**M E D I C A L  U N I V E R S I T Y - P L E V E N**

**Faculty of Health Care**

**Department of Clinical Laboratory, Clinical Immunology and Allergology**

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***Antioxidant status in patients with hyperplasia and carcinoma of the prostate gland***

# ABSTRACT

## of a dissertation

## for the award of the degree of Doctor of Education and Science

## in the doctoral program "Clinical Laboratory"

**Scientific supervisor: Prof. Dr Adelaida Lazarova Ruseva, MD, PhD**

**Reviewers: Prof. Dr Milena Georgieva Velizarova, MD, PhD**

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**Pleven**

**2025**

The dissertation is written on 155 standard pages and illustrated with 64 figures, 61 tables, and 4 appendices.

The references include 183 titles - 5 in Cyrillic and 178 in Latin alphabet.

The author is a regular PhD student at the Department of Clinical Laboratory, Clinical Immunology and Allergology, Faculty of Health Care, Medical University – Pleven. She is a physician - specialist in Clinical laboratory and a member of the team of the department of the Clinical Laboratory at the UMBAL "St. Marina"in the town of Pleven.

There are 3 publications and 3 participations in scientific forums in Bulgaria related to the dissertation.

The dissertation was discussed, accepted and proposed for public defence at a meeting of the Council of the Department of Clinical Laboratory, Clinical Immunology and Allergology, Faculty of Health Care at Medical University - Pleven, held on 14.03.2025.

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The public defence of the dissertation will be held on 13.06. 2025 at 12.00 o'clock in the Aleksander Fleming Hall of the Faculty of Pharmacy at Medical University - Pleven.

The materials for the defence are published on the website of Medical University - Pleven.

**CONTENTS**

**ABBREVIATIONS USED**

| | |
|---|---|
| BPH | Benign prostatic hyperplasia |
| BMI | Body mass index |
| LEPG | Laparoscopic enucleation of the prostate gland |
| OS | Oxidative stress |
| PC | Prostate cancer |
| RARP | Robot-assisted radical prostatectomy |
| TAS | Total antioxidant status |
| TURP | Transurethral resection of the prostate |
| ABTS | 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid |
| ANOVA | Analysis of Variance (ANOVA) |
| BMI | Body Mass Index |
| CRP | C-Reactive Protein |
| Df (df) | Degree of freedom |
| Leuc. | Leucocytes |
| Mean | Mean value |
| Me | Median value |
| PSA | Prostate-specific antigen |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| Xmax | Maximum value of the variable |
| Xmin | Minimum value of the variable |
| p-value | Guarantee probability value |
| WBC | White blood cells |

# I. Introduction

The prostate gland is among the most commonly affected organs by diseases in men, with benign prostatic hyperplasia (BPH) and prostate cancer (PC) being two of the most prevalent and socially significant pathological conditions. These two diseases affect many men worldwide, including in Bulgaria, where current research and strategies for prevention and treatment are of utmost importance.

One area that has attracted increasing attention from the scientific community is the role of oxidative stress (OS) and antioxidant status in developing and progressing these diseases. Determination of total antioxidant status (TAS) by various laboratory methods can provide valuable information on the state of antioxidant defence in the body and indicate its potential role as an indicator in BPH and PC.

Various studies have highlighted the role of OS in the pathogenesis of these diseases, focusing on investigating various markers, including TAS, in different pathologies. Still, there is not a large number of data evaluating TAS in these two prostate diseases.

However, there are still some unresolved questions and issues on the topic that are essential for the understanding, early diagnosis and successful treatment of these conditions:

1. Variability in TAS measurement methods:

    - There are different methods of determining TAS, which can lead to inconsistencies in results. The lack of standard methodologies makes it challenging to compare different studies and limits the ability to establish consensus on reference TAS values in patients with BPH and PC.

2. The effect of concomitant factors:

    - Multiple factors, including age, diet, lifestyle, and comorbidities, can influence OS and antioxidant status. These factors are often not sufficiently taken into account in studies, which can lead to variable results and make data difficult to interpret.

3. Long-term clinical implications:

- Although many studies focus on instantaneous levels of TAS, few studies address the long-term clinical implications of changes in antioxidant status. There is a lack of data on the impact long-term TAS lowering has on the progression of BPH and PC, as well as on patients' general health.

4. Effectiveness of antioxidant therapy:

- Despite the potential of antioxidants as therapeutic agents, it is not yet clear whether additional antioxidant intake can significantly affect the progression of BPH and PC. Further clinical studies are needed to establish the effectiveness and safety of such therapy.

Resolving these issues is essential for developing more effective diagnostic and therapeutic approaches to improve patients' prognosis and quality of life.

Additional clinical research evaluating the effectiveness of antioxidant therapy may open new avenues for preventing and treating these two prostate diseases. It may have a significant impact on improving the quality of life of affected patients and may lead to a reduction in morbidity and mortality from these two prostate diseases.

No similar study has been conducted in Bulgaria, which makes this research new, relevant and essential for the medical community. Evaluation of TAS and its relationship with other biomarkers and clinical indicators may provide valuable information on the pathogenesis of BPH and PC.

In conclusion, our work has the potential to contribute significantly to the understanding of the role of antioxidant status in patients with BPH and PC, providing new perspectives on the diagnosis, prevention and treatment of these common and important diseases in men.

## II. AIM AND OBJECTIVES

### AIM:

The thesis aims to analyse TAS levels in prostate cancer and benign prostatic hyperplasia and its correlations with: 1) more widely available markers for assessing antioxidant statuses such as bilirubin, uric acid, and albumin; 2) levels of markers of inflammation (CRP, WBC); and 3) levels of the tumour marker PSA. It also aims to identify changes in antioxidant status according to disease stage and before and after surgical treatment.

### OBJECTIVES:

1. To determine the role of certain lifestyle factors in developing the respective disease in patients with PC and BPH.

2. To compare serum TAS levels in healthy controls and patients with PC and BPH.

3. To establish the level of correlation between the results obtained for TAS and other parameters of antioxidant significance.

4. To look for an association between TAS and the major markers of inflammation.

5. To determine the influence of the type of surgical technique on the results of TAS, other indices of antioxidant significance and markers of inflammation.

6. To compare and analyse the mean levels of TAS, other indices of antioxidant significance, and markers of inflammation in patients with PC and BPH before and after surgical intervention.

7. To look for a correlation between PSA levels in patients with PC and BPH and TAS levels.

8. To determine whether there is a change in TAS levels according to the PC stage and the presence of metastases.

## III. MATERIALS AND METHODS

The study was prospective and comparative, using a control group.

### 1. MATERIAL

#### 1.1 Inclusion and exclusion criteria for forming the target groups

**Inclusion criteria:** Men aged 50 years and older with histologically proven BPH or PC and no evidence of other malignancy who completed a questionnaire regarding the study were included.

**Exclusion criteria:** Men under 50 years of age; no evidence of BPH, PC, or other inflammatory disease.

#### 1.2. Inclusion and exclusion criteria to form the control group

**Inclusion criteria:** men over 50 without clinical evidence of prostate gland disease and no evidence of malignancy who completed a questionnaire. The selection of controls followed the principle of matching each case by age to the patients in the target group.

**Exclusion criteria:** men under 50 without malignancy and/or other inflammatory process.

#### 1.3. 1.2. Formation of the two groups - target and control

For the period October 2022 to June 2023, a total of 135 men, aged 54 to 86 years, who fulfilled the inclusion and exclusion criteria of the study were studied.

The target group of patients consisted of 90 men with histologically proven prostate gland disease who underwent surgery at the urology clinic of St. Marina University Hospital - Pleven. The number of patients with PC was 45, with a mean age of 69.87±6.233 years, and 45, with proven BPH, with a mean age of 70.47±7.322 years. All study participants signed informed consent.

The control group consisted of 45 clinically healthy men meeting the inclusion criteria, with a mean age of 69.44±6.621 years, who visited the University Hospital “St. Marina”-Pleven for a prophylactic examination and laboratory tests.

All study participants followed the recommendations in the WHO Ethical Issues in Patient Safety Research: Interpreting Existing Guidance. Procedures were performed following the

ethical standards of the Commission on Human Research and the Declaration of Helsinki. All men signed an informed consent for the medical activities administered to them during their hospital stay and had a questionnaire completed. The study was approved by the Research Ethics Committee (REC) at MU-Pleven.

All participants had venous blood drawn from the cubital vein, 5 ml in a vacutainer containing Vacuette separating gel by Greiner, and a second vacutainer containing K2EDTA for complete blood count analysis twice: on admission and before discharge from the urology clinic. The control group had venous blood drawn once during their hospital stay for a prophylactic examination. The blood was taken in the morning on an empty stomach after a 12-hour meal break. After standing for 30 minutes at room temperature, the blood was centrifuged for 15 minutes at 3000g to separate serum, and the vacutainer containing $_{K2}$EDTA was gently mixed 8-10 times and used to determine the total leukocyte count. Then 600 µl of the serum was transferred to an Eppendorf-type tube, which was numbered with the appropriate ID, using an automatic pipette with a disposable plastic tip. The analysis did not include samples with haemolysis, icterus and lipemia because this would have interfered with the results obtained. The separated sera were stored at -20℃ for a maximum of 14 days before analyses. Samples remaining after the 14th day of storage were removed.

All laboratory tests were performed at the Clinical Laboratory of St. Marina University Hospital-Pleven.

## 2. METHODS

**2.1. Standard interview**. The questions were asked orally to the patients, and the interviewer recorded the answers in the questionnaire. The questionnaire for all patient groups consisted of 11 closed-ended questions with preformulated answers reflecting the main elements of the patients' style and lifestyle relevant to the study.

**2.2 Clinical Methods**. All patients in the study underwent an initial urological examination before laboratory testing between October 2022 and June 2023.

**2.3**. **Histopathological examination** for evidence of BPH and PC.

**2.4 Laboratory investigations.** The requirements of the Medical Standard for Clinical Laboratory and the International Standard for Quality and Competence of Medical Laboratories ISO15189 are met.

### 2.4.1. Determination of TAS

A quantitative in vitro assay was used to determine total antioxidant status (TAS RANDOX UK) in plasma, serum, wine, beer, and fruit juice, with catalogue number NX 2332. The system used is a Beckman Coulter AU 480.

a/ **Principle of the method.** The spectrophotometric method applied.

ABTS is incubated with peroxidase (metmyoglobin) and H2O2 to produce the radical cation ABTS-+. It has a relatively stable blue-green colour, which is measured at 600nm wavelength. The antioxidants in the added sample suppress the coloration to an extent proportional to their concentration.

b/ Reagent composition includes: R1 Buffer phosphate buffered saline, which is stable to expiration at storage +2 to +8°C; R2 Chromogen -Metmyoglobin, ABTS. Chromogen reconstituted is stable for two days at +2 to +8°C or 8 hours at +15 to +25°C.; R3 Substrate-hydrogen peroxide (in stabilised form). Dissolved, it is stable for 24 hours from +2 to +8°C.

Cal Standard 6-hydroxy-2,5,7,8-tetramethylchroman -2-carboxylic acid is lot specific (NX 2332), with a manufacturer's stated concentration of 2.55 mmol/l. It is reconstituted with a stability of two days at +2 to +8°C or 1 month at -20°C.

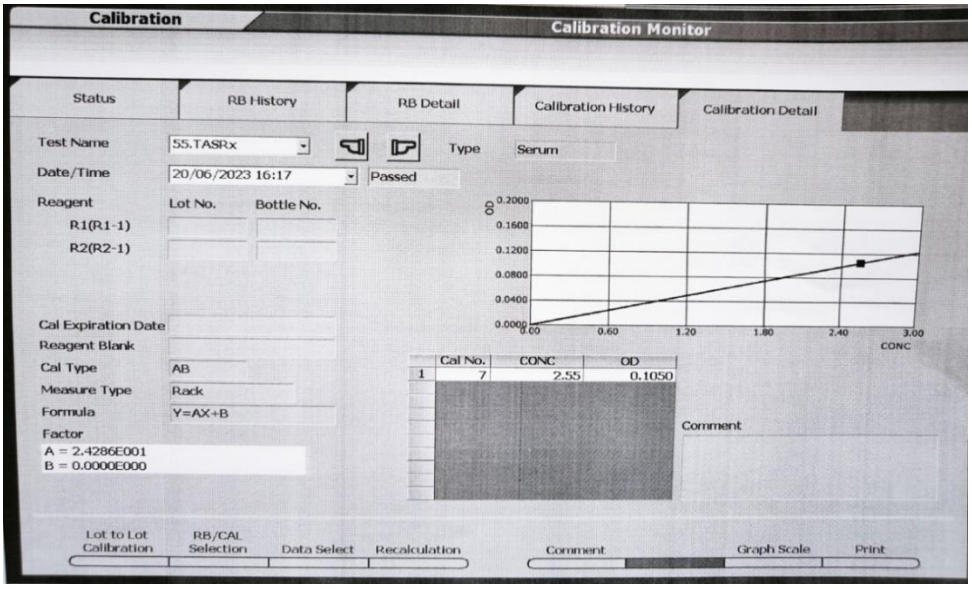



***Figure 1. Determination of the TAS calibration curve***

**TAS program for Beckman Coulter AU 480 biochemical analyser**

| | | | |
|---|---|---|---|
| TEST NUMBER | | | * |
| TEST NAME | | | TAS |
| SAMPLE TYPE | | | SERUM/PLASMA |
| SAMPLE VOLUME | | | 2 µl |
| DILUENT VOLUME | | | 10 µl |
| REAGENT VOLUME (R1) | | | 120 µl |
| DILUENT VOLUME | | | 0 µl |
| REAGENT VOLUME (R2) | | | 24 µl |
| DILUENT VOLUME | | | 0 µl |
| WAVELENGTH MAIN | | | 600 nm |
| WAVELENGTH SUB | | | 0 nm |
| METHOD | | | END |
| REACTION | | | - |
| POINT 1 | FIRST | | 0 |
| | LAST | | 21 |
| POINT 2 | FIRST | | 0 |
| | LAST | | 10 |
| LINEARITY | FIRST | | |
| | SEC | | |
| NO LAG TIME | | | |
| MINIMUM OD | L | | |
| MAXIMUM OD | H | | |
| REAGENT O.D. LIMIT | | | |
| | FIRST | L | -0.1 |
| | | H | 2.5 |
| | LAST | L | -0.1 |
| | | H | 2.5 |
| DYNAMIC RANGE | | L | 0.0 |
| | | H | 2.5 |
| CORRELATION FACTOR | | A | 1.0 |
| | | B | 0.0 |
| ON BOARD STABILITY PERIOD | | | 1 |
| **CALIBRATION** | | | |
| CALIBRATION TYPE | | | AB |
| FORMULA | | | Y = AX + B (1) |
| COUNT | | | * |
| PROCESS | | | CONC |
| 1 POINT CALIBRATION POINT | | | * |
| MB TYPE FACTOR | | | - |
| CALIBRATOR STABILITY PERIOD | | | 1 |

* Data entered by operator

d/ The Randox Total Antioxidant Control (Cat. No. NX 2331) internal control was used with the manufacturer's setpoints.

e/ Linearity of method: Samples with concentrations above 2.5 mmol/l should be diluted with 0.9% NaCl and re-analysed, which may result in a 20% increase in values. Most samples do not require dilution because they have values below 2.5 mmol/l.

Reference range: 1.30-1.77 mmol/L plasma. This range was measured in a European working population, and it is recommended that each laboratory develop its own reference range according to the age, sex, diet, and geographic location of the population.

f/ Sample requirement: freshly collected serum or heparinised plasma without haemolysis.

Blood samples are centrifuged after collection to separate serum from blood cells. The sample can be stored for up to 36 hours at +2 to +8°C. Plasma/serum can be frozen for up to 14 days.

### 2.4.2. Determination of uric acid

a/ **Principle of the method:** enzymatic colorimetric test.

The in vitro Uric Acid test (MEDICON lot 2243) was used to quantify the concentration of uric acid. Calibrator MEDICAL LOT 1085.

Uricase converts uric acid to allantoin and hydrogen peroxide. The colour intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance at 552 nm. Reference ranges for males: 202-420 µmol/l.

b/ **Uric acid calibration curve:**

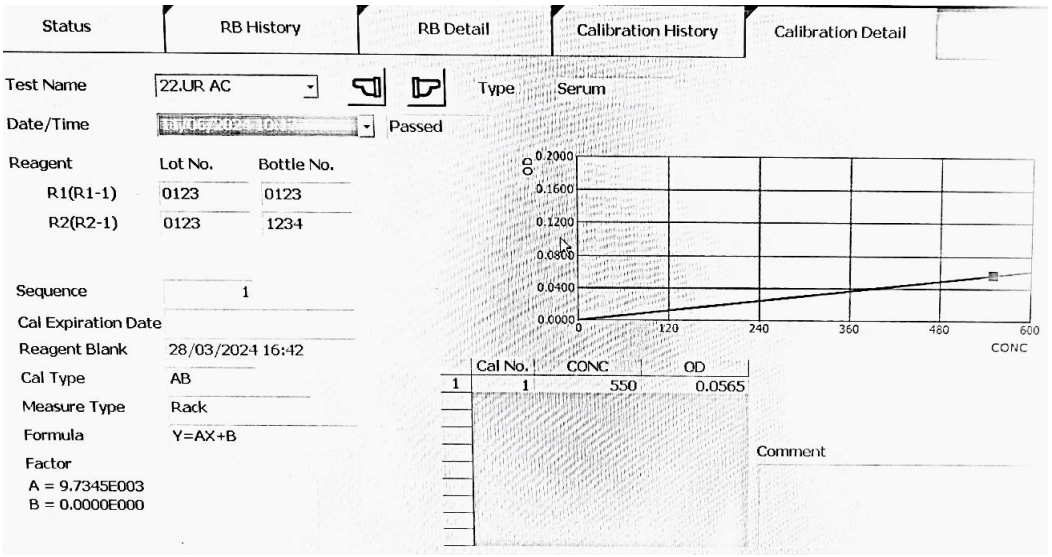



***Figure 2. Determination of the uric acid calibration curve***

c/ Sample requirement - serum free of haemolysis, icterus and lipaemia.

d/ MEDICON CLINICAL CHEMISTRY CONTROL Lot 1221.

### 2.4.3. Determination of albumin

a/ **Principle of the method** - colorimetric test for quantifying albumin in human serum or plasma for in vitro diagnostics (MEDICON Lot 2139), calibrator MEDICAL Lot 1085.

