positive biopsy findings according to ISUP grade groups in Table 1 and compare these strata in Table 2 according to your comment

We added as follows (page 8 , line 144)

The ISUP grades and other variebles are shown in Table 2.

6. The point of concern are patients simultaneously having BPH and small csPCa, where PSAD would probably be lower than cutoff value. How can these patients be identified and diagnosed in timely manner?

We wholly agree with the reviewer's opinion .

As a result of careful consideration, We decided to put this in discussion. (page 15, line304-305)

Lastly, this study could not accurately describe for patients simultaneously having BPH and small csPCa, where PSAD would probably be lower than cutoff value.

7. Since PSAD is a biomarker, I would suggest that besides PHI, at least several other promising contemporary PCa biomarkers are mentioned and compared to PSAD in the discussion. Maybe a table comparing ROCs or senstivity/negative predictive values would be most informative for the reader.

Thank you for pointing this out.

We have added more details to this at page14, line 283 and Table 6.

Some biomarkers have been developed to predict the diagnosis of PCa (Table 6).