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Peer Review File

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Reviewer Comments:

This paper examines the diagnostic utility of serum DACT-2 promoter methylation as a diagnostic marker for PCa. A diagnostic marker that outperforms PSA is highly desirable, as PSA has low specificity and sensitivity. Thus, the question addressed is clinically relevant.
The authors claim that DACT-2 is superior to PSA levels as a screening test for PCa. However there are major methodological issues, the most important being

Comment 1: the PSA cut-off they selected is not the one used in clinical practice

Reply 1: This is an interesting question. Prostate specific antigen (PSA) is commonly used to detect prostate cancer. However, Only 26% of patients with a PSA within the grey zone of 4.1–9.9 mg/mL have prostate cancer (Arthritis Res Ther. 2019 May 2;21(1):111; Int J Clin Pract, 2016 Nov;70(11):950-959). Therefore, the diagnosis of patients in the "grey zone" is controversial. In the present study, 66 patients with PCa, 22 patients with benign prostatic hyperplasia and 47 healthy controls were consecutively enrolled in this prospective observational study. Based on the differential serum level of PSA in patients with PCa and BPH, and the healthy person, ROC curve analyses of the serum PSA levels was performed. The serum PSA levels differentiated PCa patients from BPH patients and healthy person (AUC = 0.59; CI = 0.45–0.73; P = 0.258) in this group of samples analyzed. For the discrimination between PCa and Non-cancer patients, the optimal cut-off point of PSA was 7.56, with a sensitivity of 80.1% and a specificity of 59.4%.

Comment 2: Biopsy not available for BPH and health controls

Reply 2: This is a very good question. Indeed, Biopsy was not available for BPH and health controls. In the present study, men that were diagnosed with benign prostatic hyperplasia were used color Doppler ultrasound examinations as benign group. Men with normal findings both at physical and color Doppler ultrasound examinations were included in the healthy group which has been added to the Materials and Methods as list in lines 108-111.

Comment 3: PSA levels were not available for healthy subjects.

Reply 3: This is a constructive suggestion. We had detected the PSA level of healthy subjects and added the results to the table1.

Comment 4: A control group of people with both PSA levels and (negative) biopsy results would be informative.

Reply 4: This is a good suggestion. Ideally, everyone enrolled in this study undergone PSA examination and biopsy. However, biopsy, an invasive test, is not acceptable to everyone, especially those with normal findings both at physical and color Doppler ultrasound examinations.

In the present study, PSA level of healthy subjects was available, whereas no information of biopsy was available for healthy subjects.

Comment 5: Line 1 Abbreviations are not appropriate for the title. Please revise PCa.

Reply 5: Thank you for the comment. We have revised the title as "**Diagnostic Value of DACT-2 Methylation in Serum of Prostate cancer Patients".**

Comment 6: line 36 'And we further evaluated' It is not grammatically correct to start a sentence with and. In addition/furthermore or something similar would be better. This has been done several times throughout the manuscript. Please revise accordingly.

Reply 6: Thank you for the critical comment. This is our mistake. We have revised 'And we further evaluated' as 'Further, we evaluated'.

Comment 7: Line 39 Do you mean PSA levels?

Reply 7: This is our mistake. Thank you for the critical comment. We have revised in the article.

Comment 8: Line 57 Abbreviations should be written in full when first mentioned in the text

Reply 8: Thank you for the comment. We revised PCa as **Prostate cancer.**

Comment 9: Lines 67-68 Not sure what the authors want to say here. Is PCa screening performed less frequently due to the combination of PSA screening and random biopsy? Not sure if that is true. There are guidelines that may advise against PSA screening but it is not PSA

perse that is causing the decline (if any).

Reply 9: Thank you for the comment. We have been revised in the article, we want express that the sensitivity and specify of PSA screening and random biopsy is not high.

Comment 10: Line 73 Methylation of promoter regions leads to reduced expression of the gene (reduced access of polymerase) not downregulation of the gene and definitely not deletion of the gene. Please revise.

Reply 10: Thank you for the comment. We have been revised in the article.

Comment 11: Line 110 Uterine myoma in men? Are those the BPH patients? Also what do you mean pathologically? As in transurethral resection? Was there a biopsy done in BPH patients and in health subjects?

Reply 11: I am so sorry about this mistake. Thank you for the critical comment. We have revised in the article. These are the BPH patients. BPH patients and health subjects were diagnosed with color doppler ultrasound examinations.

Comment 12: Line 117 Define treatment (radical prostatectomy?). Please also clarify if Gleason scores were taken from biopsies or prostatectomies and also which system was used for Gleason scoring (i.e. was the 2005 ISUP recommendations taken into account? What about the 2014 recommendations?)

Reply 12: I am so sorry for our unclear description. After color doppler ultrasound examinations，blood samples were collection from patients with BPH and healthy controls. Blood samples of patients with PCa were obtained on the day before treatment including radical prostatectomy, pre-operative chemotherapy and radiotherapy. And the pathological parameters, Gleason scores were taken from biopsies or prostatectomies. We have revised in the article.

Comment 13: Line 118 Why did you use the 1997 TNM system? The 7th edition of the AJCC staging system was published in 2010.