At pH 4.2, albumin shows sufficient cationic charge to bind to bromocresol green (BCG), an anionic dye, to form a blue-green complex. Absorbance is measured bi-chromatically (600/800nm). The colour intensity is directly proportional to the albumin concentration in the sample and is measured photometrically. Reference ranges for adults: 35-52g/l.

b/ ***Albumin calibration curve:***

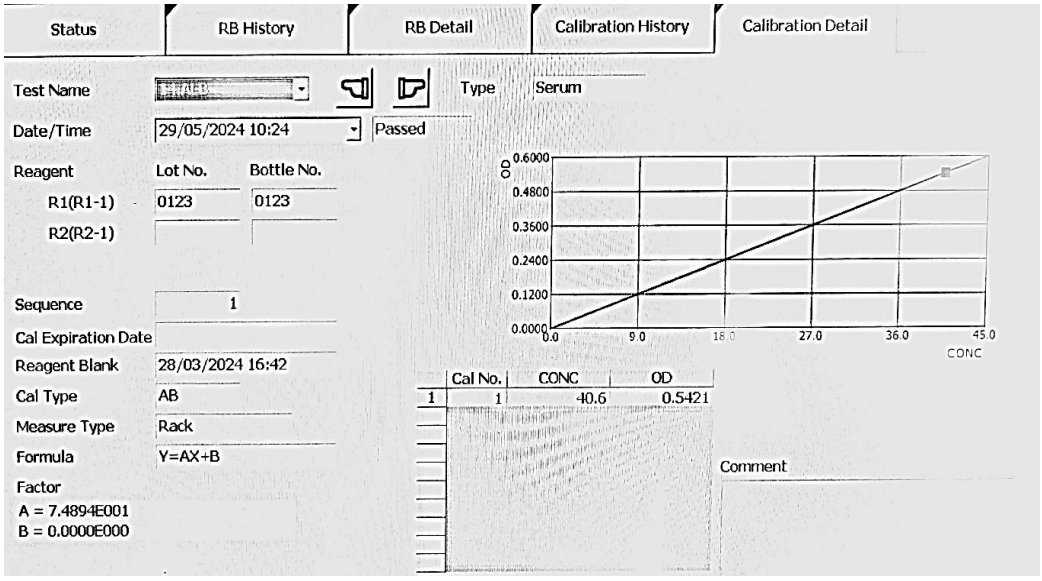



***Figure 3. Determination of the albumin calibration curve***

c/ Sample requirement - serum free of icterus, haemolysis and lipaemia

d/ Internal control MEDICON CLINICAL CHEMISTRY CONTROL Lot 1221

### 2.4.4. Determination of Total Bilirubin

a/ **Principle of method** - colorimetric test for quantifying total Bilirubin (Beckman Coulter OSR6212) in human serum or plasma for in-vitro diagnosis.

Stabilised diazonium salt (DPD) reacts directly with conjugated bilirubin and with unconjugated bilirubin in the presence of a catalyst to form azo-bilirubin. Absorption at 540nm is proportional to the concentration of total bilirubin.

A separate blank test is performed to reduce endogenous serum interference.

b/ Beckman Coulter System Calibrator cat. number 66300 was used.

c/ Control material lot specific Controls ODC0004 with values determined by Beckman Coulter.

Adult reference ranges: 5-21 µmol/l.

d/ Sample requirement - serum free of icterus, haemolysis and lipaemia.

**Calibration curve of total bilirubin**

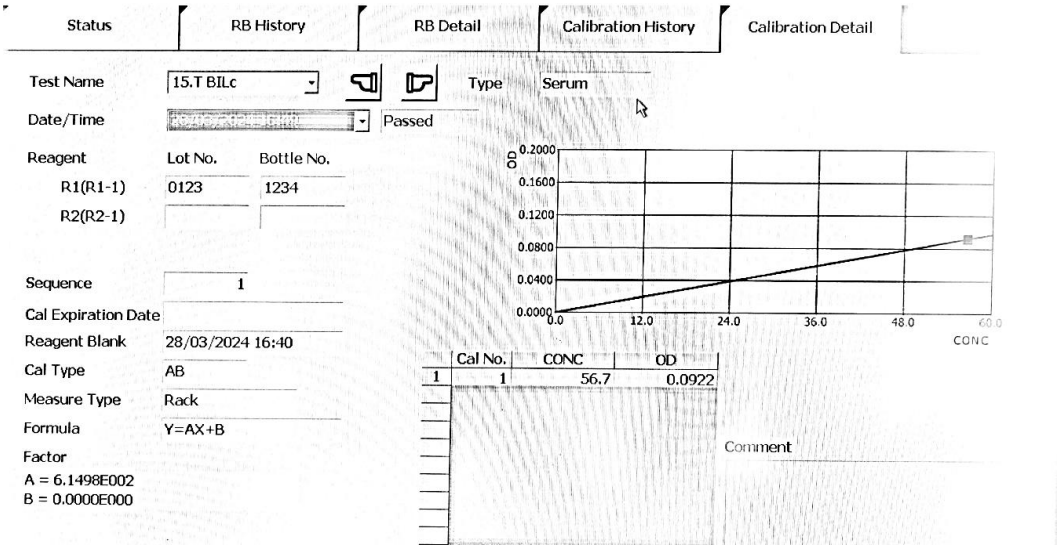



***Figure 4. Determination of total bilirubin calibration curve***

### 2.4.5. Determination of C-reactive protein (CRP)

a/ **Principle of the method** - immunoturbidimetric test for quantifying CRP (ELITech ICRP-0400) in human serum or plasma for in-vitro diagnostics only. When a sample is mixed with R1 buffer and R2 latex suspension, CRP interacts specifically with anti-human CRP antibodies on latex particles and forms insoluble aggregates. Absorption of these aggregates is proportional to the concentration of CRP in the sample. Reference ranges < 5 mg/l

b/ ELITech CRP IP Calibrator Set Ref : ICRP-0043, Lot 22-4003, with manufacturer's stated calibrator values, was used.

c/ ELITech control material REF 0047 with manufacturer's declared values.

d/ Sample requirement - serum free of icterus, haemolysis and lipaemia.

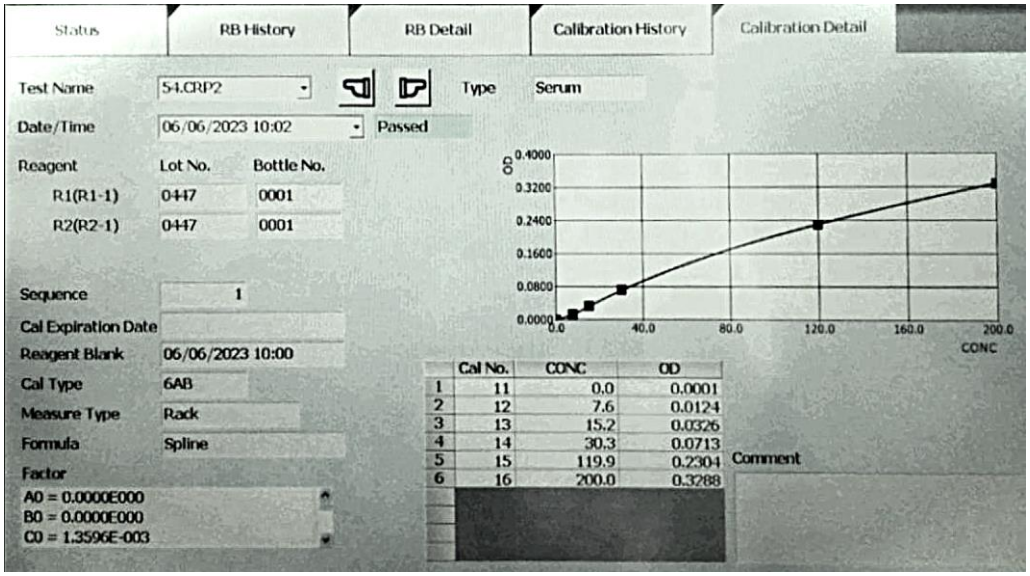



***Figure 5. Determination of C-reactive protein calibration curve***

### 2.4.6. Determination of PSA

The ST AIA PACK PSA in vitro assay (TOSOH) was used to quantify PSA concentrations in human serum or heparinised plasma using the TOSOH AIA 360 automated immunological assay platform.

a/ **Test principle.** The AIA-PACK PSA assay is a two-step immunoenzymometric assay kit with a chemiluminescent assay (CLEIA). The assay is performed in the AIA-PACK PSA TEST CUP. The PSA in the test sample binds to an anti-PSA mouse monoclonal antibody immobilised on magnetic microparticles in a single cell (Cell-I). After a first incubation, the magnetic microparticles were washed to remove unbound material, and then a volume of enzyme-labelled anti-PSA mouse monoclonal antibody was added to Cell-I, which was diluted in another cell (Cell-II). After the second incubation, the magnetic microparticles were rewashed to remove the unbound enzyme-tagged monoclonal antibody and incubated with a chemiluminescent substrate, DIFURAT®. The amount of enzyme-labelled monoclonal antibody that binds to the magnetic microparticles was directly proportional to the PSA concentration in the test sample. A standard curve was constructed from which the unknown sample concentrations were calculated.

***b/ PSA calibration curve (Figure 6):***

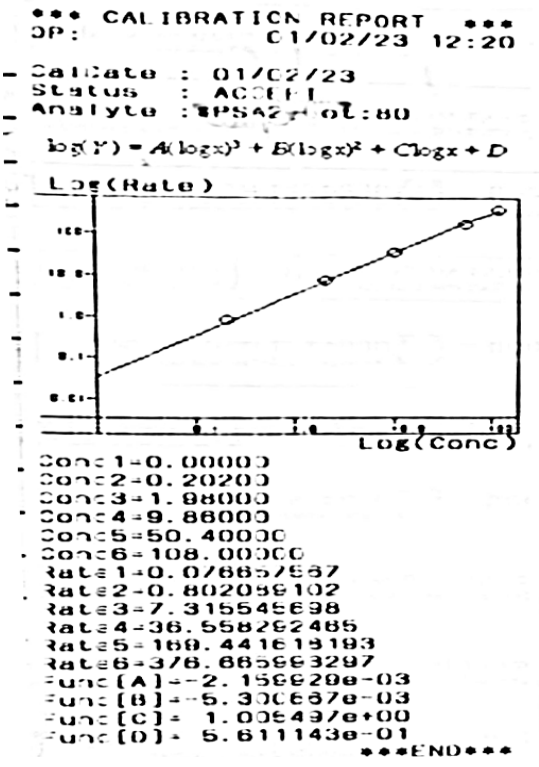



***Figure 6. Determination of PSA calibration curve***

c/ Calibrator: - batch specific

d/ Control material AIA PACK MULTI ANALYTE CONTROL (MAC) lot A607

PSA reference values for adult males: < 4 ng/ml

e/ Sample requirement - serum free of icterus, haemolysis and lipaemia.

### 2.4.7. Determination of total leukocyte count (WBC)

A fully automated 3-type leucocyte differentiation haematology analyser, Medonic M- Series M32 (Boule Medical AB, Sweden), designed for in vitro diagnostic testing of human blood samples in the laboratory, was used to determine total leucocyte count.

a/ The principle of WBC measurement is impedance: the number of cells to determine WBC values were counted from a 1:400 dilution ratio whole blood suspension.

The linearity is 0.5-130.0 $\times 10^9$ /L.

The reference interval for adult males is 3.5-10 $\times 10^9$ /L.

b/ Study material: whole blood- venous or capillary containing anticoagulant K2EDTA to prevent clotting. Clotted samples were not allowed. After collection, the blood was carefully mixed several times with the anticoagulant and then analysed for leucocyte count.

c/ A batch-specific calibrator and controls with manufacturer-specified values were used.

### 2.5 Statistical methods

The study data was processed using IBM SPSS Statistics v. 25 software package and Excel 2016 for Windows.

The following statistical methods were applied:

**1. Descriptive (descriptive) statistics** - methods for calculating the summary characteristics of the variables included in the study:

- **for qualitative (categorical) variables:** absolute number of cases in groups and subgroups, frequency distribution in percentages; tabular presentation of data using multivariate tables;

- **for quantitative variables**: determination of the type of frequency distribution (symmetric or asymmetric); calculation of central tendency indicators (mean, median, mode) and indicators of dispersion (standard deviation, minimum and maximum values of the variables, spread).

**2. Deductive statistics (inference and inference statistics):**

Ø **Parametric methods for hypothesis testing:**

- **Two-tailed t-test -** to test hypotheses of the significance of differences when comparing means in two samples with normal distribution;

- **One-factor analysis of variance** (One-way ANOVA) - to test hypotheses of the significance of differences when comparing means in more than two samples;

Ø **Non-parametric methods for hypothesis testing** in asymmetric distributions:

- **Mann-Whitney U test** - to test hypotheses about the significance of differences between two independent samples;

- **Kruskal-Wallis test**- to test hypotheses about the significance of differences between three or more independent samples;

- **Wilcoxon Signed Ranks Test -** to test hypotheses about the significance of differences between two dependent samples.

- **Friedman test -** to test hypotheses of the significance of differences between more than two dependent samples.

Ø **Methods for establishing dependence:**

- **For qualitative variables: χ2 test (Chi-square test)** to establish a relationship between two qualitative variables; **Phi (φ)** and **Cramer`s V** to assess the strength of a relationship between two qualitative variables;

- **For quantitative variables, the Pearson r correlation coefficient was used to establish a relationship between two quantitative variables; the coefficient of determination R2** was

used to determine the role of a particular independent variable on changes in a dependent variable.

Differences and dependencies were considered **statistically significant** at a confidence level of $p<0.05$.

Ø **Graphical analysis -** using a wide range of graphs to illustrate established relationships and trends: line graphs, histograms, bar and bar charts, scatter plots, pie charts, etc.

## IV. RESULTS AND DISCUSSION

### Characteristics of the study groups by age

In the three groups studied, the highest relative proportion was that of men aged 70-79, followed by men aged 60-69 (**Table 1** and **Fig. 7)**. The two extreme age groups (under 60 and over 80) are represented by insignificant relative shares. There was no statistically significant difference in the age distribution between the three study groups (chi-square=5.026; df=6; p=0.540.

***Table 1. Distribution of persons in the compared groups by age group (number and %)***

| Age groups | | Under 60 | 60-69 years | 70-79 yeas | ≧ 80 years | Total |
|---|---|---|---|---|---|---|
| **PC** | N | 4 | 17 | 23 | 1 | 45 |
| | **%** | **8.9** | **37.8** | **51.1** | **2.2** | **100.0** |
| **BPH** | N | 3 | 17 | 20 | 5 | 45 |
| | **%** | **6.7** | **37.8** | **44.4** | **11.1** | **100.0** |
| **Controls** | N | 4 | **17** | 23 | 1 | 45 |
| | **%** | **8.9** | **37.8** | **51.1** | **2.2** | **100.0** |

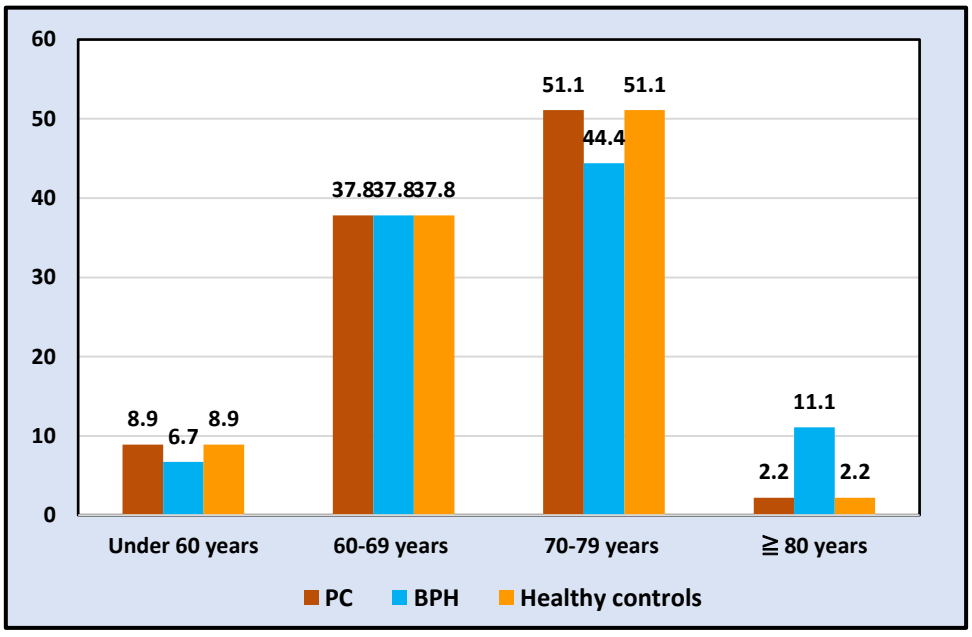



***Figure 7. Distribution of persons in compared groups by age (%)***

**Table 2** presents the main descriptive characteristics of the groups according to the variable "age": mean, standard deviation, confidence interval, median, minimum and maximum values, range, and coefficient of asymmetry (skewness). These descriptive characteristics had exactly

the same values in PC and healthy subjects; therefore, the selection of healthy subjects met the basic methodological requirement in case-control studies to match controls by age to PC cases.

***Table 2. Descriptive characteristics of individuals in the compared groups by age***

| Groups | Mean±SD | Me | 95% CI | Min | Max | Range | Skewness |
|---|---|---|---|---|---|---|---|
| **PC** | 69.87±6.233 | 70 | 67.899 – 71.834 | 57 | 82 | 25 | - 0.330 |
| **BPH** | 70.47±7.322 | 71 | 68.499 – 72.434 | 54 | 86 | 32 | 0.016 |
| **Controls** | 69.44±6.621 | 70 | 67.410 – 71.345 | 55 | 80 | 25 | - 0.302 |

The lack of significant differences in age distribution between the three groups was supported by the application of univariate analysis of variance (F=0.301; p=0.741). This fact is important because the differences in the individual indicators found in the further analysis would thus not be influenced by age.

Our data fully confirm the results of many other studies. According to Siegel R. et al. (2024), PC and BPH occur most frequently in older men over 55 years of age and with a peak between 70-75 years, after which this age peak gradually decreases. According to Dyba T. et al. (2021), the incidence of PC varied, with 19.4% of PC occurring in the 45-64 age group and 25.3% in individuals over 65 years. The cumulative risk of being diagnosed before 75 is 8.2%, while the risk of death from PC before 75, according to the European Association of Urology, is 1% in Europe - Uroweb (2024).

**Task 1. To identify the role of some lifestyle factors of patients with PC and BPH on developing the respective disease.**

### 1. Role of fruit and vegetable consumption

As shown in **Fig. 8**, the most frequent responses for fruit and vegetable consumption in all three groups were "two to three times a week". The proportion of daily consumers was much lower in the PC patients (24.4%) compared to the BPH and controls (40% and 35.6%, respectively). The differences were non-significant (chi-square=2.644; df=4; p=0.610). The correlation was weak and insignificant (Cramer's V=0.100; p=0.610).

