In Prague on January 2, 2024

<u>**Opponent Review of Doctoral Dissertation**</u>

Author: Joana Isabel Do Carmo Silva, MD

Title: Diagnostic and Prognostic Ability of Selected Markers of Prostate Cancer in the Serum and Urine

Opponent: Assoc. Prof. Otakar Čapoun, MD, PhD, FEBU, First Faculty of Medicine, Charles University, Prague

Topic, title, objectives

The topic is of utmost importance, as prostate cancer is one of the most common malignancies in men in developed countries and serious diagnosis with respect to deaths from malignant diseases. Current diagnostic algorithm combines serum markers (mostly prostate specific antigen), imaging methods (multiparametric resonance of prostate) and nomograms. This approach is still burdened with low accuracy, leading to many cases of non-lethal prostate cancer and the risk of overdiagnosis and overtreatment. Looking for new, cheap, and non-invasive markers to improve the prediction of a positive prostate biopsy is therefore one of the most actual topics in oncourology.

The title of the Thesis is well designed, as an ideal marker of prostate cancer should have a strong predictive (probability of positive prostate biopsy) and prognostic (probability of disease recurrence, metastatic spreading, or risk of prostate cancer specific death) power.

The Thesis is divided in three separate research Projects and Objectives, that are well selected, defined and appropriately described. The established hypotheses are reasonable, measurable and correspond to the subjects of individual Projects and their results. Unfortunately, I lack any continuity across individual Projects when each work deals with a different way of diagnosing prostate cancer or a different stage of the disease.

Structure and methodology

Author proved high standards at both theoretical and methodological level. The Introduction briefly covers basic information about prostate cancer diagnosis, staging and clinical management. More space is properly devoted to the description of currently used biomarkers. However, I would also expect at least a brief mention of the theory of biomarkers, the definition of the "ideal" marker and pitfalls in the diagnosis of cancer in general using markers in serum or urine. Some of the paragraphs in the text are mentioned repeatedly (e.g. 1.8.1.1. and 1.8.5.2.).

The Material and methodology of individual projects are described in full in attached articles, here in the Thesis, information about methodology is sufficient and clear. Statistical methods correspond with the input data and expected results. Study groups of all Projects are well designed and the whole Thesis gives evidence about the long-term research of prostate cancer biomarkers at the author's research department.

Results and discussion

The Results are described clearly regarding their potential clinical use. The data in tables are clearly arranged, graphs and figures are properly prepared with clearly recognizable results. The Discussion is well constructed with explanation of rationale for each Project and clinical view of expected results. The author does not hide any negative results and makes an appropriate commentary towards the limitations of each Project, which is mostly a relatively small sample or retrospective design.

Formal standards

Reviewed Thesis has high formal standard. The structure follows recommendation for doctoral thesis. All used sources are cited correctly according to citation norm.

Questions to the author

a) How could you explain that the level of EN2 was after DRE lower than before DRE (although not statistically significant)? I would rather expect a trend similar to that after prostatic massage.

b) The second Project used prospectively collected blood samples under precisely defined rules, including processing and storage of a sample before laboratory analysis. Are there any pitfalls regarding using the ultrasensitive PSA test and assessing such low PSA values in a daily clinical praxis? I mean possible differences in a way of processing of a sample after taking of blood, different time from sampling to analysis, transport, different laboratories etc.

c) What is a rationale for assessing the risk of BCR before radical prostatectomy (Project 3), when both surgeon and patient agree with the procedure, and it will be done regardless of the prediction?

Overall assessment

Presented dissertation of dr. Joana Isabel Do Carmo Silva by its methodological approach and achieved results fulfils the criteria of dissertations of a doctoral study program. It answers research questions and verify hypotheses, which have been set, and by its contents it actually does bring an innovated knowledge related to the research topic. The candidate proves an acceptable theoretical and methodological background, and ability to apply it in practical application. As far as the presented dissertation fulfils the criteria, **I recommend** it to be defended.

Otakar Čapoun, MD, PhD