Reply 13: Thanks for your constructive suggestion. According to the 7th edition of the AJCC staging system, PCa patients were re-evaluated for pathological stage.

Comment 14: Line 145 Which instrument?

Reply 14: this instrument is An ABI 7500 system (Applied Biosystems). We have revised in the article.

Comment 15: Line 150 level of 7.56 is somewhat high to be used a cut off. 4ngr/ml is the widely accepted cut off for PSA.

Reply 15: This is an interesting question. A lot of clinical practice have confirmed that 4-10ng/ml is the gray zone for PSA in the prostate cancer diagnosis(Arthritis Res Ther. 2019 May 2;21(1):111; Surgeon. 2014 Dec;12(6):323-7). In the present study, 66 patients with PCa, 22 patients with benign prostatic hyperplasia and 47 healthy person were consecutively enrolled in this prospective observational study. Based on the differential serum level of PSA in patients with PCa and BPH, and the healthy person, ROC curve analyses of the serum PSA levels was performed. The serum PSA levels differentiated PCa patients from BPH patients and healthy person (AUC = 0.59; CI = 0.45–0.73; P = 0.258) in this group of samples analyzed. For the discrimination between PCa and Non-cancer patients, the optimal cut-off point of PSA was 7.56, with a sensitivity of 80.1% and a specificity of 59.4%.

Comment 16: Line 189 as noted above the cut off used for PSA is rather high compared to the one used in clinical practice. Do your clinicians use this cut off to decide which patients need further work up (i.e. biopsy)? You should compare the marker you are testing in regards to the standard of practice if you want to claim that the marker is better. You need to perform statistics again using a more appropriate cut off for PSA.

Reply 16: In the present study, 66 patients with PCa, 22 patients with benign prostatic hyperplasia and 47 healthy person were consecutively enrolled in this prospective observational study. Based on the differential serum level of PSA in patients with PCa and BPH, and the healthy person, ROC curve analyses of the serum PSA levels was performed. The serum PSA levels differentiated PCa patients from BPH patients and healthy person (AUC = 0.59; CI = 0.45–0.73; P = 0.258) in this group of samples analyzed. For the discrimination between PCa and Non-cancer patients, the optimal cut-off point of PSA was 7.56, with a sensitivity of 80.1% and a specificity of 59.4%.

Comment 17: Line 167 was there any follow-up on BPH and healthy controls? Was any of

them diagnosed with PCa subsequently? Especially those with DACT methylation.

Reply 17: Thank you for the critical comment. This is an important advice, but we have not taken the follow-up BPH and healthy controls who was with DACT methylation. In the future research we can do the work.

Comment 18: Paragraphs of lines 169-177 and 190-199 repeat the same results in different wording.

Reply 18: Thank you for the critical comment. In fact they are different, paragraphs of lines 169-177 described the methylation status of DACT-2 gene in serum of PCa patients using the Methylation-specific polymerase chain reaction (MSP), whereas the paragraphs of lines 190-199 revealed the rate of DACT-2 methylation in serum of PCa patients using the Real-time methylation-specific PCR.

Comment 19: Line 203 detection of PSA should be rephrased as the detection of enhanced PSA levels

Reply 19: According to your suggestion, we have revised this part in the manuscript.

Comment 20: Lines 205-207, 213-214 need rephrasing as they are not making sense

Reply 20: We are very sorry for our unclear statement, which has been revised according to the Reviewer's comments.

Comment 21: Table 1 PSA levels were not available for healthy controls. This should be clearly stated in the manuscript. Also if you did a subgroup analysis in the <4ngr/dl group of patients, would the addition of methylation analysis provide any benefit?

Reply 21: According to your suggestion, we have revised the table 1. And we try to do a subgroup analysis in the <4ng/ml group of patients, No methylation of DACT-2 gene were observed.

Comment 22: Figure 2 Is there a statistical difference in the AUC between PSA and DACT2?

Reply 22: ROC curve analyses shown that the DACT-2 methylation rate differentiated PCa from non-cancer patients (AUC = 0.86; CI = 0.72–0.95; $P<0.001$), with a more favored ROC

than serum PSA levels (AUC = 0.59; CI = 0.45–0.73; P = 0.258) in this group of samples analyzed. The optimal cut-off point of the optimal cut-off point of DACT-2 methylation rate was 0.745 with a sensitivity of 81.8% and a specificity of 75.0%, whereas the serum PSA value cut-off was 7.56(ng/ml), with a sensitivity of 80.1% and a specificity of 59.4%.These results suggest that the DACT-2 methylation rate can distinguish PCa from non-cancer patients, and that the DACT-2 methylation rate is a better parameter than serum PSA level measurement for the differential diagnosis of these prostatic diseases.

Comment 23:There are many grammatical and syntactic errors. A revision by a professional language editor or a native English speaker is highly advised. Some examples include
Line 63 'which tends to diagnostic….' this sentence does not make sense. Please revise.
Line 66 'they overrate the low risk of disease and undervalue the risk…' this sentence does not make sense. Please revise.

Reply 23: This is a good suggestion. The whole text has been revised and edited by English writing assistant system "grammarly" and a professional English editing company.