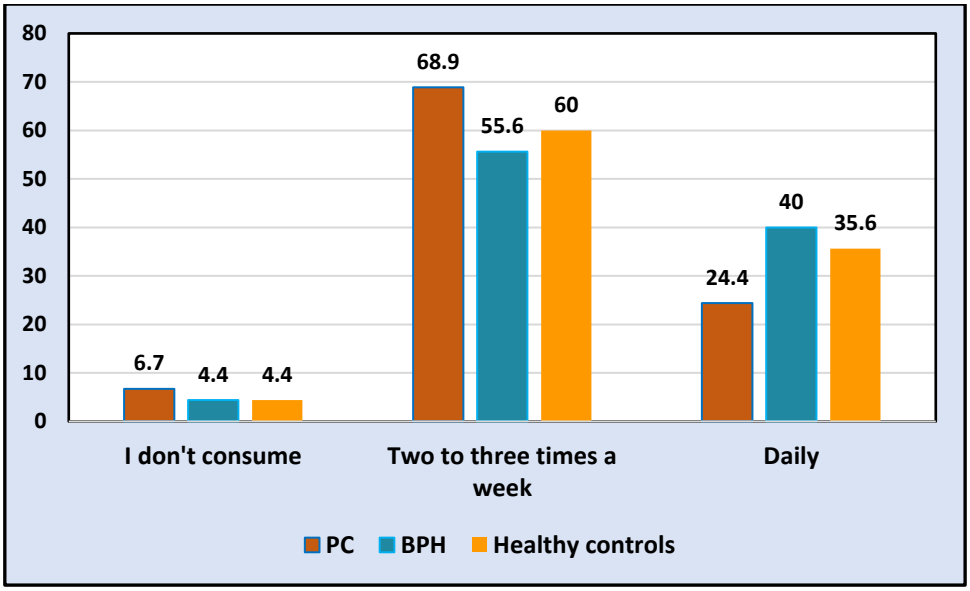



***Figure 8. Distribution of individuals in the compared groups according to fruit and vegetable consumption (%)***

### 2. Role of red wine consumption

The data in **Fig. 9** show that 40% of the persons with PC indicated "did not consume", while this proportion was 3 times lower in the controls (13.3%). Conversely, over half (53.3%) of the healthy individuals indicated drinking one glass daily and one-third (33.3%) drinking more than one glass daily. In the PC group, only 15.6% consumed more than one glass daily, compared with a two-fold higher proportion (33.3%) of healthy individuals.

The differences in the frequency of red wine consumption in the compared groups were significant (chi-square=10.454; df=4; p=0.033). The correlation, although weak, was statistically significant (Cramer's V=0.197; p=0.033).

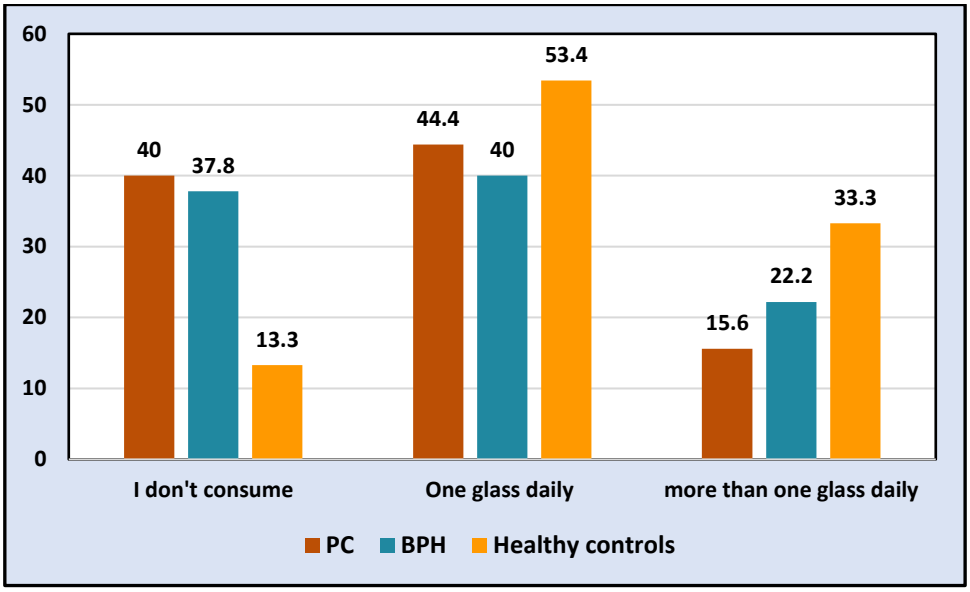



***Figure 9. Distribution of persons in the compared groups according to the frequency of red wine consumption (%)***

### 3. Role of smoking

As shown in **Fig. 10**, the proportion of non-smokers in the three groups we compared was very low, 20% for the PC group and 15.6% each for BPH and healthy persons. The most frequent response was "I used to smoke, but I quit": 57.8% each for the PC and healthy persons and 46.7% for the BPH. The number of smokers at the time of the survey was highest in the BPH group (37.8%), followed by the controls (26.7%), whilst the PC group had the lowest number of smokers (22.2%). Differences between the three groups were non-significant (chi-square=3.033; df=4; p=0.552). The correlation was very low and insignificant (Cramer's V=0.106; p=0.610).

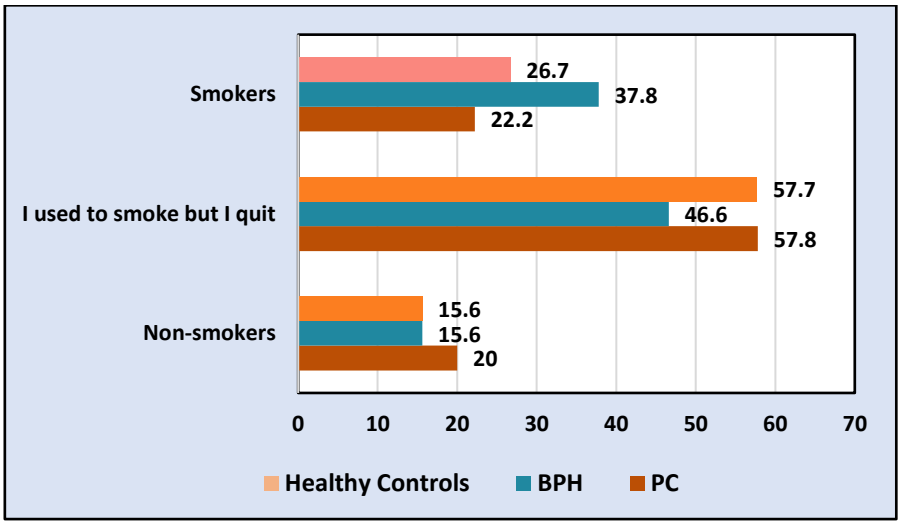



***Figure 10. Distribution of individuals in the compared groups according to smoking prevalence at the time of the survey (%)***

## 4. Role of exercise

More than half of the patients with PC (51.1%) and BPH (53.3%) chose the answer "I exercise 2-3 times a week"; one-third indicated that they did not exercise at all (33.3% and 31.1%) and the proportion of those who exercised every day was even lower: only 15.6% in both the PC and BPH groups (**Fig. 11)**, probably due to age and comorbidities. The differences were statistically insignificant (chi-square=2.470; df=4; p=0.650), and the correlation was very low and non-significant (Cramer's V=0.096; p=0.650).

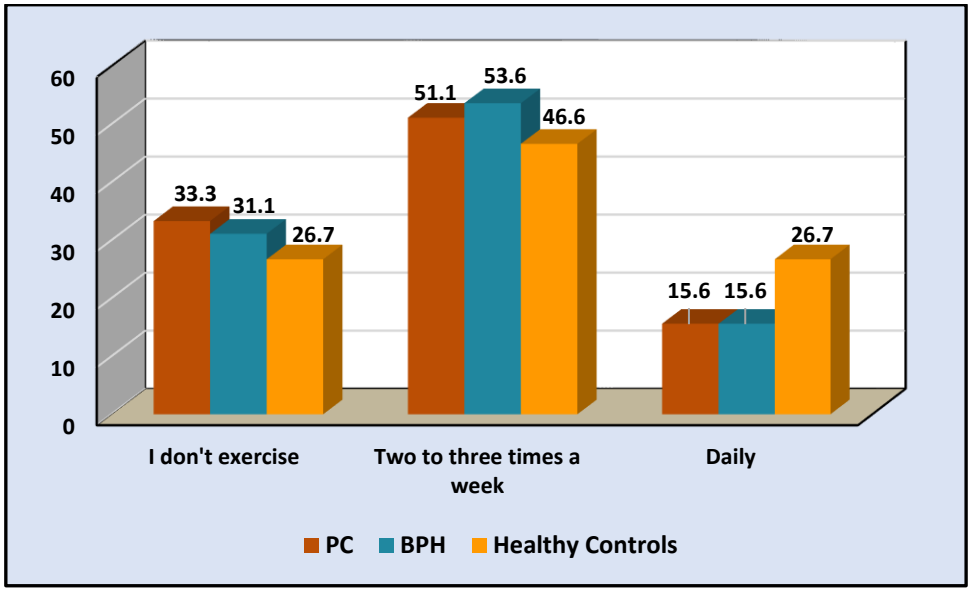



***Figure 11. Distribution of persons in the compared groups according to the frequency of playing sports (%)***

## 5. Role of body weight

We evaluated the body mass index (BMI) of each examined person according to the WHO scale: underweight - BMI <18.5; normal weight - BMI 18.5 - 24.99; pre-obese - BMI 25.0 - 29.99; obese - BMI ≥ 30.0

There were no underweight men in the observed groups. The highest proportion of individuals was in the "pre-obese" category - 80% in the PC group, 48.9% - in BPH, and 53.4% in healthy individuals). Of the PC patients, 13.3% were obese, whereas this relative proportion was only 2.2% for healthy controls (**Fig. 12**). The differences were highly significant (chi-square=20.650; df=4; p=0.000) and they were supported by a moderate and significant correlation (Cramer's V=0.277; p=0.000).

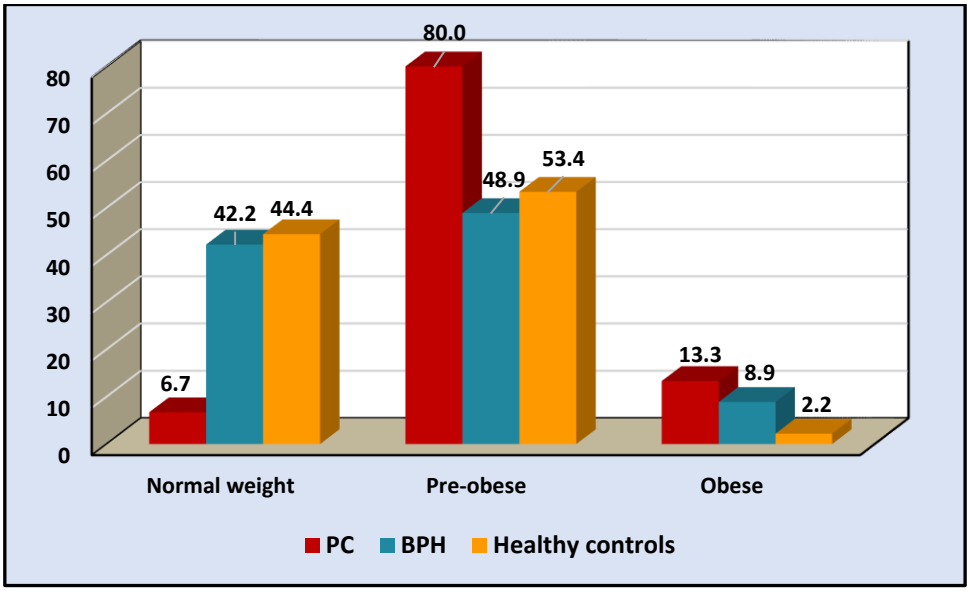



***Figure 12. Distribution of persons in the compared groups according to the degree of obesity (%)***

The highest mean BMI value was observed in patients with PC (27,653±2,359), followed by patients with BPH (26,770±4,197), and the lowest BMI was observed in healthy individuals (24,647±2,235), as shown in **Fig. 13.**

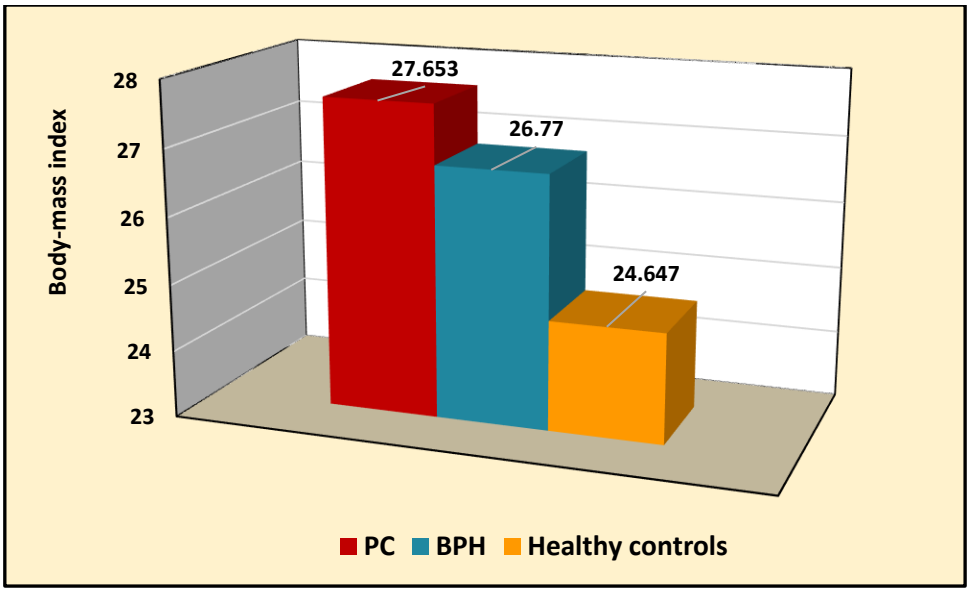



***Figure 13. Mean BMI values in the three groups compared***

The subgroup differences in BMI, demonstrated by comparing the median values using the Mann-Whitney U-test for two independent samples, were statistically significant (**Table 3).** The significance level of the differences was p=0.000 for the groups "PC - healthy", p=0.014 for "BPH and healthy persons". Significant difference in BMI between the PC and BPH groups was also significant (p=0.012).

*Table 3. Statistical significance levels of differences in BMI in subgroup comparison*

| Groups compared | Mann-Whitney U test | z | p |
|---|---|---|---|
| **PC - Healthy controls** | 352.50 | 5.326 | 0.000 |
| **BPH – Healthy controls** | 708.50 | 2.453 | 0.014 |
| **PC – BPH** | 700.00 | 2.522 | 0.012 |

**6. Intake of antioxidants**

A very small number of individuals in the three groups compared indicated that they were taking antioxidants. There were no significant differences (chi-square=2.368, df=2, p=0.306; extremely weak correlation Cramer's V=0.132).

**7. Presence of relatives in the family with malignancy**

A total of 36 individuals reported the presence of malignancy in relatives in the family (**Table 4**): 15 individuals (33.3%) in the PC group, followed by 10 patients (22.2%) in the BPH group and 11 cases (24.4%) in the healthy group. There was no statistically significant difference between the three groups compared (chi-square=1.591; df=2; p=0.451), and the degree of correlation was extremely weak (Cramer's V=0.109).

***Table 4. Distribution of persons in the compared groups according to the answers to the question "Are there any relatives with malignant disease in the family*? *(number and %)***

| Answers / Groups | | Yes | No | Total |
|---|---|---|---|---|
| **PC** | N | 15 | 30 | 45 |
| | **%** | **33.3** | **66.7** | **100.0** |
| **BPH** | N | 10 | 35 | 45 |
| | **%** | **22.2** | **77.8** | **100.0** |
| **Controls** | N | 11 | 34 | 45 |
| | **%** | **24.4** | **75.6** | **100.0** |
| **Total** | N | 36 | 99 | 135 |
| | **%** | **26.7** | **73.3** | **100.0** |

**8. Presence of diabetes**

There were 5 cases of diabetes mellitus (11.1%) in the PC group. Another 6 cases were recorded in the group of patients with BPH (13.3%). There were no cases of diabetes in healthy persons. Although the number of persons with diabetes in both patient groups was very low, the differences were statistically significant (chi-square =6.136; df=2; p=0.047), but the correlation was weak (Cramer's V=0.213).

**9. Presence of rheumatoid arthritis**

A total of 5 individuals in the groups we compared indicated that they had rheumatoid arthritis: 2 patients with PC and 3 patients with BPH. These data do not allow us to conclude about the association of PC and BPH with the presence of rheumatoid arthritis **(**chi-square=2.908; df=2; p=0.234; very weak correlation - Cramer's V=0.147).

From the analysis of the role of some lifestyle factors of patients with PC and BPH on the development of the respective disease, statistically significant differences and correlations were demonstrated for the following factors:

- age over 70 years was the age with the highest risk of developing PC and BPH;

- systematic moderate consumption of red wine can be considered a protective factor against the development of PC and BPH;

- pre-obesity and obesity are significantly more frequent in patients with PC and BPH and can be considered a significant risk factor for the development of the disease;

- the presence of diabetes increases the likelihood of developing PC.

Our data confirm the positive influence of some lifestyle factors. Patients with PC and BPH reported the most frequent consumption of fruit and vegetables 2-3 times a week. The highest percentage of patients also consumed one glass of red wine per day, and its positive effects on health are still the subject of many studies. Some authors even have opposite opinions on its usefulness, but most literature supports its antioxidant properties for the body.

Physical activity positively affects health, but we did not obtain a statistically significant correlation with this lifestyle indicator, which is probably due to the age of the patients.

We also evaluated smoking in different groups as a risk factor, with the highest percentage of patients with PC and BPH indicating that they were ex-smokers. Still, it remains one of the critical risk factors for the accumulation of OS in the body and an increased risk of urological cancer progressing with a more aggressive course and a high risk of recurrence. For PC, many studies by other authors, such as Mari et al. (2017), confirmed higher Gleason score levels in smokers, more advanced tumour stage and extracapsular invasion. Smoking cessation, although not thoroughly studied, provides a protective effect. According to R. Kumar et al. (2023) and Cacciamani et al. (2021), smoking leads to worse outcomes after therapy. R. Tellini (2021) and P. Pietro (2022) assume that mortality, infection rates and complications are increased in patients who smoke.

