Peer Review File

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[Reviewer A]

The authors review the diagnostic value of free to total PSA, as well as PSA density and testosterone to PSA ratios in the diagnosis of prostate cancer. They have appropriately included clinically significant prostate cancer (Gleason >=7) as an additional analysis. They do not observe any significant differences in low and normal testosterone groups for free to total PSA, though the AUC was different for the PSAD.

Comment 1: This study mirrors a similar recent American study and so is not novel, and the results are limited by a small sample size. Nonetheless, the analyses and reporting is appropriately done. Overall, the study provides very small incremental information to assist clinical decisions.

Reply 1: Thank you for the comments. We agree with your opinion. However, although a few studies have reported on the role of various PSA parameters in predicting Pca, no studies have been performed in Asia, where the incidence and characteristics of Pca are somewhat different from those in Western countries. Therefore, our study would be a useful reference for physicians considering prostate biopsy and testosterone replacement therapy in Asian men with gray-zone PSA levels. We thank the Editor for these comments, and we have amended our description in the Discussion section.

Changes in the text 1: See page 13, lines 1–3

Our study would be a useful reference for physicians considering prostate biopsy and testosterone replacement therapy in men with gray-zone PSA levels.

[Reviewer B]

The authors report retrospective results regarding the predictive ability of PSA parameters stratified

by testosterone-levels. The study is of interest and fills a gap in the growing evidence about the use of PSA and basically report a negative result.

(Major issues)

Comment 1: The ROC curves are (I assume) based on the logistic regression models. The authors state that they have performed multivariate analysis (under the statistical analysis section). But I fail to see the results of such an analysis (or perhaps more appropriately a multivariable analysis) and there are only unadjusted results reported. It would nevertheless be interesting so see if testosterone can improve a multivariable model's ability to discriminate cases from non-cases and preferably both with dichotomized testosterone levels as well as with continuous T- levels. And if not – provide a rational for choice of statistical methodology.

Reply 1: Thank you for your valuable comments. We apologize for the error in Supplementary Table 1 (i.e., multivariable logistic regression). The aim of our study was not to find the predictors of Pca but to compare the diagnostic value of various PSA parameters in men with low and normal testosterone levels and gray-zone PSA levels. Therefore, we deleted the logistic regression in the Methods and Results sections, and Supplementary Table 1.

Changes in the text 1: See page 6, line 21; page 7, line 14; page 19

(Minor issues)

Comment 2: The title implies that the article will focus on free/total PSA in combination with testosterone levels when it in fact focuses on several PSA parameters

Reply 2: Thank you for your comment. We agree with your point. Accordingly, we have amended the title, abstract, and conclusions of the manuscript.

Changes in the text 2: See page 1, lines 1–3; page 2, lines 2–4; page 2, lines 18–19; page 13, lines 18–

(Title) Do prostate-specific antigen parameters have a similar role in predicting prostate cancer regardless of serum testosterone levels in men with gray-zone prostate-specific antigen levels?

Background: To evaluate whether various prostate-specific antigen (PSA) parameters have a similar diagnostic value in predicting prostate cancer (Pca) in men with gray-zone PSA levels (4.0–10.0 ng/mL) depending on different serum testosterone levels.

Conclusions: The analyzed PSA parameters showed a similar diagnostic value in predicting Pca regardless of testosterone levels in men with gray-zone PSA levels.

Conclusions

The diagnostic performance of various PSA parameters in predicting Pca was not significantly different between the low testosterone and normal testosterone groups of men with gray-zone PSA levels.

Comment 3: Page 3 line 12 refers to conflicting results regarding T and Pca in 4 studies. The newest reference is 21 y.o. there are several much more contemporary investigations regarding androgens and prostate cancer development/progression.

Reply 3: We agree with your point. Thank you for the comments. We have inserted the following references into the manuscript.

Changes in the text 3: See page 17, lines 7–13

12. Kaplan AL, Hu JC, Morgentaler A, Mulhall JP, Schulman CC, Montorsi F. Testosterone therapy in men with prostate cancer. Eur Urol 2016;69:894-903.

13. Mearini L, Zucchi A, Nunzi E, Villirillo T, Bini V, Porena M. Low serum testosterone levels are predictive of prostate cancer. World J Urol 2013;31:247-52.

14. Kim M, Byun S-S, Hong SK. Testosterone replacement therapy in men with untreated or

treated prostate cancer: do we have enough evidences? World J Mens Health 2021;39:705.

Comment 4: In the discussion section the authors state that "It is unclear whether the same cutoff value of PSA can be sufficiently sensitive and specific for detecting Pca in men with low testosterone levels compared with men with normal testosterone levels or all men". A statement substantiated by referring to the REDUCE trial (ref 26). I am unable to draw the same conclusions from the article by Andriole et al.

Reply 4: Thank you for the comments. In accordance with your comments, we have deleted the article by Andriole et al. and amended our description in the Discussion section.

Changes in the text 4: See page 11, line 22–page 12, line 3

Although several studies have investigated the diagnostic value of various PSA parameters in predicting Pca, there is still limited evidence on whether the same cutoff value of each PSA parameter can be sufficiently sensitive and specific for detecting Pca in men with low testosterone levels compared with men with normal testosterone levels or all men.

