Author’s response to reviews

Title: Clinical evaluation of prostate cancer gene 3 score in diagnosis among Chinese men with prostate cancer and benign prostatic hyperplasia

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Author’s response to reviews:

Sep 22, 2015
Dr. Homayoun Zargar, MD
Editor-in-Chief
BMC Urology
Washington University
RE: Ms. Ref. No.: BURO-D-15-00013

Clinical evaluation of prostate cancer gene 3 score in diagnosis among Chinese men with prostate cancer and benign prostatic hyperplasia

Dear Dr. Homayoun Zargar,

Attached please find the revised manuscript entitled “Clinical evaluation of prostate cancer gene 3 score in diagnosis among Chinese men with prostate cancer and benign prostatic hyperplasia” for your review. The representativeness of subjects in our study was mentioned in the discussion section. The potential value of PCA3 as a predictor of clinically significant disease was explored and the analysis results was added to the results section. We also clarify the reason for using prostate tissue samples rather than urine samples for PCA3 detection. In addition, we have carefully considered and responded to all other comments from the reviewers and editors. All changes have been detailed in the point-to-point responses to reviewers listed on the following pages. My coauthors and I would like to thank the reviewers for their expert opinions. We believe that this manuscript has been improved by this revision. Thank you very much for agreeing to review our revised manuscript.
Response to Reviewer Comments:

BURO-D-15-00013

Clinical evaluation of prostate cancer gene 3 score in diagnosis among Chinese men with prostate cancer and benign prostatic hyperplasia

Jin Huang; Kathleen H. Reilly; Hui-Zhen Zhang; Haibo Wang

BMC Urology

Dear Mr Wang,

Your manuscript "Clinical evaluation of prostate cancer gene 3 score in diagnosis among Chinese men with prostate cancer and benign prostatic hyperplasia" (BURO-D-15-00013) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Urology.

Their reports, together with any other comments, are below. Please also take a moment to check our website at

http://buro.edmgr.com/l.asp?i=2121&l=1RPLXVRK for any additional comments that were saved as attachments. Please note that as BMC Urology has a policy of open peer review, you will be able to see the names of the reviewers.
If you are able to fully address these points, we would encourage you to submit a revised manuscript to BMC Urology. Once you have made the necessary corrections, please submit online at:

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Please include a cover letter with a point-by-point response to the comments, describing any additional experiments that were carried out and including a detailed rebuttal of any criticisms or requested revisions that you disagreed with. Please also ensure that all changes to the manuscript are indicated in the text by highlighting or using track changes.

Please also ensure that your revised manuscript conforms to the journal style, which can be found at the Instructions for Authors on the journal homepage.

A decision will be made once we have received your revised manuscript, which we expect by 10 Oct 2015.

I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Best wishes,

Homayoun Zargar

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Reviewer reports:

Reviewer #1: This is an interesting retrospective review of PCA3 and its correlation with PSA on a Chinese population as a detection tool for prostate cancer. PCA3 was a predictor of prostate cancer but interestingly did not show any correlation with high-risk (Gleason \( \geq 8 \)) prostate cancer.

There are several major issues that need to be addressed:

1) This is not a true screening population. These were patients selected because of a clinical concern for prostate cancer. The median age of the study population is also slightly older
than a typical screening population. Additionally, PSA levels are much higher than a typical screening population. Therefore, the conclusions of this paper are only valid in a Chinese population with high clinical suspicion for prostate cancer and not in the general population. This selection bias and limitation should be mentioned by the authors.

The selection bias and limitation have been mentioned in discussion section as follows:

The study population was referred to a PCA3 test for several reasons (i.e., a high PSA level or suspicious prostate cancer), therefore, those who were selected to have a PCA3 test because of a clinical concern for prostate cancer may differ from screening populations referred to triage testing. These subjects in the study had a median age of 70 years (IQR 66-77) with a relatively high PSA level (median 13.67; IQR 7.98-29.02), which is higher than that of a typical screening population. The subjects recruited with high clinical suspicion for prostate cancer could not represent the population in China, more unlikely to reflect the actual situation in China.

