EVALUATION OF LABORATORY TEST RESULTS IN SUSPECTED PROSTATE CANCER.

PhD thesis

Zoltán Tóth

Semmelweis University Doctoral School

Theoretical and Translational Medicine Division

Supervisor: Béla Gyarmati, MD, PhD

Official reviewers: Domokos Máthé, MD, PhD
Béla Köves MD, PhD

Head of the Complex Examination Committee: György Reusz D.Sc

Members of the Complex Examination Committee: Gellért Karvaly Ph.D
Szabó László Ph.D

Budapest
2024

1. INTRODUCTION

My research work is focusing on three specific topics related to prostate cancer (PCA).

Topic 1. Prostate-specific antigen (PSA) levels are commonly used as a laboratory marker for PCA screening. An elevated PSA level raises the suspicion of PCA and thus indicates biopsy sampling. The introduction of personalised PSA reference ranges could avoid unnecessary biopsies: it would be worthwhile to take into account in the assessment those conditions that may affect PSA levels independently of prostate pathology. Well known factors are age, lifestyle, some drugs and high body mass index, but probably other factors also exist.

Topic 2. If PCA is suspected, biopsy is necessary to be performed. To avoid biopsy-related infections antibiotic treatment (2*500 mg of ciprofloxacin daily and 2*150 mg of clindamycin daily for 5 days starting from the day of biopsy) is routinely given in our practice.
The human microbiome is a complex community of microorganisms. The dynamic interaction between the microbiome and the host is a key player in health and disease including PCA. There is limited data on the short-term impact of antibiotic regime applied to prevent biopsy-related prostatitis on gut microbiome, however.

Topic 3. Both local and systemic inflammation may influence tumour development, promotion and metastatic progression. Systemic inflammation indicated by complete blood count (CBC) and alterations in rates of CBC parameters were predictive for survival in many cancers. It is not known, however, this is the case in a general PCA population.

2. OBJECTIVES

2.1. To test the interrelationship between PSA levels and laboratory markers of liver and kidney disease, low levels of systemic inflammation, thyroid abnormalities or vitamin D deficiency.

2.2. To investigate the short term changes in gut microbiome as the result of ciprofloxacin and clindamycin combination administered with the goal to avoid biopsy-related prostatitis.

2.3. To assess whether blood counts and related parameters determined at the time of PCA diagnosis can help predict patients' long-term overall survival.

3. METHODS

Objective 1.

For a retrospective analysis of association between PSA and laboratory parameters in a large database of Department of Laboratory Medicine, Semmelweis University, we used selected the results of men who had a PSA level measurement and had any of the following parameters available within ±1 month: estimated glomerular filtration rate (eGFR), alanine transaminase (ALT), C-reactive protein (CRP), vitamin D & thyroid-stimulating hormone (TSH).

We created subgroups and cohorts according to ALT, eGFR and CRP and calculated PSA values of 5, 25, 50 (median), 75 and 95 percent. In addition, logistic regression analysis of logarithmic data was used to determine the independent effects of eGFR, ALT and CRP, lnTSH and age on PSA levels. The direct correlation between age and each analyte was also tested. For the analyses, we performed logarithmic transformations of PSA and laboratory parameters to obtain a near-normal distribution in each dimension.

We also created subgroups according to vitamin D levels and age. We examined the proportion of subjects with elevated PSA (>4 μg/L) in each vitamin D subgroup. Logistic regression analysis was used to determine the independent effect of vitamin D level and age on PSA levels.

Objective 2

The study included 9 patients who underwent TRUS-guided transrectal prostatic needle biopsy for suspected PCA. Patients received 2*500 mg of ciprofloxacin and 2*150 mg of clindamycin from the day before the procedure. The combination therapy lasted until day 5 after the biopsy; thereafter, patients received only 2*150 mg clindamycin daily for three more days.

