February 13, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8190-review.doc).

Title: Alternative mechanisms for PSA elevation: a prospective analysis of 222 TURP patients

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The manuscript has been improved according to the suggestions of reviewers. The comments have been added, a summary has been provided and the references have been adjusted according to the journal requirements.

Thank you again for publishing our manuscript in the *World Journal of Clinical Urology.*

Sincerely yours,

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Answers to the reviewers

Reviewer 00505708

1. Description of statistical analysis too simple and does not reflect all results in the tables

   We agree with the reviewer. We have therefore updated the section on the statistical analysis so that it is more in line with the results shown in the tables.

2. Inflammation is a major factor studied. Why is inflammation not included in Table 1 and Table 2?

   We cannot include a categorical variable in Table 1 or 2. As an alternative we could provide a table with the frequencies of all levels of inflammation (cfr. table 1 for continuous variables) and a table with means and standard deviations of log(PSA) levels for all different categories (cfr. Correlation between two continuous variables)

ACUTE	N	Mean	Std Dev	Minimum	Maximum
0	25	-0.061	0.855	-1.609	2.018
1	91	1.031	0.969	-1.204	2.981
2	88	1.323	0.975	-0.511	3.481
3	14	0.994	0.994	-0.511	3.266
CHRONIC	N	Mean	Std Dev	Minimum	Maximum
0	4	-0.088	0.550	-0.511	0.693
1	109	0.853	1.048	-1.609	3.463
2	91	1.268	1.009	-1.204	3.481
3	14	1.048	0.885	-0.511	2.874

   From the above table, one can conclude the frequencies of all different levels of inflammation together with the corresponding mean values for log(PSA).

   In case PSA values are preferable, the table below can be used.

ACUTE	N	Mean	Std Dev	Minimum	Maximum
0	25	1.413	1.633	0.200	7.520
1	91	4.363	4.338	0.300	19.700
2	88	5.923	6.365	0.600	32.500
3	14	4.631	6.623	0.600	26.210
CHRONIC	N	Mean	Std Dev	Minimum	Maximum

ACUTE	N	Mean	Std Dev	Minimum	Maximum
0	4	1.037	0.655	0.600	2.000
1	109	4.036	5.038	0.200	31.900
2	91	5.672	5.848	0.300	32.500
3	14	4.156	4.413	0.600	17.700

3. Multiple regression analysis:
   - What were the criteria in the selection of the variables

     The selection criteria were based on the AIC and R-squared adjusted. This is now better explained in the text (updated statistical analysis). The following section has been added to the manuscript: 'Selection of the variables included in the model for log PSA was based on the AIC criterion and the adjusted r-square. With respect to the variable active inflammation, it might seem not realistic from a clinical point of view to assume that the difference between any consecutive levels of the active inflammation was the same or the increase was linear in nature. For this reason the choice was made to treat inflammation as a categorical variable rather than a continuous. Furthermore this choice resulted in a better fit of the model.'

   - Why was active inflammation treated as a categorical variable

     With respect to the variable active inflammation it might seem not realistic from a clinical point of view to assume that the difference between any consecutive levels of the active inflammation was the same or the increase was linear in nature. For this reason the choice was made to treat inflammation as a categorical variable rather than a continuous. Furthermore this choice resulted in a better fit of the model.

     The following section has been added to the manuscript: 'Selection of the variables included in the model for log PSA was based on the AIC criterion and the adjusted r-square. With respect to the variable active inflammation, it might seem not realistic from a clinical point of view to assume that the difference between any consecutive levels of the active inflammation was the same or the increase was linear in nature. For this reason the choice was made to treat inflammation as a categorical variable rather than a continuous. Furthermore this choice resulted in a better fit of the model.'

4. Adjusted R-square = 0.38 , which suggests that a substantial proportion of PSA variation in this study population is caused by other factors. This contribution should be briefly discussed.

   It is certainly possible that the inclusion of any other information in this analysis might increase the value of R-square and R-square adjusted indicating that indeed such factors also explain part of the variation in PSA levels. However, since we did not have any

further information to be used other than the information we used in this analysis, it might be part of further experiments to try and collect any additional information which might from a clinical perspective result in statistical models explaining more of the variation in PSA levels.

In literature, it can be found that PSA is directly correlated to age and prostate volume, as well as prostate volume is related with age (JAMA 1993 Aug 18;270(7):860-4: Serum PSA in a community based population of healthy men. Estabishment of age specific reference ranges: Oesterling et al). Age related PSA values could be useful in refining PSA testing. However, age specific values can result in missing up to 60% of cancers in men older than 60 yrs (Urology 2000; 56: 255-260: Comparison of percent free PSA, PSA density and age specific PSA cut-offs for prostate cancer detection and staging: Catalona et al). Moreover, prostate biopsy need was not safely eliminated in men 60-79 yrs by referring to age ajusted PSA values (J Urol 1998; 159 (2): 444-448: Age specific PSA reference ranges: population specific: Borer et al). On the other hand, PSA density that correlates PSA with prostate volume is a more reliable way of fine tuning PSA evaluation (J Urol 1992; 147:817-821: The use of PSA density to enhance the predictive value of intermediate levels of serum PSA; Benson et al).