The highest statistical significance concerning BMI was not found. It has a significant role as a risk factor in these patient groups; many other authors have confirmed this. Obesity increases the risk for many chronic diseases, including type 2 diabetes and cardiovascular disease. It was estimated that in 2019, about 4.6% of adult cancer deaths were attributable to overweight and obesity, accounting for about 462,550 deaths worldwide. According to some researchers, the associations between obesity and various cancers reported in different studies are gender, site, and histological subtype-specific. Various mechanisms have been proposed to link obesity and cancer. A study by Pan J. et al. (2021) demonstrated the association between BMI and serum albumin levels. Patients with high BMI but lower serum albumin levels have a higher risk of death, in contrast to patients with low BMI but higher albumin levels.

Diabetes and rheumatoid arthritis also play an essential role in the development and prognosis of prostate diseases, but we cannot draw a conclusion about them because of the small number of patients with these comorbidities.

The presence of a family history of malignancy in one-third of PC patients (33.3%) supports evidence from a number of other studies that hereditary predisposition and family history are important risk factors for the development of PC.

**Task 2. To compare serum TAS levels in healthy controls and in patients with PC and BPH.**

As can be seen from ***Table 5 and Fig. 14***, the mean TAS levels at admission in the PC and BPH groups were significantly lower than the mean in healthy controls: in PC, 1.69±0.1131; in BPH, 1.72±0.1176; in healthy controls, 1.93±0.1198. Similarly, the median TASs in PC and BPH (1.70 and 1.74 mmol/l) were significantly lower than in healthy controls (1.95 mmol/l). The significance of these differences was confirmed by univariate analysis of variance (F=53.988; p=0.000) and by comparing the medians using the Kruskal-Wallis test (H=62.306; df=2; p=0.000).

***Table 5. Descriptive characteristics of TAS on admission in subjects in the three groups compared (reference values 1.3 - 1.77 mmol/l)***

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | 1,6931 ± 0,1131 | 1.70 | 1.659 – 1.728 | 1.35 | 1.87 | - 0.931 |
| **BPH** | 1.7224 ± 0.1176 | 1.74 | 1.688 – 1.757 | 1.50 | 1.89 | - 0.332 |
| **Healthy controls** | 1.9280 ± 0.11985 | 1.95 | 1.894 – 1.962 | 1.52 | 2,10 | - 1.420 |

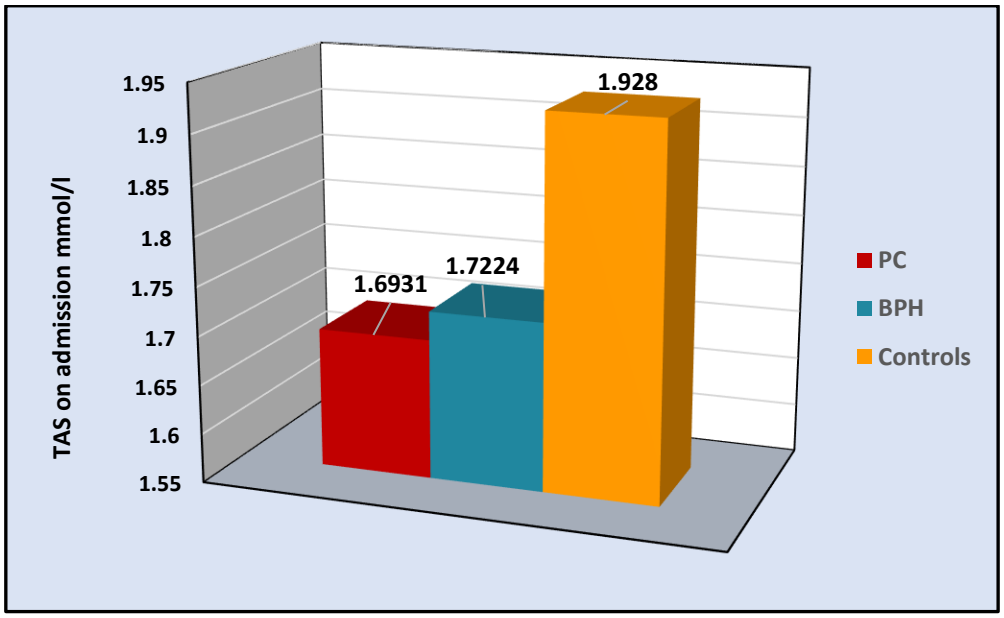



***Figure 14. Mean TAS values on admission in the three groups compared***

When comparing the TAS for each person against the reference TAS values (1.3 - 1.77 mmol/l), we did not find any cases below the lower limit of the reference interval. While 42 individuals

(93.3%) in the control group had TAS values above 1.77 mmol/l, only 11 patients (24.4%) in the PC group had TAS values above 1.77 mmol/l. The differences were highly significant ($\chi^2$=49.269; df=2; p=0.000) and strongly significantly correlated (Phi=0.604; p=0.000).

Subgroup comparison of median TAS in healthy controls and patients with PC and BPH using the Mann-Whitney U criterion for independent samples (**Table 6**) showed the presence of a highly significant difference between TAS in PC patients and healthy controls (z=7.019; p=0.000) and BPH and healthy controls (z=6.539; p=0.000), whereas there was no statistically significant difference between the two patient groups (PC and BPH) (z=1.110; p=0.267).

***Table 6. Statistical significance levels of differences in subgroup comparison of TAS at admission***

| Groups compared | Mann-Whitney U test | z | p-value |
|---|---|---|---|
| **PC - Healthy controls** | 143.00 | 7.019 | 0.000 |
| **BPH – Healthy controls** | 202.500 | 6.539 | 0.000 |
| **PC – BPH** | 875..00 | 1.110 | 0.267 |

Some authors have also confirmed that TAS levels, as well as levels of key antioxidant enzymes, are lower in patients with malignancy compared to healthy individuals. Also, each laboratory should set its own reference limits depending on patients' geographical distribution, race, and certain lifestyle factors because reference limits can vary considerably from region to region.

The levels of individual antioxidants, as well as some vitamins, are reduced in the blood of patients with BPH and PC, but the overall antioxidant status of these patients has not been assessed. There is little recent literature on TAS in patients with BPH and PC, but there is data on TAS in other malignancies such as colon cancer, breast cancer, etc.

Our results confirm that TAS is significantly lower in the selected two groups of patients compared to the control group and decreases with advancing disease stage. There is still much discussion and controversy on this topic, as high ROS levels may more likely result from neoplastic transformation rather than its cause, and high ROS levels are known to lead to increased OS and, hence, decreased TAS.

**Task 3. To establish the level of correlation between the results obtained for TAS and other parameters of antioxidant significance.**

### 3.1. Correlation between TAS and uric acid on admission

The results presented in **Table 7**, which describe the characteristics of the compared groups convincingly, show that mean uric acid values were significantly lower in patients with PC and BPH compared to healthy subjects. Because the frequency distribution of uric acid values in all three groups was asymmetric (right-weighted for PC and BPH and left-weighted for healthy subjects), we also compared the medians, which better characterise the central tendency in asymmetric distributions. We observed significantly lower median values for uric acid in patients with PC and BPH compared with healthy controls. The differences were at a high level of significance (one-factor ANOVA F=6.67; p=0.002; Kruskal-Wallis H=17.808; df=2; p=0.000).

***Table 7. Descriptive characteristics of uric acid on admission in the comparison groups reference ranges 202-420 mkmol/l)***

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | 340.11 ± 83.636 | 325.00 | 318.165 – 362.058 | 215 | 612 | 1.105 |
| **BPH** | 353.58 ± 84.127 | 343.00 | 331.631 – 375.524 | 169 | 531 | 0.439 |
| **Healthy controls** | 395.09 ± 50.451 | 405.00 | 373.142 – 417.035 | 273 | 491 | -0.622 |

Subgroup comparisons of medians by Mann-Whitney U test were also statistically significant (**Table 8**): between PC patients and healthy subjects - z=4.148; p=0.000); between BPH and healthy subjects - z=2.950; p=0.003. The difference between the two patient groups was non-significant (z=0.864; p=0.348).

***Table 8. Statistical significance levels of differences in subgroup comparison of uric acid at admission***

| Groups compared | Mann-Whitney U test | z | p-value |
|---|---|---|---|
| **PC – Healthy controls** | 498.500 | 4.148 | 0.000 |
| **BPH – Healthy controls** | 647.00 | 2.950 | 0,003 |
| **PC – BPH** | 905.500 | 0.864 | 0.388 |

The correlation between TAS values and uric acid in patients with PC is weak and non-significant (Pearson r=0.17; p=0.265) - **Fig. 15**.

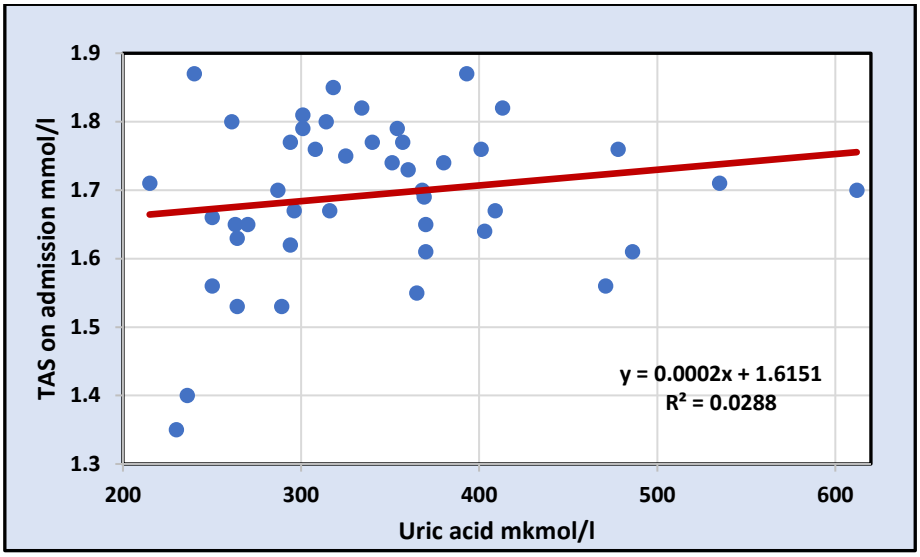



***Figure 15. Correlation between TAS and uric acid on admission in PC patients***

For the BPH group, the correlation between TAS and uric acid was strong and highly significant (r=0.509; p=0.000) as shown in **Figure 16.** The coefficient of determination $R^2$=0.2592 means that 25.9% of changes in TAS can be associated with changes in uric acid level.

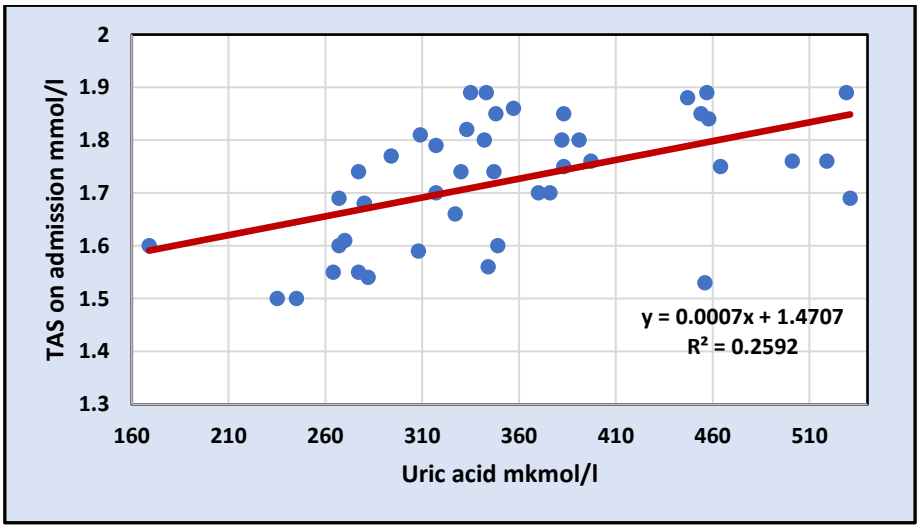



***Figure 16. Correlation between TAS and uric acid on admission in patients with BPH***

An even higher degree of correlation between TAS and uric acid was found in healthy subjects (Pearson r=0.706; p=0.000). The coefficient of determination $R^2$=0.4987 means that almost 50% of changes in TAS can be associated with changes in uric acid (**Fig. 17**).

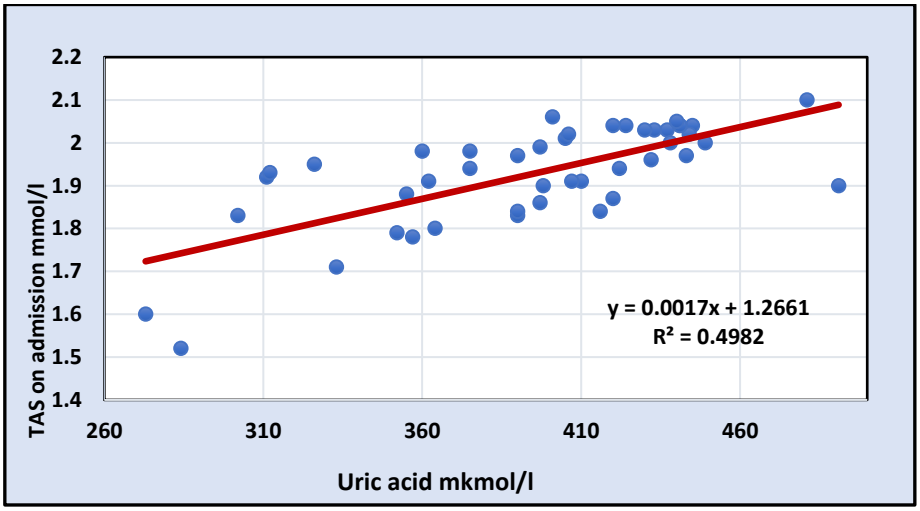



***Figure 17. Correlation between TAS and uric acid in healthy subjects***

### 3.2. Correlation between TAS and albumin at admission

In the patients with PC and BPH groups, mean albumin levels and medians had very similar values, but in both groups, they were significantly lower than the corresponding albumin values in healthy subjects (**Table 9**). The differences in mean albumin levels in the three groups compared were highly significant (univariate ANOVA - F(2,132)=13.186; p=0.000; Kruskal-Wallis - H=23.655; df=2; p=0.000).

***Table 9. Descriptive characteristics of albumin in the groups compared at admission***

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | 43.491 ± 4.987 | 44.300 | 42.336 – 44.646 | 31.6 | 50.0 | - 0.580 |
| **BPH** | 43.027 ± 3.825 | 43.500 | 41.872 – 44.182 | 32.9 | 48.2 | - 1.011 |
| **Healthy controls** | 46.909 ± 2.551 | 46.900 | 45.754 – 48.064 | 39.1 | 53.4 | - 0.316 |

There were highly significant subgroup differences between albumin levels in PC and BPH compared to healthy subjects (**Table 10**). The difference between the two patient groups was statistically insignificant (p=0.307).

*Table 10. Levels of statistical significance of differences in subgroup comparison of albumin at admission*

| Groups compared | Mann-Whitney U test | z | p-value |
|---|---|---|---|
| **PC – Healthy controls** | 635.000 | 3.43 | 0.002 |
| **BPH– Healthy controls** | 369.000 | 5.194 | 0.000 |
| **PC – BPH** | 886.000 | 1.021 | 0.307 |

The correlation between TAS and albumin values in all three study groups was very weak and non-significant (**Table 11).** The correlation was even inverse in the PC patients and healthy subjects, i.e., as albumin values increased, TAS values decreased.

*Table 11. Correlation between TAS and albumin in the compared groups and significance of the correlation*

| Groups | Pearson r | p-value |
|---|---|---|
| **PC** | - 0.195 | 0.198 |
| **BPH** | 0.037 | 0.807 |
| **Healthy controls** | - 0.133 | 0.383 |

### 3.3. Correlation between TAS and total bilirubin at admission

When comparing the mean total bilirubin values in the three groups, there was a slight trend towards lower total bilirubin values in patients with PC and BPH compared to healthy subjects (**Table 12**), but the differences were statistically unreliable (Kruskal-Wallis H=1.723; df=2; p=0.423).

*Table 12. Descriptive characteristics of total bilirubin in the compared groups at admission*

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | 43.491 ± 4.987 | 44.300 | 42.336 – 44.646 | 31.6 | 50.0 | - 0.580 |
| **BPH** | 43.027 ± 3.825 | 43.500 | 41.872 – 44.182 | 32.9 | 48.2 | - 1.011 |
| **Healthy controls** | 46.909 ± 2.551 | 46.900 | 45.754 – 48.064 | 39.1 | 53.4 | - 0.316 |

Subgroup comparisons of mean total bilirubin values by Mann-Whitney U-test also revealed statistically non-significant differences in all three pairs of groups compared: PC – Healthy controls (p=0.183); BPH – Healthy controls (p=0.397) and PC - BPH (p=0.759).

The correlation between TAS values and total bilirubin in PC patients was weak and inverse (Pearson r=0.044; p=0.778); in BPH patients, it was even weaker and inverse (Pearson r=0.015; p=0.922). Only in healthy subjects there was a significant moderate correlation (Pearson r=0.307; p=0.04).

The results of our study show that of the investigated parameters with antioxidant significance, uric acid plays the most significant role in changes in TAS. Its mean values in PC and BPH patients were significantly lower than those in healthy subjects. The correlation between TAS and uric acid in PC was weak and of low significance. A moderate and significant correlation was found only for patients with BPH.

There are conflicting opinions in the literature regarding uric acid levels and its protective role in the development of these two diseases. Some authors even claim that its levels are positively associated with the risk of developing PC. In our opinion, uric acid has been shown to be a potent antioxidant, which is evident in the healthy subjects group and TAS levels.

The mean albumin levels in the PC and BPH groups were nonsignificantly lower compared with healthy subjects, and there was no significant correlation between TAS and albumin levels in PC and BPH patients.

There were no statistically significant differences in the mean TAS and total bilirubin values in all three groups compared. The correlation between TAS and total bilirubin in PC and BPH was extremely low and inverse. A reliable moderate straight correlation was found between TAS and total bilirubin only in healthy subjects.

**Task 4. To look for a relationship between TAS and major markers of inflammation.**

### 4.1. Correlation between TAS and C-reactive protein at admission

We found significantly higher mean CRP values in patients with PC compared to healthy subjects, nearly 5-fold higher mean values and nearly 3-fold higher median values (**Table 13**).