All participants took stool samples themselves at home 1 day before the antibiotics were given and 14 days after the biopsy. The samples were processed at uBiome processing laboratory (uBiome, San Francisco, Ca, USA); the results generated were used to compare microbiome components before and after biopsy at strain, order and genus level. Only those strains, orders and genera that had a median frequency of at least 1.0% of the total microbiome before and/or after biopsy were considered for analysis. The detection level was considered as an abundance ratio of 0.1%; abundance values below this level were used as '0' in the statistical analysis. Differences in abundance ratios of the microbiome components before and after biopsy were analysed using the Wilcoxon test.

Objective 3.

PCA patients diagnosed between 2000 and 2005 were included in the analysis. The major CBC parameters and their relative ratio, PSA level, TNM score, histological result (Gleason score) and co-morbidities at the time of diagnosis were extracted from the patients' medical records. We retrieved the date of death from theHungarian National Heath Fund database based on the patients' social security number. If this date was not available, the patients were classified as living. Statistical analysis was performed using descriptive statistics to characterize the clinical parameters of living and non-living patients. The effect of blood count parameters, Gleason score, PSA level, comorbidities and age of death was evaluated by Cox regression analysis.

4. RESULTS

Objective 1.

A significant correlation was found between PSA and CRP when the association was adjusted for age. The majority of patients (56 and 22%) had moderate or severe vitamin D deficiency, respectively. There was no relationship between PSA and vitamin D levels.

A relationship was found between PSA and lnTSH levels; a 10% decrease in TSH levels leads to a 0.42% increase in PSA levels. In a direct comparison of hyperthyroid and euthyroid patients, PSA levels were higher in hyperthyroid (n = 405) compared to euthyroid patients (n = 6698) (median, interquartile range) (PSA level: 1.118 [0.639-2.338] vs. 0.920 [0.508-1.826] μg/L, p = 0.016). The estimation of PSA levels that would be associated with different TSH values in the same patient suggests that in hyperthyroidism, measured PSA levels can rise to levels close to or well above the cut-off of 4 μg/L in euthyroidism in patients with euthyroid PSA levels of 2 and 4 μg/L, respectively

Objective 2.

4 strains were detected at all time points with a median frequency of at least 1.0%. The abundance of Actinobacteria and Firmicutes decreased, while that of Bacteroides and Proteobacteria increased after antibiotic therapy. The Firmicutes:Bacteroides ratio reversed (from 2.81 to 0.74, p=0.035). Of 7 orders with a median abundance of at least 1%, Bacteroides and Clostridia together accounted for 75% and 91% of the total microbiome before and after treatment, respectively. The abundance of Bacteroidales and Veillonellales increased, while that of Clostridiales and Coriobacteriales decreased after antibiotic therapy.

We detected 15 genera with a relative frequency of at least 1% median at any time point and in at least 3 patients. These were responsible for 61.87% and 79.89% of the total microbiome, respectively. Four genera showed significant changes in abundance; of these, Bacteroides increased, while Roseburia, Faecalibacterium and Collinsella decreased dramatically.

Objective 3.

In our analysis, we processed data from 97 patients; of those, 82 patients died and 15 patients were still alive at the time of analysis. CBC parameters determined at the time of PCA diagnosis did not differ between the two groups; in contrast to Gleason score, age of patients, PSA levels, and palliative treatment.

In the group of patients who died, a significant effect of age (p=0.004) and PSA and Gleason score (both p=0.033) on overall survival was died without any effect of CBC parameters.

5. CONCLUSIONS

Objective 1. The evaluation of PSA reference range in patients with comorbidities

In the case of general laboratory parameters suggestive of liver disease, kidney disease or vitamin D deficiency, no modification of the reference range is justified. PSA levels rise in inflammation. It is therefore recommended to measure PSA levels when CRP levels are low (i.e no overt inflammation is present). In hyperthyroidism, PSA levels rise. In such a case, clinical judgement should be made only on the basis of the PSA level, with increased caution.