[Reviewer C]

Comment 1: This article concluded that the free-to-total prostate-specific antigen has a similar role in predicting prostate cancer regardless of serum testosterone levels in men with gray-zone prostate-specific antigen levels. However, in this article, it seemed to conclude that PSAD had more diagnostic value. Hence, I do not consider this title to be appropriate.

Reply 1: We agree with your points. Thank you for the comments. As mentioned above, we have amended the title of the manuscript.

Changes in the text 1: See page 1, lines 1–3

Do prostate-specific antigen parameters have a similar role in predicting prostate cancer regardless of

serum testosterone levels in men with gray-zone prostate-specific antigen levels?

Comment 2: P4, line 16. “Clinically significant Pca (csPca) was defined as a Gleason score of ≧7.” It should show the references for this sentence.

Reply 2: In accordance with your comments, we have inserted the following reference into the manuscript.

Changes in the text 2: See page 17, lines 20–21

17. Oon SF, Watson RW, O’Leary JJ, Fitzpatrick JM. Epstein criteria for insignificant prostate cancer. BJU Int 2011;108:518-25.

Comment 3: P9, line 7. ″Similarly, in the present study, the low~ .” However, as you probably know, reference 24 does not target the gray zone. Therefore, it seems that the word "similarly" is not appropriate.

Reply 3: Thank you for the comment. We have amended our description in the Discussion section.

Changes in the text 3: See page 11, lines 15–20

Previously, Shin et al. reported that patients with lower serum testosterone levels had a higher risk of Pca than those with higher serum testosterone levels, although low testosterone level was not associated with a higher grade of Pca (28). In the present study, the low testosterone group showed a higher incidence of Pca than the normal testosterone group (45.5% vs. 35.5%, p = 0.030), although the incidence of csPca did not differ between the groups.

Comment 4: P10, line 11. “A randomized clinical trial with a larger cohort is needed to confirm our results.” It should describe the plans and directions for future research.

Reply 4: In accordance with your comment, we have inserted the following sentence into the Discussion section.

Changes in the text 4: See page 13, lines 13–15

A randomized, prospective study with a larger sample size is warranted to verify the diagnostic value of PSA parameters depending on the serum testosterone levels.

Comment 5: Supplementary Table 2. Total number of free-to-total PSA ratio and PSA density in the category of positive of Pca in low testosterone men was 206 and 150, respectively. However, the total number seems to be 220, what do you think? The reason why the number does not match should be mentioned. If there is no reason, the data must be corrected.

Reply 5: Thank you for the meticulous review of our paper. We apologize for the mistake in creating the table. We have amended the supplementary table and the Results section.

Changes in the text 5: See page 8, lines 9-10; pages 20-21

A contingency table evaluating the accuracy of free-to-total PSA ratio and PSA density in diagnosing Pca and csPca is presented in Supplementary Table 1.

Supplementary Table 1. Contingency table evaluating the accuracy of free-to-total PSA ratio and PSA density in predicting Pca and csPca

	Pca in men with low testosterone level		Total	Pca in men with normal testosterone level		Total
	Positive	Negative		Positive	Negative	
Free-to-total PSA ratio < 16.25†	134	86	220	36	43	79
Free-to-total PSA ratio ≥ 16.25†	86	177	263	18	55	73

Total	220	263	483	54	98	152
PSA density ≥ 0.197 ng/mL^{2†}	136	73	209	37	40	77
PSA density < 0.197 ng/mL^2	84	190	274	17	58	75
Total	220	263	483	54	98	152

	csPca in men with low testosterone level		Total	csPca in men with normal testosterone level		Total
	Positive	Negative		Positive	Negative	
Free-to-total PSA ratio < 16.38	90	136	226	23	56	79
Free-to-total PSA ratio ≥ 16.38†	42	215	257	9	64	73
Total	132	351	483	32	120	152
PSA density ≥ 0.195 ng/mL^{2†}	87	130	217	22	55	77
PSA density < 0.195 ng/mL^2	45	221	266	10	65	75
Total	132	351	483	32	120	152

†The cutoff values of free-to-total PSA ratio and PSA density for Pca are 16.25 and 0.197 ng/mL^2, respectively, and those for csPca are 16.38 and 0.195 ng/mL^2, respectively.

Pca, prostate cancer; csPca, clinically significant prostate cancer, PSA, prostate-specific antigen

Comment 6: In each category, sensitivity and specificity were examined, but I suggest that further examination of PPV, NPV, and accuracy would improve this article.

Reply 6: We agree with you. Thank you for the comments. We did not describe the mentioned data because they can be affected by the prevalence of Pca in a specific lesion, which was described as a limitation. In accordance with your suggestion, we added the PPV, NPV, and accuracy for each variable (limited to Pca) in the Results section.

Changes in the text 6: See page 7, line 22–page 8, line 3

The cutoff value of free-to-total PSA ratio and PSA density with the best sensitivity and specificity for predicting Pca was 16.25 (sensitivity, 62.04%; specificity, 64.27%; accuracy, 63.3%; positive predictive value [PPV], 56.86%; and negative predictive value [NPV], 69.05%) and 0.197 ng/mL^2 (sensitivity, 63.14%; specificity, 68.98%; accuracy, 66.3%; PPV, 60.48%; and NPV, 71.06%), respectively.