***Table 13. Descriptive characteristics of CRP in the compared groups at admission***

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | 12.180±16.7210 | 5.100 | 8.934 – 15.426 | 0.2 | 67.2 | 1.981 |
| **BPH** | 6.587±8.7034 | 4.000 | 3.341 – 9.833 | 0.8 | 47.2 | 2.939 |
| **Healthy controls** | 2.528±2.8487 | 1.800 | - 0.718 – 6.574 | 0.5 | 18.5 | 4.316 |

In patients with BPH, mean CRP levels were lower as compared to PC patients but also remained significantly higher compared to healthy subjects (**Fig. 18)**. The differences in mean CRP levels between the three groups were generally at a high level of significance (Kruskal-Wallis H=18.481; df=2; p=0.000).

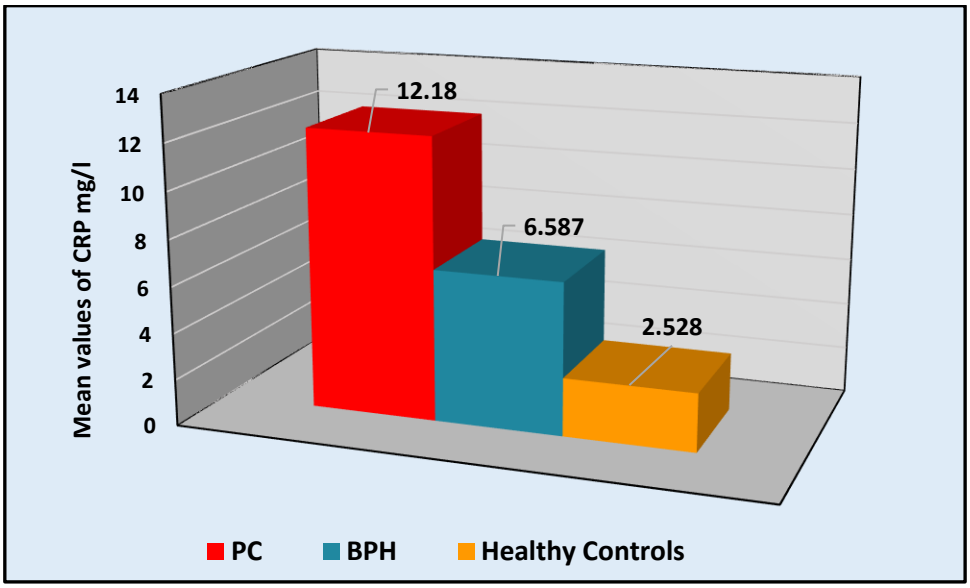



***Figure 18. Mean CRP values in the compared groups at admission***

Because the distribution of CRP in the three groups was highly asymmetric, we performed subgroup comparisons of medians using the Mann-Whitney U test (**Table 14**). Differences between the PC and healthy subjects (z=3.947; p=0.000) and between the BPH and healthy subjects (z=3.262; p=0.001) were highly significant. There was no significant difference between the PC and BPH groups (z=1.227; p=0.22).

*Table 14. Levels of statistical significance of differences in subgroup comparison of CPR at admission*

| Groups compared | Mann-Whitney U test | z | p-value |
|---|---|---|---|
| **PC – Healthy controls** | 523.500 | 3.947 | 0.000 |
| **BPH – Healthy controls** | 608.500 | 3.262 | 0.001 |
| **PC - BPH** | 860.500 | 1.227 | 0.,220 |

The correlation between TAS and CRP at admission for patients with PC was weak and non-significant (r=0.226; p=0.136). There was no correlation between patients with BPH and healthy subjects (r=-0.032 and r=-0.061, respectively).

### 4.2. Correlation between TAS and leukocytes on admission

The mean leukocyte levels at admission in the groups compared tended to show marked differences (**Table 15 and Fig. 19**). The mean and median values were the highest in the PC group ($8.551x10^9 \pm 2.9048$), followed by the BPH group ($8.073x10^9 \pm 2.5322$). Significantly lower mean leukocyte values were found in healthy subjects ($6.847x10^9 \pm 1.7603$).

*Table 15. Descriptive characteristics of leukocytes in the compared groups at admission*

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | $8.551x10^9 \pm 2.9048$ | 7.900 | 7.830 – 9.272 | 4.4 | 18.08 | 3.033 |
| **BPH** | $8.073x10^9 \pm 2.5322$ | 7.300 | 7.362 – 8.795 | 4.7 | 15.8 | 0.441 |
| **Healthy controls** | $6.847x10^9 \pm 1.7603$ | 6.600 | 6.125 – 7.658 | 4.1 | 11.6 | 0.336 |

In the PC patients, leukocyte values had a significant range (from $4.4x10^9$ to $18.08x10^{9)}$ and a marked asymmetric distribution (Skewness=3.033)

Differences in mean values of leukocytes between the three groups were statistically significant, as confirmed by one-factor ANOVA (F=5.814; p=0.004) and by the non-parametric Kruskal-Wallis test (H=10.940; df=2; p=0.004).

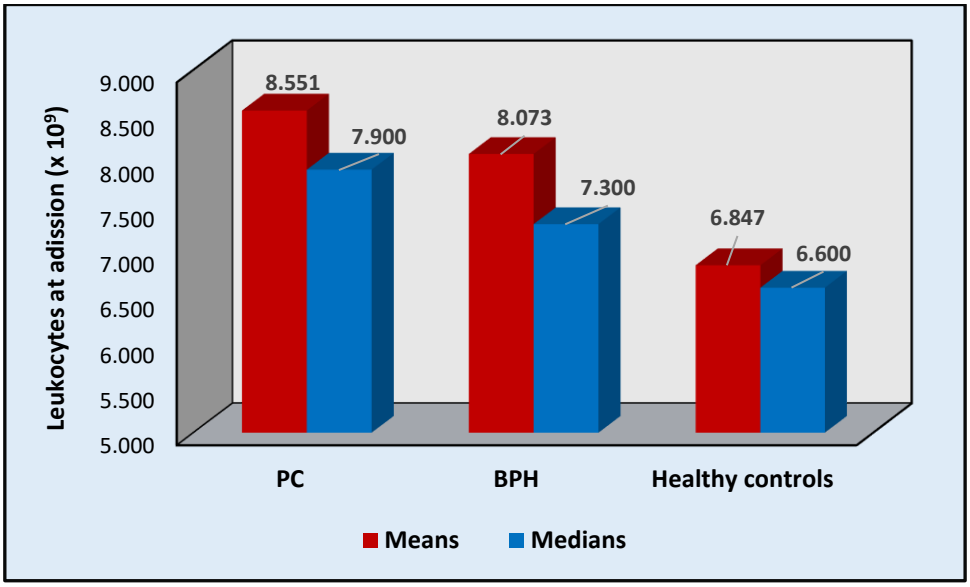



***Figure 19. Values of means and medians for leukocytes at admission in the compared groups***

Pooled comparisons by the Mann-Whitney U test (**Table 16**) showed significant differences for PC patients and healthy subjects (z=3.314; p=0.001) and BPH patients and healthy subjects (z=2.217; p=0.027). As with CRP, subgroup comparison of mean leukocyte values between the two patient groups (PC - BPH) showed a statistically unreliable difference (z=0.726; p=0.468).

***Table 16. Levels of statistical significance of differences in subgroup comparison of leukocytes at admission***

| Groups compared | Mann-Whitney U criterion | z | p |
|---|---|---|---|
| **PC – Healthy controls** | 602.000 | 3.314 | 0.001 |
| **BPH – Healthy controls** | 738.500 | 2.217 | 0.027 |
| **PC - BPH** | 922.500 | 0.726 | 0.468 |

When the degree of correlation was examined, there was no correlation between TAS level and preoperative levels of leukocytes in PC group (r=0.024; p=0.875) and BPH group (r=0.042; p=0.785). A very weak inverse correlation (Rearson r = - 0.163) was established in healthy subjects, which was statistically in significant (p=0.286).

The data from our study convincingly demonstrate that both inflammatory markers examined (CRP and leukocytes) have significantly higher values in patients with PC and BPH compared to healthy subjects. The differences were at a high level of significance.

However, there was no statistically significant correlation between TAS at admission and the levels of the two markers of inflammation (CRP and leukocytes) in all three groups.

These results confirm the data of other authors that patients with PC and BPH have higher levels of these markers of inflammation compared with the control group. This finding makes the investigation of CRP and WBC necessary for these patients, as their higher values are a risk factor for the development and prognosis of BPH and PC, and their changes should be monitored dynamically. High levels of inflammatory markers are associated with increased OS and lower TAS levels, although in our study, there was no statistically significant correlation between TAS and CRP or between TAS and WBC.

Other authors have found that chronic inflammation can cause damage to DNA over time and reduce the action of cells to repair DNA, which would cause uncontrolled cell growth, development of neoplastic cells, and tumour growth. Various cancers are associated with chronic inflammation, not just PC. That is why testing for more inflammatory markers and evaluating them is very important for patients with BPH and PC. There are also conflicting opinions about CRP and its relationship with PC, which confirms the need for further studies on this topic.

**Task 5. To determine the influence of the type of surgical technique on the results of TAS determination, other parameters with antioxidant significance and markers of inflammation.**

### 5.1. Changes in TAS before and after surgery in patients with PC and BPH

We sought to answer the question of what changes in TAS occur after surgery by first comparing individual TAS values in each patient at admission and discharge. We grouped the results into 3 categories: decreasing, no change, and increasing (**Fig. 20**). In total, 30% of patients in both groups had no improvement in TAS value. In 26.7%, there was even a decrease in the TAS value at discharge compared with admission (35.5% for patients with BPH and 17.8% for PC). A more significant improvement in TAS level at discharge was observed in the patients with PC, in 35 patients (77.8%) compared to 28 patients with BPH (62.2%). A more detailed analysis found that TAS elevation was most often in the minimal range of 0.01 to 0.10 mmol/l or 0.11-0.20 mmol/l. This result may be explained by the short stay of the patients in the clinic and the early remeasurement of TAS. There was no significant difference in the changes in TAS levels that occurred in the two groups of patients (chi-square=3.778; df=2; p=0.151; Phi=0.205).

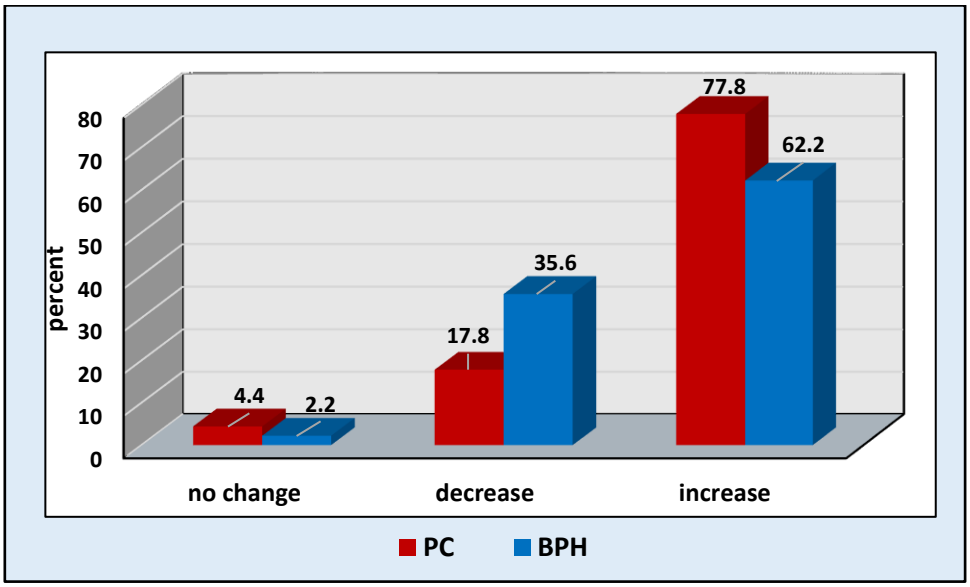



***Figure 20. Changes in TAS level in patients with PC and BPH after surgical intervention (%)***

**What changes occurred in each group? Did the removal of the tumour neoplasm affect TAS levels?**

Despite the short length of stay in the clinic, TAS at discharge in both compared patient groups showed an increase in mean values (**Fig. 21**). The increase was from 1.6931 to 1.7398 in

patients with PC, and it was smaller in patients with BPH - from 1.724 to 1.7631. The mean TAS values at admission and discharge were higher in patients with BPH than those with PC.

We tested the significance of differences in TAS levels in patients with PC and BPH before and after surgery by using Wilcoxon Sign Ranks Test in dependent samples. In both patient groups, the differences were highly significant. The significance of differences was higher for the group of patients with PC (z=3.783; p=0.000) compared to BPH (z=2.572; p=0.01).

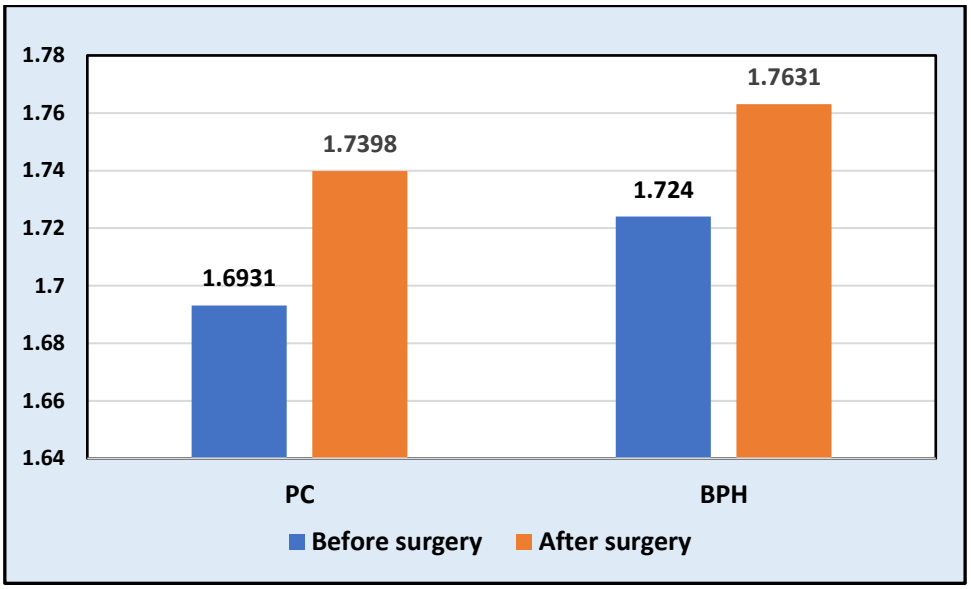



***Figure 21. Mean TAS values in patients with PC and BPH before and after surgical intervention***

**Therefore, the removal of the tumour mass contributed to an increase in TAS levels in both groups of patients.**

This conclusion is supported by the significant correlations between pre- and postoperative TAS values for each group separately. There was a strong correlation with a very high level of significance (Pearson r=0.737; p=0.000) in the PC patients - **Fig. 22**. The coefficient of determination $R^2$=0.5432 means that 54% of the changes in TAS that occurred were due to the surgical intervention.

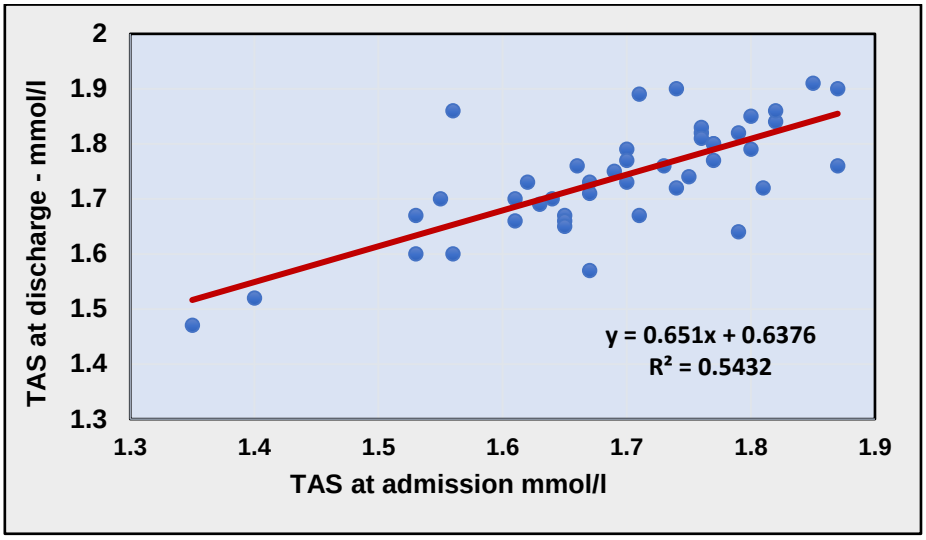



***Figure 22. Correlation between TAS values before and after surgical intervention in patients with PC***

In the group of patients with BPH, the degree of correlation was moderate (r=0.597) but also had a high level of significance (p=0.000) - **Fig. 23**. The coefficient of determination r2=0.3564 means that more than one-third (35.6%) of the changes in that occurred TAS were related to the surgical intervention.

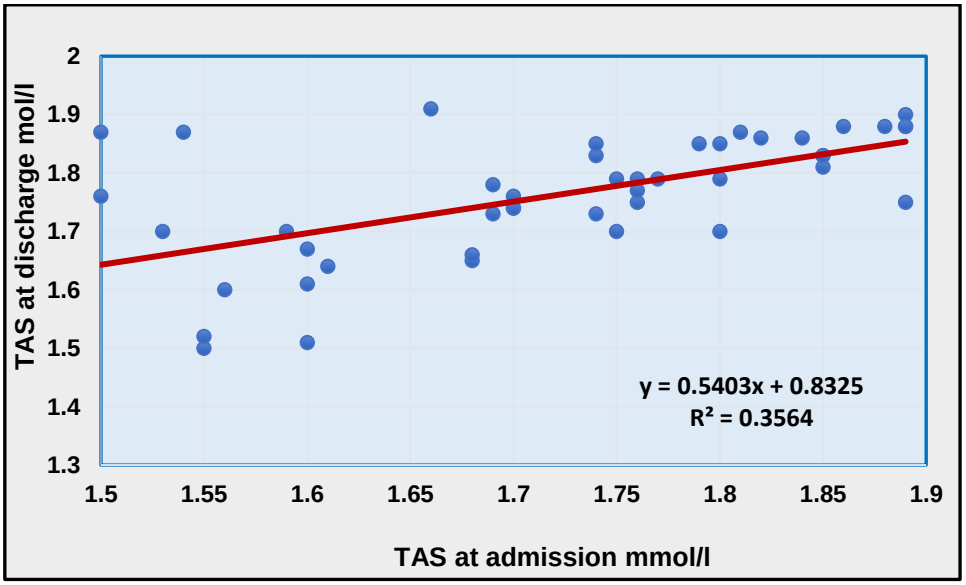



***Figure 23. Correlation between TAS values before and after surgical intervention in patients with BPH***

It is evident that TAS values increase after tumour removal. This change is associated with a decrease in inflammation in the body, a decrease in OS, and increased antioxidant protection, which supports the assumptions of other authors. However, the mechanism by which surgical

intervention affects TAS should be further investigated in the future, which is essential for early diagnosis, successful treatment and disease control in these patients.