5. Consistent observation that inflammatory conditions increase levels of PSA. The innovative aspect of this study needs to be discussed in more detail.

   Elevated and/or rising PSA levels with regard to underlying prostate cancer and prostatic inflammations are well known. In this paper, we showed that PSA could also be indicative of BOO, not only with respect to observational data but also with regard to statistically relevant correlations between PSA and PdetQmax. Therefore, PSA levels should be taken into account or could even be used as a biomarker when a treatment strategy is determined or executed for patients suffering from clinical BPH. Additionally, the results obtained in this study indicate that inflammation can be correlated with PSA levels. This implies that elevated PSA levels should also be considered as predictive for prostate inflammation as well as for prostate cancer.

Reviewer 00505652

1. Statistical analysis:
   - What is the null hypothesis and what test was used to compare all parameters

     There is not one single null hypothesis in this experiment but when focusing on the multiple regression analysis we are interested in whether a certain parameter is significantly different from 0. This yields several hypotheses in the format H_0: $\beta = 0$ versus H_1: $\beta \neq 0$. In order to test for this hypothesis the t-test and the F-test was used.

   - Multivariate analysis should be carried out

     A multiple regression analysis was performed and the details about this analysis are now better explained. A multivariate analysis is not preferable here since this means we are investigating several response variables which is not the case here.

2. I think that an accurate microbiological analysis (Meares-Stamey test) is needed in order to correlate the grade of flogosis and eventually pathogens found. Please discuss it.

   Meares-Stamey culturing was not performed on a routine basis in our population since we are studying a TURP population, for the same reason STD patients do not occur in this patient population. We consider Meares-Stamey culturing as probably interesting in a chronic bacterial prostatitis population that is completely different from the population we analysed (Asian J Androl 2009; Jul; 11(4):461-77: Semen analysis in chronic bacterial prostatitis: diagnostic and therapeutic implications; Magri et al).

3. What about patients with suspected STDs

   For an answer to this question, we refer the reviewer to the answer of the previous question.

Reviewer 02446005

All correlations were performed without correction for age and other confounders. I personally think that such correlations are essential. I suggest to perform the statistical analysis in this way and draw conclusions accordingly.

We agree with the reviewer. We feel that the previous description of the statistical analysis did not correspond 100% with the results and this might have caused some difficulties for the reviewers. We indeed performed all correlations using simple correlations between any 2 variables where calculating a correlation was applicable. This was only done as an exploratory step since later on indeed, as the reviewer suggested, we carried out a multiple regression analysis which investigates the relation between a response variable and several potential explanatory variables of confounders. Therefore, we updated the description of the analysis in the manuscript explaining this strategy in more detail.

We can also add to the following to this discussion: PSA is directly correlated to age and prostate volume, as well as prostate volume is related with age (JAMA 1993 Aug 18;270(7):860-4: Serum PSA in a community based population of healthy men. Estabishment of age specific reference ranges: Oesterling et al). Age related PSA values could be useful in refining PSA testing. However, age specific values can result in missing up to 60% of cancers in men older than 60 yrs (Urology 2000; 56: 255-260: Comparison of percent free PSA, PSA density and age specific PSA cut-offs for prostate cancer detection and staging: Catalona et al). Moreover, prostate biopsy need was not safely eliminated in men 60-79 yrs by referring to age ajusted PSA values (J Urol 1998; 159 (2): 444-448: Age specific PSA reference ranges: population specific: Borer et al). On the other hand, PSA density that correlates PSA with prostate volume is a more reliable way of fine tuning PSA evaluation (J Urol 1992; 147:817-821: The use of PSA density to enhance the predictive value of intermediate levels of serum PSA; Benson et al).

Reviewer 00505691

In the materials and methods section as well as in Table 1 there is some interesting data mentioned which is subject to scientific criticism e.g. min Pdet Qmax was 10 cm H_2O (but that is not evidence of BOO!!), the maximum size of prostate removed during tURP was 189 g (difficult to believe in the time constraints of a TURP) as well as the operation time (do the authors mean resection time or total operation time since the maximum 110 min are well over the advisable 60 min resection time for TURP-was the resection with monopolar/glycine or bipolar/Saline???). This needs to be clarified.

According to Griffiths Pdet Qmax of 10 cm H_2O is indeed not considered to be obstructive, this patient was however a patient with recurrent urinary retention and did not respond in the past to alpha 1 blockers (Neurourol Urodyn 16:1-18: Standardization of terminology of lower urinary tract function. International society subcommitte on standardization of terminology of pressure flow studies; Griffiths et al).

Our centre is a high volume centre considering TURP procedures. In case of prostates with high expected volume, special precautions are made especially by the anesthesiology department (for example I.V. Furosemide and administration of NaCl I.V.) The same goes for longer resection times. Procedures are done monopolar/glycine since this allows us to use a 28 Charriere resectoscope, which makes “shorter” resection time possible. Bipolar/saline has a maximum loop of 24 Charriere which will result in an even longer resection time.