Objective 2. Impact of ciprofloxacin – clindamycin therapy on gut microbiome

This antibiotic regime routinely used before prostate biopsy has a marked and potentially detrimental effect on the gut microbiome. It increases the proportion of Bacteroides strains and decreasing the proportion of Firmicutes strains and has an overall profound impact on the compostion of microbiome.

Objective 3. Predictive value of blood count parameters in prostate cancer

When diagnosing PCA, conventional blood count parameters do not predict long-term mortality from any cause.

ACKNOWLEDGEMENTS

This work could not have been accomplished without the support and guidance of my esteemed mentors**: Dr. Barnabás Ruszinkó** and **Dr. József Varga**. Special thanks to **Dr. Michael Figge**, chief urologist at Urban Hospital in Berlin.

Respect and gratitude to **Dr. Béla Gyarmati**, my supervisor, who propelled my work forward with specific medical advice. I owe my gratitude to **Dr. Barna Vásárhelyi** (Semmelweis University), who introduced me to the intricacies of scientific work.

I say special thanks to **Dr. Ákos Tordé** urologist; **Dr. Attila Bezzegh** laboratory expert**; Dr. Béla Kovács**, urologist; **Dr. Andrea Ficzere**, director of Uzsoki Hospital; **Prof. László Hangody** traumatologist; **Jenő Mészáros** informatician; **Veronika Láng**, administrative colleague and **Dr. Tamás Szabó** biostatistician for their specific contribution.

Lastly, but certainly not at least, I express my deepest gratitude to my partner, **Tünde**, for her patience and understanding, which contributed to creating the balanced, peaceful environment necessary for completing this thesis.

6. LIST OF OWN PUBLICATIONS

6.1 PUBLICATIONS ON WHICH THE DISSERTATION IS BASED

1. **Tóth Z**, Szalay B, Gyarmati B, Jalal DA, Vásárhelyi B, Szabó T. Vitamin D deficiency has no impact on psa reference ranges in a general university hospital - a retrospective analysis. EJIFCC. 2020 Sep 29;31(3):225-230.
2. **Tóth Z**, Gyarmati B, Szabó T, Vásárhelyi B. An inverse significant association between thyroid stimulatory hormone (TSH) and prostate specific antigen (PSA) blood levels in males 40-75 years of age 40-75 years of age. Orv Hetil. 2019 Sep;160(35):1376-1379.
3. **Tóth Z**, Bezzegh A, Tordé Á, Vásárhelyi B, Gyarmati B. Short term ciprofloxacin and clindamycin combination antibiotic therapy before and after transrectal ultrasound scan and prostate biopsy: Its impact on major components of gut microbiome. Mol Cell Probes. 2022 Dec;66:101874.
4. Toth Z, Szalay B, Gyarmati B, Jalal DA, Vasarhelyi B, Szabo T. Prostate specific antigen serum levels in patients with different levels of hepatic or renal impairment and in those with systemic inflammation in a university hospital. A retrospective analysis of 10 years of laboratory data. Open Access J Urol Nephrol 2020, 5(3): 000184.

6.2. OWN PUBLICATIONS NOT ON THE SUBJECT OF THE THESIS

1. Jalal DA, Vásárhelyi B, Blaha B, **Tóth Z**, Szabó TG, Gyarmati B. Interrelationship of hemoglobin A1c level lipid profile, uric acid, C-reactive protein levels and age in a large hospital database. Mol Cell Probes. 2023 Dec;72:101933.
2. Vásárhelyi B, Dlovan AJ, Blaha B, **Tóth Z**, Szabó GT, Gyarmati B. Relationship between red blood cell parameters and hemoglobin A1c levels based on a retrospective evaluation of 10 years of data. Orv Hetil. 2024 Feb 18;165(7):243-248.
3. Karvaly G, Kovács K, Gyarmatig M, Gerszi D, Nagy S, Jalal DA, **Tóth Z**, Vasarhelyi B, Gyarmati B. Reference data on estrogen metabolome in healthy pregnancy. Mol Cell Probes. 2024 Mar 4;74:101953