According to Bialecki JT et al. (2020), in a study of patients who underwent laparoscopic surgery for inguinal hernia, divided into two groups depending on the surgical technique, TAS levels were found to decrease on the first day after surgery, after which TAS continued to be lower on the fourth day in one group of patients and increased in the second group of patients. It was confirmed that the patients treated with the less invasive technique had higher TAS compared to the other group, in which the trauma of surgery was more significant.

### 5.2. Changes in values of antioxidant parameters before and after surgery in patients with PC and BPH

#### 1. Changes in uric acid values before and after surgery

Comparison of mean uric acid values before and after surgery shows different trends in the two patient groups (**Fig. 24**). The mean preoperative uric acid value was 340.11±83.636 mkmol/l in the PC group and was lower than in the BPH group *(*353.58±84.127 mkmol/l). After surgery, it increased by 7.45 mkmol/l compared to the preoperative level, but the difference was statistically insignificant (Wilcoxon Z=0.601; p=0.548). An opposite trend was observed in patients with BPH: the mean uric acid level after surgery decreased by 5.85 mkmol/l compared to the mean preoperative level and was equal to that of PC after surgery. The differences were also statistically insignificant (Z=1.129; p=0.259).

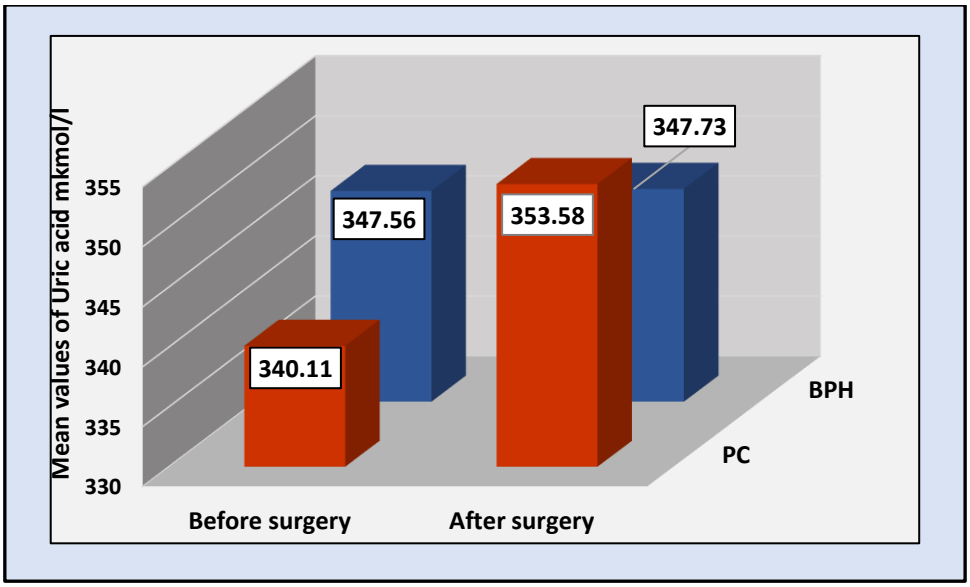



***Figure 24. Mean pre- and postoperative uric acid levels in patients with PC and BPH***

We investigated the correlation between changes in TAS after surgery and changes in uric acid. In patients with PC, the correlation between TAS and uric acid before surgery was weak and non-significant (r=0.17; p=0.236), and after surgery, it changed to moderate and significant (r=0.299; p=0.046). In patients with BPH, the correlation between TAS and uric acid was significant both preoperatively (r=0.508; p=0.000) and postoperatively (r=0.597; p=0.000).

**2. Changes in albumin values before and after surgery**

Albumin showed a tendency to decrease its mean values after surgery in both compared patient groups (**Fig. 25**). In PC patients, the mean albumin value after surgery decreased insignificantly (only by 0.807 g/l), and the difference in values before and after surgery was statistically unreliable (Z = 0.817; p = 0.414). In BPH patients, the decrease in mean albumin value after surgery was more pronounced (by 3.96 g/l), and the difference was highly significant (Z=4.922; p=0.000).

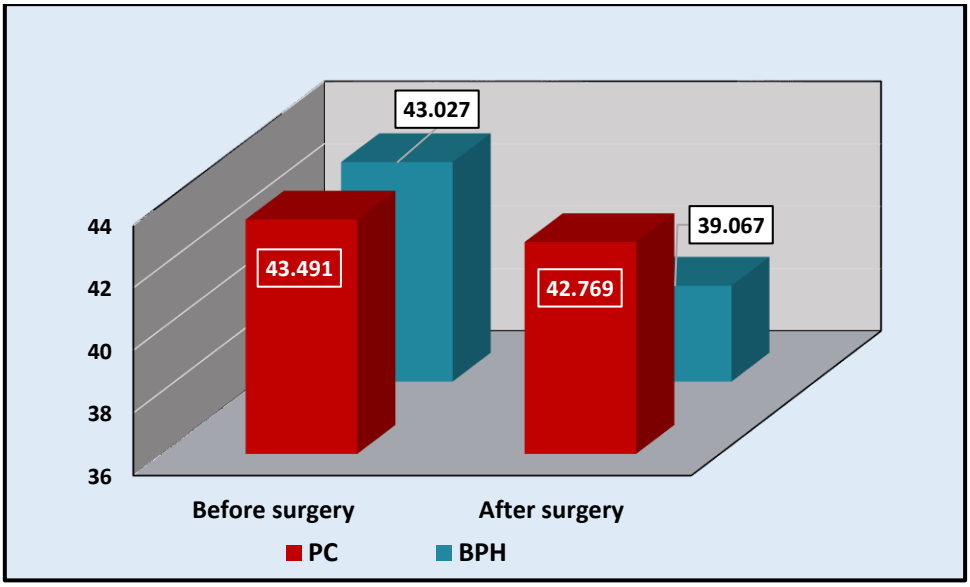



***Figure 25. Mean albumin values before and after surgery in patients with PC and BPH***

We compared the strength of the correlation between TAS and albumin before and after surgery. In both patient groups, the correlation between TAS and albumin levels before surgery was weak and non-significant, and in PC patients, the correlation was even inverse (r = -0.195; p=0.198).

Significant changes occurred after surgery. In both patient groups, the correlation between TAS and albumin increased to moderate and statistically significant: in PC, the correlation coefficient was r=0.317; p=0.032. In patients with BPH, the correlation was even stronger and at a higher level of significance (r=0.452; p=0.002).

### 3. Changes in total bilirubin values

After surgical intervention, total bilirubin in both groups showed a well-defined increase. Its mean values were almost the same in both groups of patients, both before and after surgery (**Fig. 26**). For each group separately, however, the differences before and after surgery were statistically significant: for the group of patients with PC, Wilcoxon Z=3.053; p=0.002; for the group of patients with BPH, Wilcoxon Z=2.522; p=0.010.

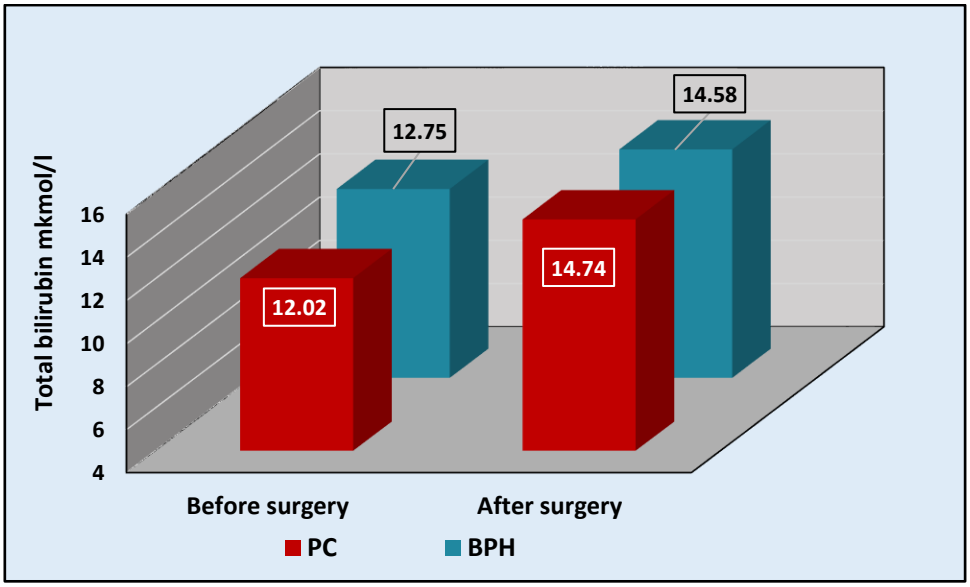



***Figure 26. Mean values of total bilirubin before and after surgery in patients with PC and BPH (µmol/l)***

Correlations between TAS and total bilirubin before and after surgery in both patient groups were extremely weak and statistically unreliable (p>0.05).

## 5.3. Changes in inflammation marker values before and after surgery in patients with PC and BPH

### 1. Changes in C-reactive protein

As can be seen from the data presented in **Fig. 27**, the mean CRP values before and after surgery in PC patients were very similar in significance, and the difference was non-significant (Wilcoxon Z=0.164; p=0.870).

In PDH patients, postoperative CRP values were almost 2-fold higher than preoperative CRP values, most likely due to some highly skewed individual postoperative CRP values. The difference was also statistically unreliable (Wilcoxon Z=1.524; p=0.127).

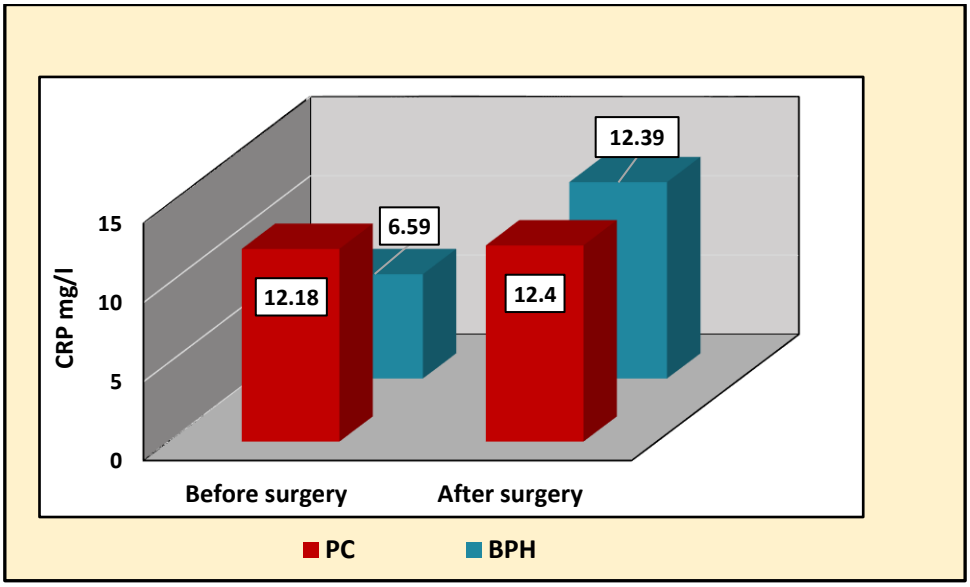



***Figure 27. Mean CRP values before and after surgery in PC and BPH***

Correlations between changes in TAS and CRP before and after surgery were very weak and unreliable. Only in PC patients after surgery, there was a moderate inverse correlation between changes in TAS and CRP (r = - 0.306; p = 0.041), which means that as TAS values increase, there is a tendency for CRP values to decrease as a marker of inflammation. This decrease can be interpreted as a positive sign of the effect of tumour removal in PC patients.

In the literature, the association between CRP values and PC patients has been demonstrated, but there is no apparent difference in the levels of this marker between PC patients and healthy men. However, other authors also confirmed that higher CRP levels are associated with an increased incidence of developing PC [124, 125].

Therefore, this marker has a reference value that should be monitored to assess the prognosis of PC. Inflammation is associated with decreased TAS levels and increased OS. Our results are consistent with the opinions of several other authors, who state that with a decrease in the levels of inflammatory markers, the antioxidant status of the organism increases and that after the removal of the tumour mass, a decrease in CRP levels occurs.

**2. Changes in leukocyte levels**

In both patient groups, there was a slight trend towards a decrease in leukocyte levels after surgery (**Table 17**). The trend was more pronounced in PC patients, where the mean value of $8.551x10^9$±2.9048 before surgery was reduced to $7x851x10^9$±2.4290 after removal of the carcinoma, but the difference was non-significant (Wilcoxon Z=1.343; p=0.179). In patients with BPH, changes in mean leukocyte levels were only marginal, from $8.073x10^9$**±2**.5322

preoperatively to $7.853x10^9$**±3**.2023 postoperatively. The difference was also not significant (Z=1.488; p=0.137).

The mean leukocyte counts of PC and BPH patients after surgery were completely equal ($7.851x10^9$±**2**.4290 for PC and $7.853x10^9$±3.2023 for BPH (p>0.05).

***Table 17. Mean values of leukocytes inPC and with BPH before and after surgery and significance levels of differences***

| **Leukocytes** | **Mean ± SD** | **Median** | **Wilcoxon** | **p-value** |
|---|---|---|---|---|
| **In patients with PC** | | | | |
| ***Before surgery*** | $8.551x10^9$ ± 2.9048 | 7.9 | **1.343** | **0.179** |
| ***After surgery*** | $7.851x10^9$ ± 2.4290 | 7.3 | | |
| **In patients with BPH** | | | | |
| ***Before surgery*** | $8.073x10^9$ ± 2.5322 | 7.300 | **1.488** | **0.37** |
| ***After surgery*** | $7.853x10^9$ ± 3.2023 | 7.200 | | |

There was no correlation between TAS level and leukocyte count in PC patients, both preoperatively and postoperatively.

There was a very weak non-significant correlation in patients with BPH (r=0.104 preoperatively and r=0.133 postoperatively).

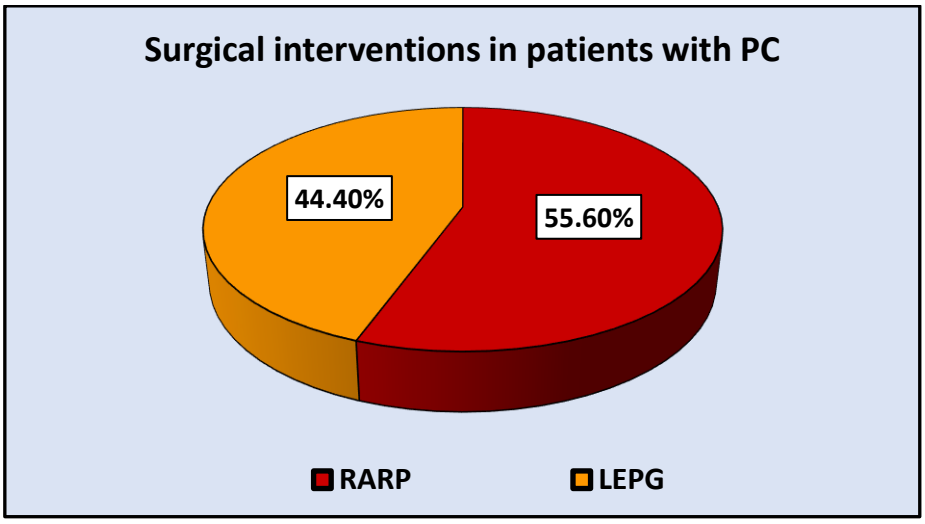
Data from a study by Bialecki JT et al. (2020) also confirm our findings that there was no significant change in leukocyte, CRP and TAS levels preoperatively between the groups compared. In our study, the levels of inflammatory markers decreased after surgery, and TAS increased slightly, but the correlation was statistically insignificant.

**Task 6. To compare and analyse the mean levels of TAS, other markers of antioxidant significance and markers of inflammation in patients with PC and BPH before and after surgery.**

The two groups of patients underwent different types of surgical interventions.

In 25 patients (55.6%) with PC, the tumour mass was removed by robot-assisted radical prostatectomy (RARP), and in 20 (44.4%), laparoscopic enucleation of the prostate gland (LEPG) was performed.

In patients with BPH, biopsy was the most common intervention - 27 patients (60%) and Fusion biopsy - 6 patients (13.3%). Transurethral resection of the prostate (TURP) was performed on 12 patients (26.7%). (**Fig. 28**)



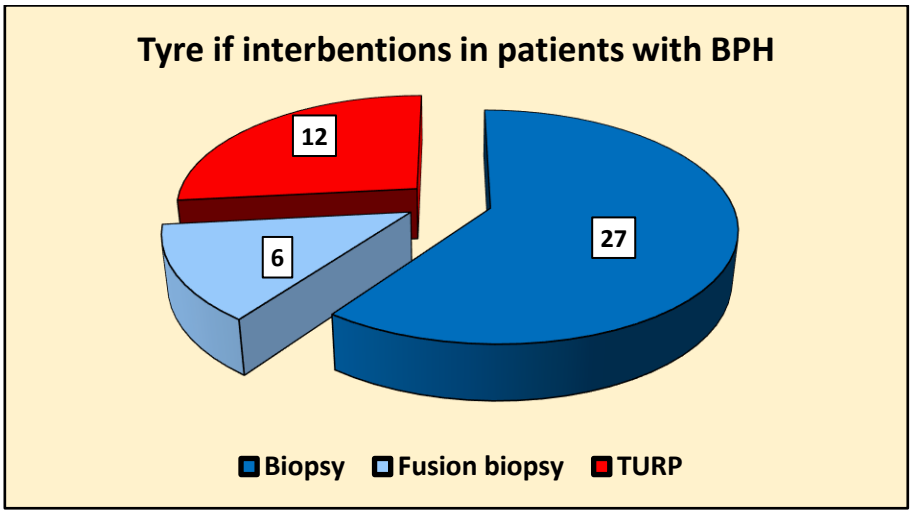




***Figure 28. Distribution of patients with PC and BPH according to the type of surgical intervention (%)***

The surgical approaches applied confirm the opinions of other authors that minimally invasive techniques are increasingly being adopted in surgical treatment methods, with an increasing percentage of robot-assisted surgery. These have many advantages over standard open surgery as they are much gentler on patients, with a quicker recovery period and better outcomes.

Due to the variation in surgical interventions used and the insufficient number of patients with some of the surgical techniques in patients with BPH, further analysis considers the impact of surgical technique only in patients with PC.