2) Did the authors look at PCA3 as a predictor of clinical significant prostate cancer (Gleason ≥7). Even though PCA3 was not a predictor of high-risk disease, a more relevant question would be to determine if PCA3 was a predictor of clinically significant disease.

We have analyzed the potential role of PCA3 as a predictor of clinically significant disease, but did not find a significant difference. We have added the relevant results in the results section as follows:

“There was also no significant difference in PCA3 scores between prostate cancer patients with biopsy Gleason score 3-6 (94.26, IQR 41.81-326.44) and patients with biopsy Gleason score ≥7 (139.02, IQR 42.66-295.69, P= 0.56).”

3) How were cut-offs determined from the ROC curve for PCA3? What methodology was used? Typically the Youden method is used to determine cut-offs points (highest combined sensitivity and specificity) but the authors do not comment on this methodology in the manuscript.

The methodology used for determining cut-offs of PCA3 in diagnosing prostate cancer was described in the statistical methods section as follows:

“The Youden index, calculated as sensitivity+specificity-1, was used for capturing the maximum vertical distance of the ROC curve and for determining cut-offs points.”

4) The authors comment that PCA3 had a better diagnostic accuracy than PSA, but this is not reflected by the results as the difference in AUC was not significant and both variables were independent predictors of prostate cancer in their multivariate model. This wording need to be changed throughout the manuscript to soften the conclusions.
We have changed the wording of this expression throughout the manuscript.

In the conclusion section it was revised as follows:

“In this population, the PCA3 score had a comparable diagnostic accuracy with PSA as there was no significant difference in ROC AUC between PCA3 score and PSA.”

However, the sensitivity and specificity comparison based on different cut-offs was kept the same in the results section as follows:

“The sensitivity of PCA3 score (≥35) was 80.4% (90/112) (95% CI 71.8-87.3) and the specificity was 62.5% (15/24) (95% CI, 40.6-81.2). The sensitivity and specificity of PSA (≥4 ng/ml) were 95.5% (107/112) (95% CI 89.9-98.5) and 16.7% (4/24) (95% CI 4.7-37.4), respectively; while the sensitivity and specificity of PSA (≥10 ng/ml) were 71.4% (80/112) (95% CI 62.1-79.6) and 58.3% (14/24) (95% CI 36.6-77.9). The sensitivity for detecting prostate cancer was comparable, but the specificity was significantly lower for PSA (≥4 ng/ml) than PCA3 score (≥35). Although without statistical significance, both sensitivity and specificity of PSA (≥10 ng/ml) were lower than PCA3 score (≥35).”

5) Did the authors considering using PSA and PCA3 as continuous variables in their model? Categorizing this variable can often result in a loss of power although it may be more clinically applicable.

We agreed with reviewer on this point. Categorizing this variable can result in a loss of power in most situations, but it is difficult to determine whether categorizing the continuous variable is better or worse. The following is the multivariate analysis result if the variable was kept in the model as continuous variable.

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA3 score</td>
<td>1.000 (1.000-1.001)</td>
<td>0.3207</td>
</tr>
<tr>
<td>PSA level</td>
<td>1.069 (1.007-1.135)</td>
<td>0.0284</td>
</tr>
</tbody>
</table>

There may be small variation in the risk of prostate cancer among men if the PCA3 score is lower than the cut-off values, but the risk had a significant increase if the PCA3 score is greater than the cut-offs. So categorizing this variable may be more suitable for this study. Moreover, the cut-offs can be used more easily in practice.

Reviewer #2: This is a relatively well-written manuscript evaluating the role of utility of PCA3
assay to predict biopsy-detected prostate cancer in Chinese men. While the authors limit the generalizability of their findings by exclusively studying Chinese men, they were upfront about this in their study objectives. Overall, they found their assay of PCA3 to be a valuable predictor for prostate cancer on biopsy, and found both PCA3 and PSA to be independent predictors of finding cancer on biopsy.

