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# Opponent's report

Dissertation thesis:

**Diagnostic and prognostic ability of selected markers of prostate cancer in the serum and urine**

Author: MUDr. Joana Isabel Do Carmo Silva, Urologická klinika 2. LF UK a FN Motol, Praha

Supervisor: doc. MUDr. Štěpán Veselý, Ph.D.

Selected topic:

The search for biomarkers of prostate cancer (PC) is still very topical, as apart from the routinely used PSA, our possibilities in terms of reliable laboratory diagnosis, follow-up and prediction of the prognosis of the disease are still limited. In the diagnosis of PC, biomarkers are replaced by very precise mpMRI of the prostate, and here the importance of biomarkers rather declines. However, when determining the risk of biochemical recurrence (BCR) or progression of the disease, the available biomarker could influence the choice of treatment and the regimen of follow-up. Genomic classifiers are a step towards personalizing the care of patients with PC, but they are not available, and the considerations of their routine use are controversial. From this point of view, the chosen topic is current, especially study No. 3 concerning the prediction of biochemical recurrence and persistence of the disease using PSA and HK2 isoforms.

Methods:

These are 3 different studies evaluating 1. the diagnostic value of urinary Engrailed 2 (EN2) biomarker, 2. the role of early ultrasensitive PSA testing in predicting BCR, and 3. the role of preoperative and postoperative PSA isoform values in predicting BCR. The research is conducted as biomarker testing on retrospectively archived samples. Project 1 and 2 was supported by AZV ČR grant 15-33910A "Laboratory and clinical testing of candidate oncomarkers of prostate cancer using a complex cryobank of samples from long-term monitored patients".

Results and new findings:

The benefit of EN2 in urine for the diagnosis of prostate cancer has not been proven. EN2 levels do not correlate with tumor clinicopathological characteristics. From the point of view of PSA testing after radical prostatectomy, the correlation of PSA levels 30 days and 60 days after surgery with BCR was demonstrated, with the cut-off values corresponding to 0.068 and 0.015 ng/ml, respectively. By using these values, it would be possible to significantly reduce the rate of overtreatment (up to 37% in the study) of patients with unfavorable pathological characteristics of the tumor.

Preoperative [-2]proPSA and PHI values are better predictors of BCR than PSA alone. Postoperative testing is not beneficial in terms of predicting BCR, PSA continues to play the most important role here.

Objectives:

The work has clearly defined goals and clearly conceived hypotheses.
Study hypothesis 1: EN2 can be used in PC diagnosis adding important information regarding tumor size and stage and prostatic manipulation will increase its urinary levels.
Study hypothesis 2: Early and multiple PSA sampling after RP will provide an optimal timing and cutoff value and allow stratification of patients for adjuvant treatment.
Study hypothesis 3: Isoforms of PSA outperform conventional PSA in predicting BCR.
The thesis fulfilled its objectives, hypothesis 1 was disproved, hypotheses 2 and 3 were confirmed.

Formal processing:

The applicant presents 3 studies and chose a parallel way of presenting the objectives, methods, results and conclusions. This disrupts the continuity of the work to a certain extent; on the other hand, it corresponds to the intention to present all three studies. It is questionable whether this was necessary in the dissertation, one or two studies in which the applicant was the first author of the published results would have been sufficient. The work thus gives the impression of a commented mini summary of publications instead of a dissertation thesis. In addition, the second thesis presented was already published in 2017. I have no objections about the formal processing of the thesis, the scope and structure correspond to a standard dissertation. The work is clear, a uniform citation style is followed, and the graphic design is exemplary.

Limitations:

A limitation of all three studies is their retrospective nature.
The author supplements the text with images taken from other publications. From the point of view of copyright, it is necessary to explicitly state (e.g. in the statement or at the end of the work) whether these images were used on the basis of a valid license allowing free distribution (e.g. Creative Commons), on the basis of authorization by the copyright center (RightsLink or other) or on the basis of the obtained consent of the publisher, or copyright owner (or a combination of the above). It is not enough to simply provide a citation.
In study 1, patients over 50 years of age with a negative oncological history and screening were selected as the control group, which does not exclude the presence of prostate cancer, moreover, these patients suffer from benign prostatic hyperplasia. A group of young patients under the age of 30 would be better, where the risk of PC and benign enlargement is completely negligible.
In study 3, the number of subjects with BCR and disease persistence is low.

Opponent's Comments:

The concept of the work as 3 studies (see comment above).
Time inconsistency of the presented research. In study 1, it is not clear what samples the research concerned (from which years and why) and when it took place, publication is in 2020, for study 2 samples from the years 2000-2014 were used, publication in 2017, for study 3 samples from the years 2013-2014, publication in 2021 (?).
The definition of BCR after radical prostatectomy has changed, it would be good to include the currently valid definition in the work from 2023.

The author could have read the definitive version after herself and removed the notes from the text (e.g. "Erro! A origem da referencia não foi encontrada" on pp. 20 and 21, and "Erro! Marcador não definido" in the list of figures, respectively). This may indicate carelessness or haste in the final editing of the text.

Questions for the author:

What is the role of EN2 in the pathogenesis of PC? Nowhere is the potential link between EN2 production and prostate disease sufficiently described (only stating that it is produced by prostate cells).

For all studies, or publication, the same approval of the ethics commission 377/13 and signature of informed consent for all patients is indicated. Does it refer to the initial storage of the samples or specifically to the three studies mentioned?

Is ultrasensitive PSA testing justified with the described PSA predictive values of 0.068 at 30 days and 0.015 at 60 days after surgery? Both values are detectable by routine testing.

Which of the PC biomarkers in urine, blood and prostate tissue, described in the theoretical part of the dissertation are available in the Czech Republic and how are they paid for (price and reimbursement)?

Conclusion:

The concept of the dissertation MUDr. Joany Isabela Do Carmo Silva "Diagnostic and prognostic ability of selected markers of prostate cancer in the serum and urine" is unusual, overall seems inconsistent and has several limitations and shortcomings. However, it brings several important findings for the diagnosis of PC and the detection of BCR after surgical treatment. EN2 does not appear to be a useful diagnostic biomarker, on the contrary, preoperative PSA isoforms may have new uses within nomograms for BCR prediction. Setting PSA threshold values at a defined time interval after surgery can help to optimize the treatment of patients at risk of BCR based on unfavorable pathological characteristics of the primary tumor. The results of the experimental part of the work (all three studies) were published in periodicals with IF. In terms of formality, the work is exemplary, clear and without grammatical errors. The work demonstrates the author's good orientation in the given issue and the ability to work with literature and publish results.
**I recommend the dissertation for defense.**

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Brno, 2.1.2024 doc. MUDr. Michal Fedorko, Ph.D., FEBU