### 6.1. Changes in TAS after surgery in patients with PC

Despite the short length of stay in the clinic, significant positive changes occurred overall in patients with PC, with an increase in mean TAS from 1.6945 mmol/l to 1.7398 mmol/l (**Fig. 29).** Increases in TAS values were observed in both subgroups of patients with PC depending on the type of surgical technique.

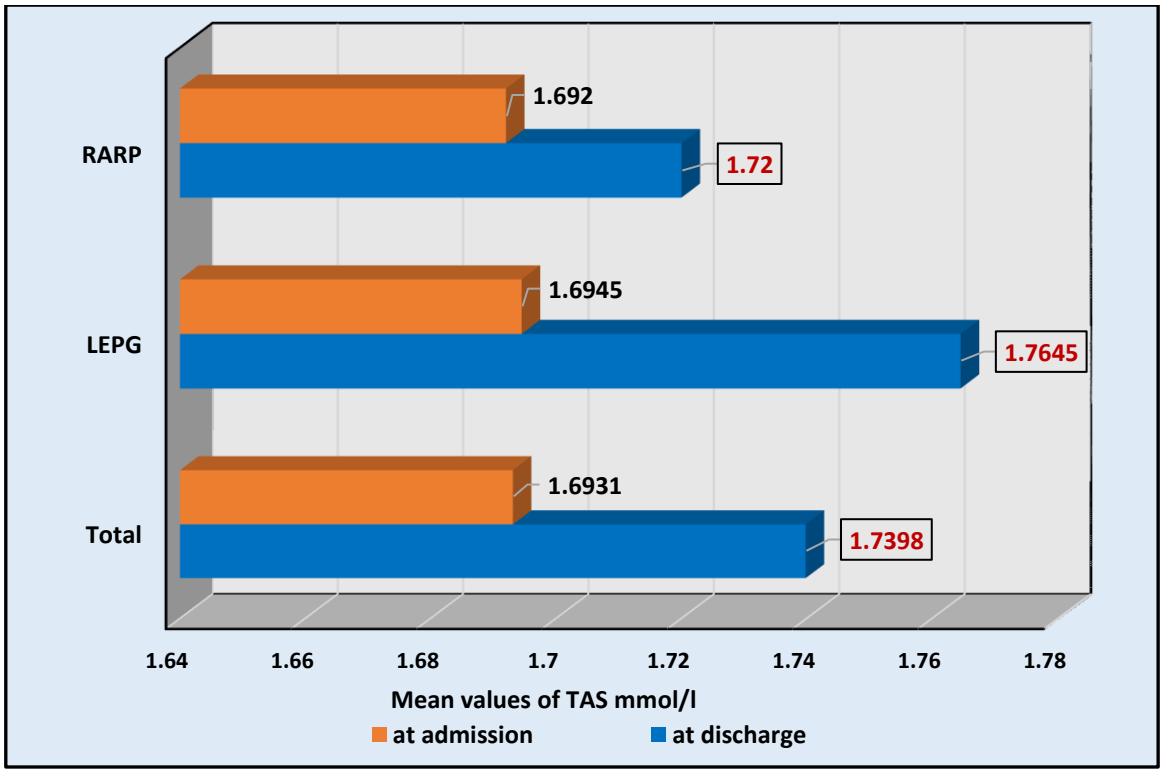



***Fig. 29 Mean TAS values in PC patients before and after surgery depending on the surgical technique (in mmol/l)***

Correlations between pre- and postoperative TAS values in the two groups according to the type of surgical technique and for the PC group are presented in **Table 18**.

The correlation was significant (r=0.617; p=0.001) in those operated with RARP. The coefficient of determination was r2=0.3807, i.e., 38% of the changes in TAS after surgery were related to the RARP operative technique. The correlation was even higher (r=0.819, p=0.000) in those operated with LEPG. The coefficient of determination was $R^2$=0.6708, i.e. 67% of the changes in TAS were related to the operative LEPG technique.

***Table 18. Correlation between TAS at admission and discharge in patients with PC depending on the type of surgical technique***

| Groups compared | TAS | Pearson r | p-value |
|---|---|---|---|
| **Robot-assisted radical prostatectomy (RARP))** | **At admission** | **0.617** | **0.001** |
| | **at discharge** | | |
| **Laparoscopic enucleation of prostate gland (LEPG)** | **At admission** | **0.819** | **0.000** |
| | **At discharge** | | |
| **Total for PC** | **At admission** | **0.737** | **0.000** |
| | **At discharge** | | |

For the entire group of patients with PC, the correlation was high (Pearson r=0.737; p=0.000). The coefficient of determination was **R2**=0.5432, i.e. a total of 54% of the changes in TAS were due to tumour removal (**Fig. 30)**.

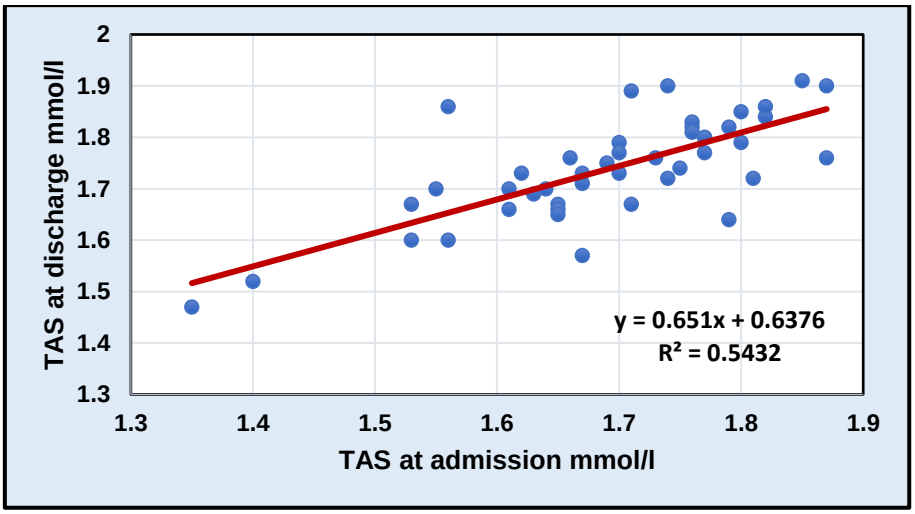



***Figure 30. Correlation between TAS values before and after surgery in all patients with PC***

As can be seen from our results and the literature data, new surgical methods lead to better patient outcomes after the respective surgical procedure and favourably affect their antioxidant status. This is probably due to the minimal invasiveness of these procedures, precision, reduced inflammatory response of the body, faster recovery and reduction of OS compared to standard

surgical techniques, and faster increase in TAS levels after surgery despite a short stay in the clinic (2 or 5 days).

According to some authors, in the first 24 hours of the postoperative period, the increase in TAS may also be due to anaesthesia, as most general anaesthetic drugs have antioxidant properties. However, more studies with dynamics are needed for this evidence, not just a single study on the first days after surgery. This is one of the limitations of our study because we only covered the period of admission and before discharge. It would be better in the future to follow up TAS in the same patients at least one month after surgery to avoid the effect of anaesthesia and medications administered. Thus, a decision could be made to administer antioxidants to patients with low TAS levels over time, which would help their recovery faster and provide a better prognosis.

### 6.2. Changes in other parameters of antioxidant significance according to surgical technique

#### 1. Changes in uric acid levels after surgery in PC

For the whole group of patients with PC and patients operated on through RARP, there was a slight increasing trend in mean uric acid levels after surgery (**Table 19**). An opposite trend was seen in patients operated by LEPG, with a slight decrease in mean uric acid values (**Table 19**). However, the changes found were non-significant (p>0.05).

***Table 19. Mean values of uric acid in PC before and after surgery depending on the type of surgical technique and the significance of differences***

| *Uric acid* | Mean ± SD | Median | Z | p-value |
|---|---|---|---|---|
| **Robot-assisted radical prostatectomy (RARP)** | | | | |
| **Before surgery** | 349.76 ± 89.769 | 340.00 | **0.928** | **0.353** |
| **After surgery** | 367.76 ± 96.978 | 346.00 | | |
| **Laparoscopic enucleation of the prostate gland (LEPG)** | | | | |
| **Before surgery** | 328.05 ± 75.790 | 316.00 | **0.121** | **0.904** |
| **After surgery** | 322.30 ± 68.079 | 312.50 | | |
| **Total for PC group** | | | | |
| **Before surgery** | 340.22 ± 83.636 | 325.00 | **0.601** | **0.548** |
| **After surgery** | 347.56 ± 87.481 | 324.00 | | |

When we followed the correlations between TAS and uric acid levels, we found no significant correlation preoperatively for the whole group of PC patients and in both subgroups according to the type of surgical technique. Postoperatively, more significant changes were observed in the patients operated with RARP, where a moderate and significant correlation was found (r=0.453; p=0.023), while in the patients operated with LEPG, the correlation was not significant (r=0.398; p=0.082).

**2. Changes in albumin level after surgery in PC**

The mean albumin levels had very similar values before and after surgery (**Table 20**). There was a slight decrease in the mean albumin level after surgery in patients with RARP, more pronounced for the medians (46.00 and 41.95, respectively). In patients with LEPG, mean albumin and median levels were almost unchanged. Thus, the changes in albumin levels before and after surgery were non-significant (p>0.05).

***Table 20. Mean albumin levels in PC before and after surgery according to the type of surgical technique and significance of differences***

| **Albumin** | **Mean ± SD** | **Median** | **Z** | **p-value** |
|---|---|---|---|---|
| **Robot-assisted radical prostatectomy (RARP)** | | | | |
| **Before surgery** | 43.644 ± 5.1860 | 46.0 | **0.871** | **0.383** |
| **After surgery** | 42.288 ± 5.8166 | 41.95 | | |
| **Laparoscopic enucleation of the prostate gland (LEPG)** | | | | |
| **Before surgery** | 43.300 ± 4.8541 | 43.8 | **0.187** | **0.852** |
| **After surgery** | 43.160 ± 4.3059 | 43.85 | | |
| **Total for the PC group** | | | | |
| **Before surgery** | 43.491 ± 4.9874 | 44.3 | **0.909** | **0.363** |
| **After surgery** | 42.769 ± 5.1189 | 43.6 | | |

Interesting data were obtained regarding changes in the correlation between TAS and albumin levels at admission and after surgery (**Table 21**).

***Table 21. Correlation between TAS and albumin before and after surgery in PC depending on the type of surgical technique***

| Groups compared | TAS and *albumin* | Pearson r | p-value |
|---|---|---|---|
| **Robot-assisted radical prostatectomy (RARP)** | **Before surgery** | - 0.007 | 0.972 |
| | **After surgery** | **0.502** | **0.012** |
| **Laparoscopic enucleation of the prostate gland (LEPG)** | **Before surgery** | 0.372 | 0.106 |
| | **After surgery** | **0.206** | **0.383** |
| **Total for the PC group** | **Before surgery** | - 0.195 | 0.198 |
| | **After surgery** | **0.317** | **0.034** |

Preoperatively, there was no significant correlation between albumin and TAS values for the whole PC group and the two subgroups. RARP and LEPJ.

After surgery, however, there was a moderately significant correlation between TAS and albumin values for the entire group of patients with PC (r=0.317; p=0.034) and a more pronounced correlation for RARP **(**r=0.502; p=0.012). The coefficient of determination $R^2$=0.2520 implies that 25% of the changes in TAS in RARP were related to the changes in albumin values that occurred (**Fig. 31**).

In LEPG, the correlation between TAS and albumin values was weak and non-significant (r=0.206; p=0.383).

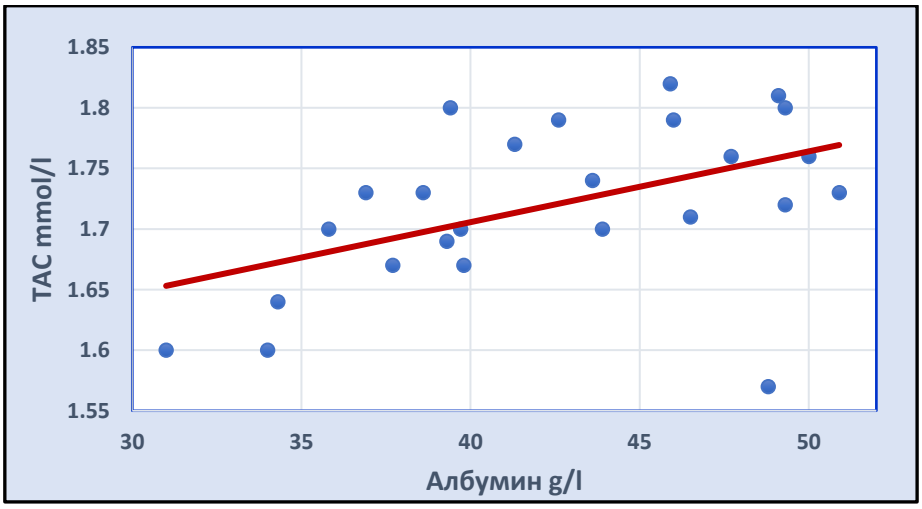



***Figure 31. Correlation between TAS and albumin values in PC patients operated by RARP***

### 3. Changes in total bilirubin level after surgery

There was a tendency for an increase in total bilirubin after surgical removal of the tumour mass for the whole group with PC in both subgroups with different surgical techniques (**Fig. 32**).

The differences were statistically significant for RARP (Wilcoxson Z=2.651; p=0.008) and for the whole group of PC patients (Wilcoxson Z=3.053; p=0.002), but the differences were non-significant for LEPJ (Wilcoxson Z=1.456; p=0.145).

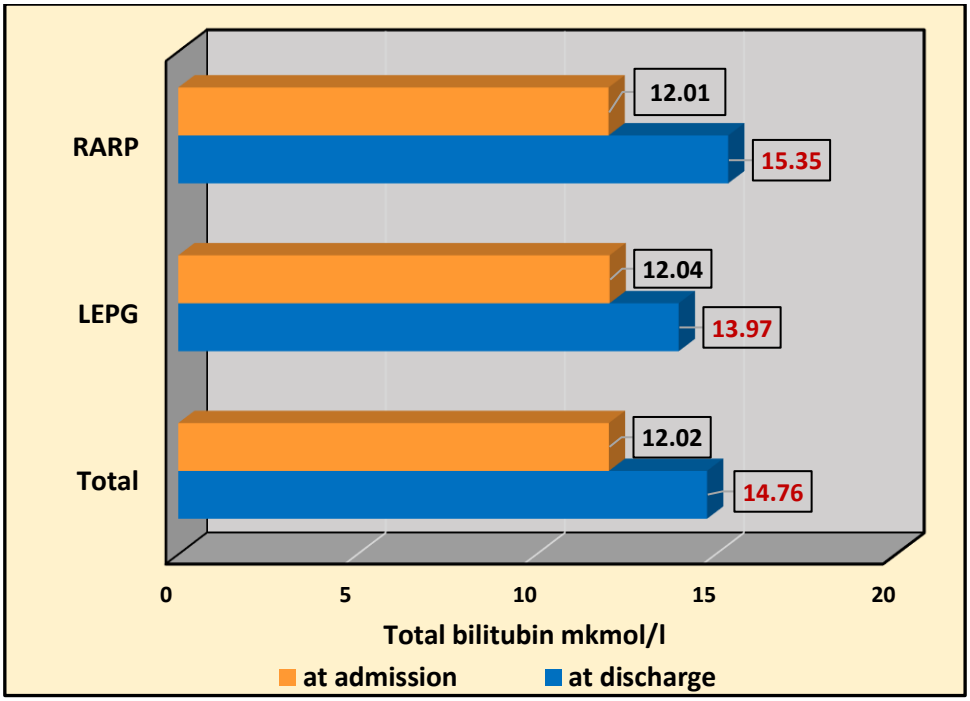



***Figure 32. Mean values of total bilirubin before and after surgery according to the type of surgical technique***

There were no significant correlations between TAS and total bilirubin levels before and after surgery depending on the surgical technique used in any of the groups compared (p>0.05).

## 6.3. Changes in markers of inflammation depending on the type of surgical technique

### 1. Changes in C-reactive protein level after surgery

The strong variation of individual values (before surgery - from min 0.2 mg/l to max 67.2 mg/l; after surgery - from min 0.9 mg/l to max 51.8 mg/l) and the pronounced asymmetric left-weighted distribution was characteristic for CRP (**Fig. 33-34**).

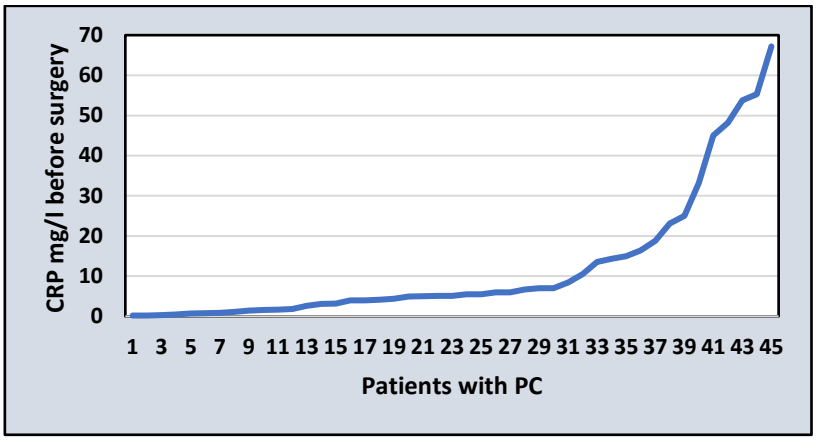



***Figure 33. Frequency distribution of CRP in patients with PC before surgery***

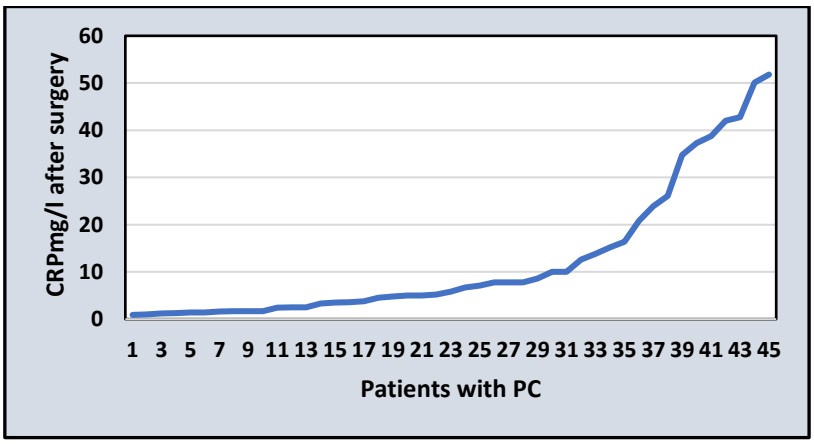



***Figure 34. Frequency distribution of CRP in patients with PC after surgery***

There were no significant changes in the mean CRP values before and after the respective surgical intervention (**Fig. 35**).