While the statistical analyses are relatively robust and well described, the most significant weakness of the manuscript comes in the description of their methods of handling and obtaining tissue. The most significant issues are as follows:

- Methods: Section 2.2 This section of the paper included an over-abundance of detail beyond what is relevant for most clinicians.

We agreed with the suggestions of reviewer. And the description on sample testing procedure has been shortened as follows:

“These samples after incubation were used for the detection of PCA3 and PSA mRNA according to the manufacturer’s instructions. The detection was based on branched DNA (bDNA) technology (DiaCarta, CA, USA), which is a sandwich nucleic acid hybridization procedure for the direct quantitation without RNA purification or reverse transcription polymerase chain reaction. The capture plate containing sample and bifunctional oligonucleotide probe sets was read on the Kodia QuantiVirus® Luminometer System. The PCA3 score was calculated as (PCA3 mRNA)/(PSA mRNA) ×1000. Transrectal ultrasound guided biopsy with at least 10 peripheral zone cores was performed, and the specimens were reviewed by local pathologists.”

However, the methods description about sample disposal was kept the same as the sample was formalin-fixed, paraffin-embedded tissue block rather than a urine sample. More detailed description is beneficial to the understanding for readers. More importantly, the sample disposal is also a necessary section in methods required by journal.

- What is lost in this methods section and subsequent discussion is that this analysis appears to be evaluating PCA3 and psa mRNA from prostate tissue that has already been collected. While this may have been relevant to early studies examining this as a biomarker, the current clinical relevance is more closely tied to serum PSA measurements, and urine PCA 3 tests to help determine the need for biopsy. In this manuscript, the authors declare utility of PCA3 as a biomarker, but are basing this on tissue samples (which presumably would only be available after a biopsy), without relating this directly to appropriate urinary biomarker results. Furthermore, they do not clearly cite or describe any evidence or literature that has previously clarified the relationship between urinary PCA3 and tissue mRNA levels. Thus, this study appears to be using information that would only be obtained after a biopsy, in attempt to inform biopsy decision making, when both PSA and PCA3 can be determined prior to biopsy through other methods.
While this study, limited to Chinese men, does suggest utility for PCA3 as a biomarker, using urine samples (which is most clinically useful) and examination of men who subsequently underwent biopsy would be a much more powerful, and clinically useful analysis.

We agree with reviewer’s viewpoint that the current clinical relevance is more closely tied to serum PSA measurements and urine PCA3 tests to help determine the need for biopsy. As the reviewer suggested that both PSA and PCA3 could be determined prior to biopsy, urine sample examination can subsequently be used to inform biopsy decision making. So it would be a much more powerful and clinically useful analysis.

However, the current study has two different purposes. First, the method in our study was an auxiliary diagnosis marker rather than a screening indicator as suggested by reviewer. The gene probe reagent approved by American FDA was used to test urine sample when the cases are still suspected after biopsy. However, more discomfort and pain would result from the massage of the prostate and sampling urine specimens after biopsy. Therefore, the unnecessary pain and discomfort can be avoided if the prostate tissue from the biopsy can be used for direct testing. Secondly, direct observation can be achieved based on biopsy prostate tissue due to visualized RNA testing methods rather than chemical signals method. Thirdly, the results of the current study could be used for the foundation of the next step. Based on the current results, we would carry out another study to explore the diagnostic significance of PCA3 in urine samples among screening population.

And we also acknowledge our limitation on this point and our next research plans in the discussion section as follows:

“Finally, PCA3 testing was based on formalin-fixed, paraffin-embedded tissue blocks collected before biopsy, therefore the clinical relevance was limited. To help determine the need for biopsy decision in screening populations, the diagnostic significance of urinary PCA3 testing will be explored in a future study.”

And we hope the results of our future study will be considered by this journal when it is completed.

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If ethics was not required for your study, then this should be clearly stated and a rationale provided.

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If individual clinical data is presented in your article, then you must clarify whether consent for publication of these data was obtained.

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