In RARP, the mean CRP value before surgery was 13.4 mg/l. It increased slightly to 14.4 mg/l after the surgical intervention.

In LEPG, the mean CRP values both preoperatively and postoperatively were lower than those in RARP, with a trend for a slight decrease in CRP from 10.6 mg/l to 9.9 mg/l postoperatively. Differences in mean CRP levels before and after surgery in both surgical intervention types were statistically insignificant ($p>0.05$).

These insignificant changes are probably due to the short length of stay of the patients and the inability to follow-up longer.

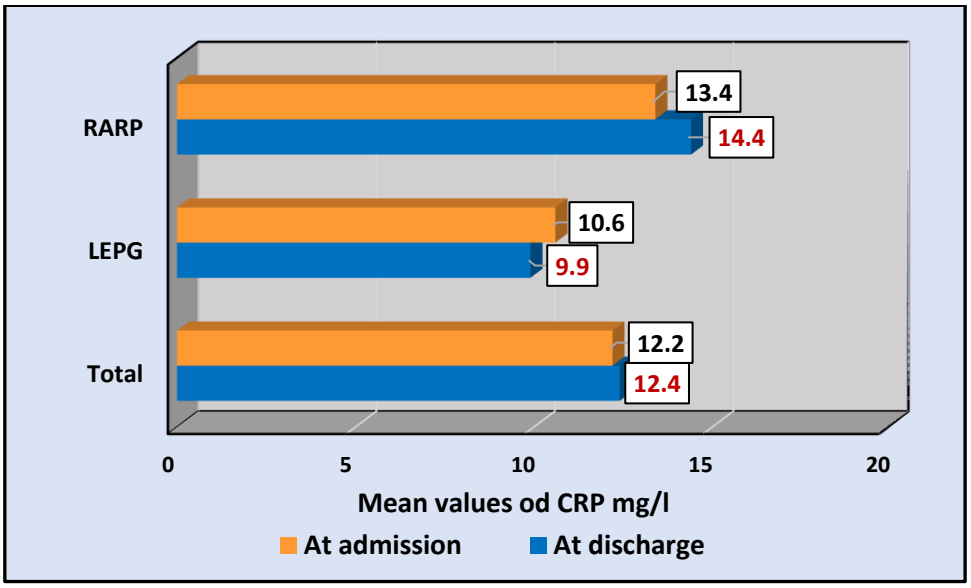



***Figure 35. Mean CRP values before and after surgery depending on the type of surgical technique***

According to some authors (Lu H. et al. 2023), inflammatory markers in robotic surgery of PC patients suggest that postoperative increase in CRP may be considered an independent factor for more significant complications. especially in patients with higher Gleason scores.

The correlations between TAS and CRP values pre- and postoperatively for both surgical techniques were non-significant (**Table 22**).

***Table 22. Correlation between TAS and CRP before and after surgery in PC according to the type of surgical technique and significance of the correlation***

| Groups compared | TAS and total bilirubin | Pearson r | p-value |
|---|---|---|---|
| **Robot-assisted radical prostatectomy (RARP)** | **Before surgery** | 0.250 | 0.335 |
| | **After surgery** | **- 0.280** | **0.167** |
| **Laparoscopic enucleation of the prostate gland (LEPG)** | **Before surgery** | 0.227 | 0.335 |
| | **After surgery** | **- 0.314** | **0.177** |

It is likely that these minor changes in CPR levels and the lack of correlation between TAS and CPR are due to the short length of stay of the patients in the clinic and the inability to follow up longer.

## 2. Changes in leukocytes after surgery

There was a slight decrease in mean leukocyte counts after tumor removal in both types of surgery (**Table 23)** but the differences were non-significant (p>0.05).

***Table 23. Mean values of leukocytes in PC before and after surgery depending on the type of surgical technique and significance of differences***

| Leukocytes | Mean ± SD | Median | Z | p-value |
|---|---|---|---|---|
| **Robot-assisted radical prostatectomy (RARP)** | | | | |
| **Before surgery** | 8.816 ± 3,0958 | 8.10 | **0,679** | **0,501** |
| **After surgery** | 8.212 ± 2.4624 | 7.60 | | |
| **Laparoscopic enucleation of the prostate gland (LEPG)** | | | | |
| **Before surgery** | 8.220 ± 2.6883 | 7.65 | **1.176** | **0.239** |
| **After surgery** | 7.400 ± 2.3702 | 6.65 | | |

Our results were in agreement with results reported by other authors that robot-assisted radical prostatectomy. compared with laparoscopic surgery, is associated with better operative and postoperative outcomes. In a study of patients with colorectal cancer, Han C et al. (2020) found that CRP levels were lower in the robot-assisted group on the first, third, and fourth days after surgery. Also, leukocyte levels were lower in the RARP group on the fourth day after surgery. but overall, according to Cuk P et al. (2021), no significant differences were observed in the postoperative period. These data regarding CRP and WBC in RARP have been confirmed by other authors as important for the prognosis of patients after surgery (Lundin ES et al. 2020).

In another extensive retrospective study of 2278 patients undergoing RARP, the role of pelvic inflammation on the development of PC was evaluated. It was found that it and increased levels of inflammatory markers lead to more aggressive PC (Chakravarty D et al. 2021).

Infection and chronic inflammation account for about 25% of malignancies worldwide. With these results, we confirm the results from studies by other authors, i.e. as the degree of inflammatory markers decreases. antioxidant status increases (Strycharz-Dudziak M et al. 2019).

**Task 7. To look for a correlation between PSA levels in patients with PC and BPH and TAS levels.**

Individual PSA levels in patients with PC were spread over an extensive range - from 0.14 ng/l to 99.7 ng/l (**Fig 36**)

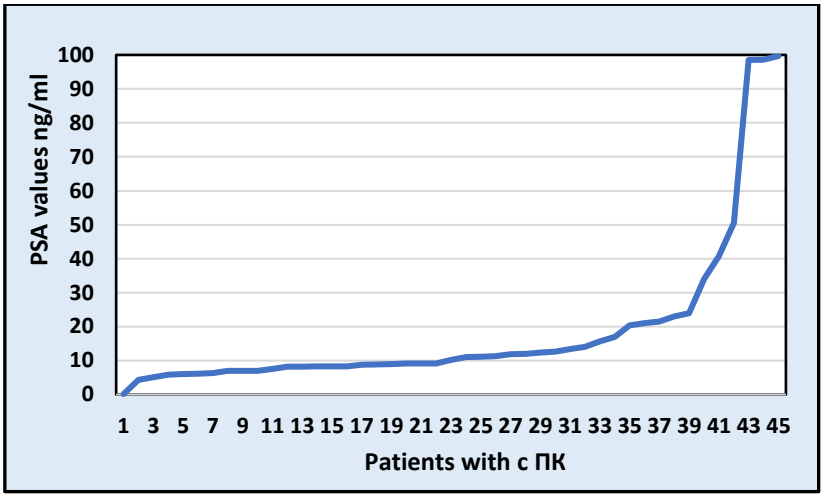



***Figure 36. Frequency distribution of PSA values in patients with PC***

In patients with BPH, the range in PSA values was relatively narrower - from 0.40 ng/l to 37.89 ng/l (**Fig. 37**). In both groups, a strongly asymmetric left-skewed distribution was formed with an asymmetry coefficient of 2.798 for the PC group and 2.355 for the BPH patients.

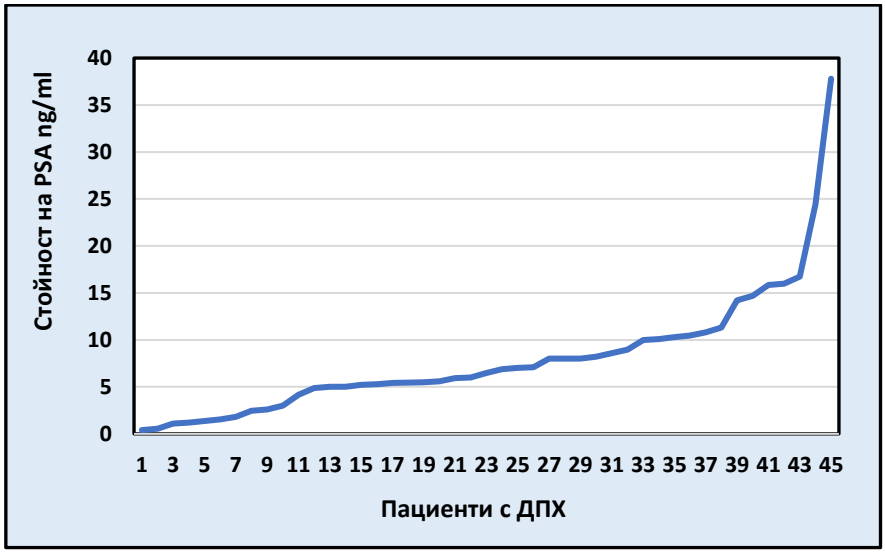



***Figure 37. Frequency distribution of PSA values in patients with BPH***

The mean PSA value in patients with PC was twice as high as that in BPH (18.7084±23.6447 ng/l in PC and 7.9838±6.7375 ng/l in BPH). Similarly, the median PSA in PC was significantly higher (10.15 ng/l) than that in BPH (6.47 ng/l) - **Table 24 and Fig. 38.**

*Table 24. Descriptive characteristics of PSA in patients with PC and BPH*

| Groups | Mean ± SD | Median | 95% CI | Min | Max | Skewness |
|---|---|---|---|---|---|---|
| **PC** | 18.7084±23.6447 | 10.15 | 14.520 – 22.897 | 0.14 | 99.70 | 2.798 |
| **BPH** | 7.9838±6.7375 | 6.47 | 3.795 – 12.172 | 0.40 | 37.80 | 2.355 |

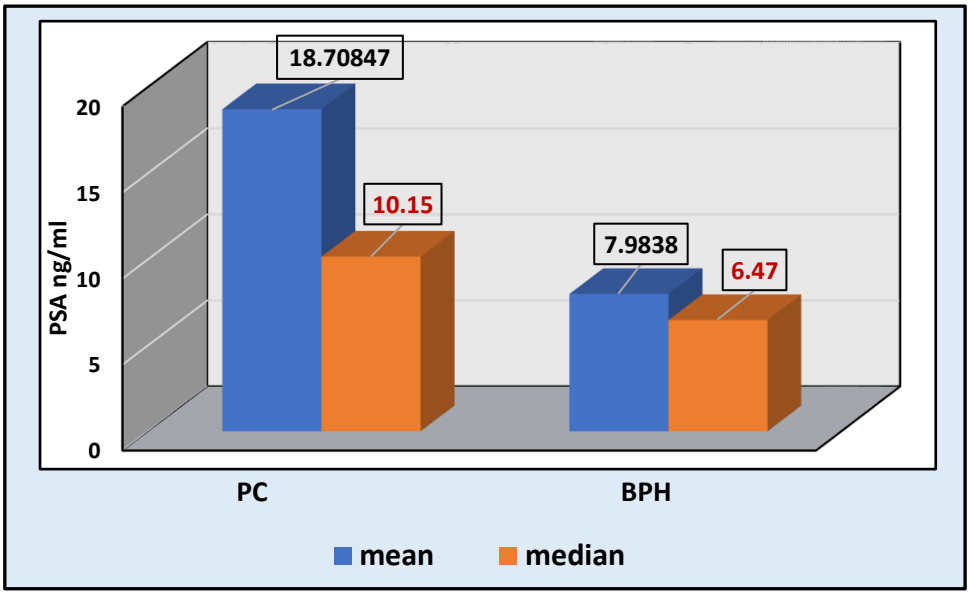



*Figure 38. Mean and median PSA values in patients with PC and BPH*

The difference between the mean PSA levels in patients with PC and BPH, assessed by the non-parametric Mann-Whitney test, had an extremely high level of significance (p=0.000).

In a prospective study conducted in the city of Benin by Ozah E. and Imasogie D. (2023) in Nigerian patients in the age range 50-85 years (mean age 70.4±8.6 years), significantly higher PSA values were found in both PC patients (79.2 ng/ml) and BPH patients (16.0 ng/ml) and statistically significant differences between the two groups. The significantly higher levels of the tumour marker PSA in that Nigerian study compared to the data from our study may be due to the different populations studied. It is also unknown how many patients in this group had metastases, which would affect the PSA results obtained. Another likely reason for these differences may be a timelier screening, prophylaxis, and better awareness among the Bulgarian population.

When the correlation between TAS and PSA values was monitored (**Fig. 39)**, it was seen that in PC patients there was a moderate significant inverse correlation between TAS and PSA

(Pearson r=-0.433; p=0.003), which means that with an increase in PSA values the TAS values decrease. The coefficient of determination $R^2$=0.1873 means that 18.7% of changes in TAS can be associated with PSA.

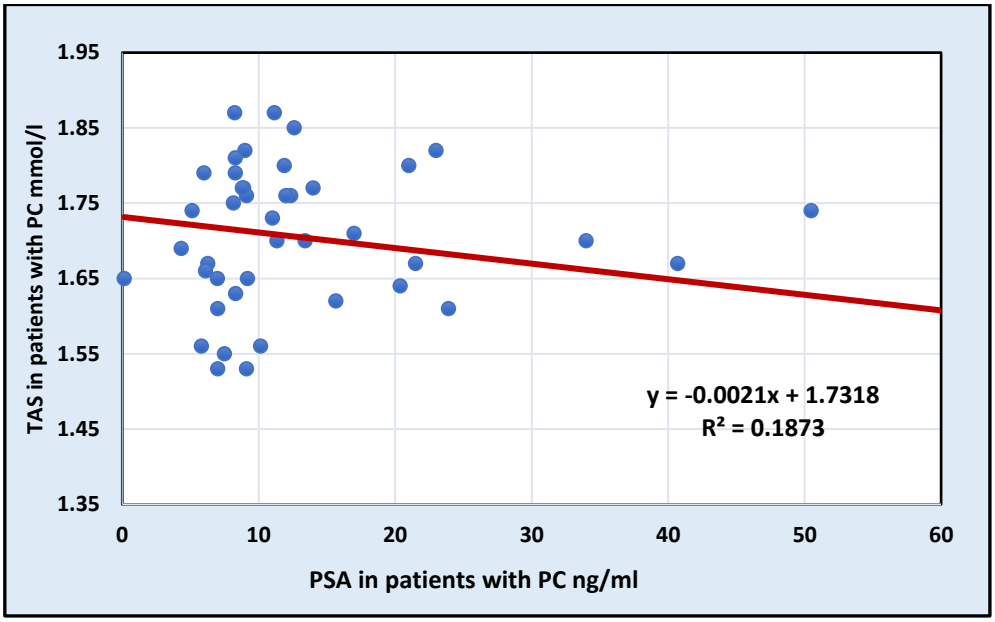



***Figure 39. Correlation between PSA and TAS values in patients with PC***

In patients with BPH, the correlation between PSA and TAS was extremely weak (virtually no correlation) and statistically unreliable (Pearson r=0.029; p=0.848) - **Figure 40.**

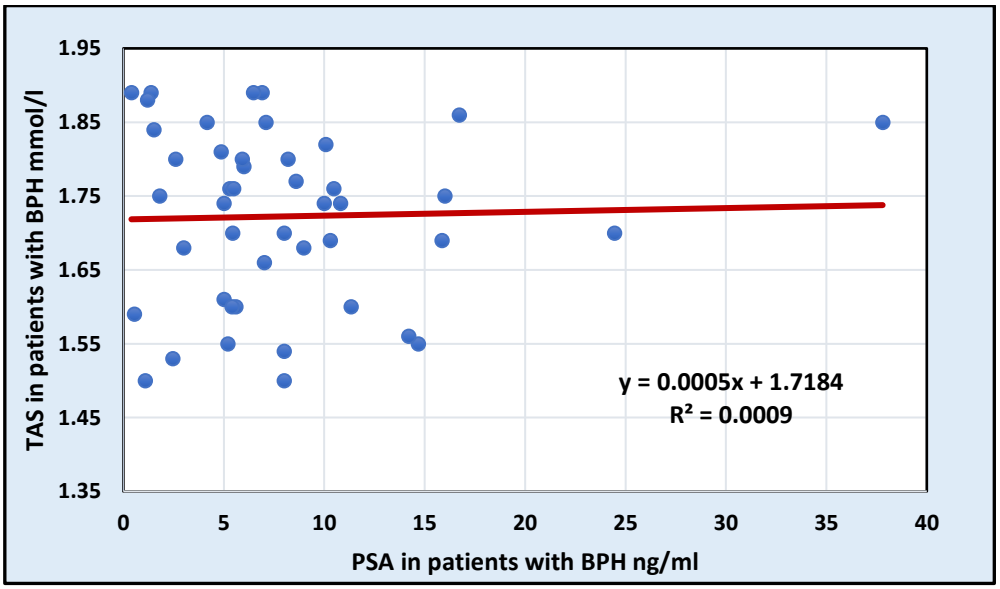



***Figure 40. Correlation between PSA and TAS values in patients with BPH***

Our results confirm the data of other authors that PSA as a tumour marker has significantly higher values in patients with PC compared to BPH. The data also demonstrate that higher tumour marker levels have lower TAS values and vice versa. This data also supports the opinion that the body's antioxidant defence levels decrease in more advanced malignancies, but this would be better monitored over time by measuring PSA and TAS levels again.

We sought to answer the question: **"What changes in PSA levels occur depending on the stage of disease and the presence of metastases?"**

Because only 2 patients had stage 1 disease, we pooled stage 1 and stage 2 patients into one group. Thus, we formed 3 groups for comparison according to disease stage (**Table 25**). The data clearly show the considerable difference in mean PSA values, median, minimum and maximum values in the patients with metastases compared to the other two groups formed. The differences in mean PSA values in the 3 groups compared are supported by a very high level of significance (Kruskal-Wallis H=10.966; df=2; p=0.004).

***Table 25. Descriptive characteristics of PSA in patients with PC according to disease stage***

| Disease stage | Number of patients | Mean ± SD | Median | Min | Max |
|---|---|---|---|---|---|
| **1st – 2nd stage** | 15 | 14.7233 ± 11.5449 | 11.87 | 0.14 | 40.70 |
| **3rd stage without metastases** | 26 | 10.5285 ± 4.1773 | 9.05 | 5.13 | 21.50 |
| **Cases with metastases** | 4 | 86.8225 ± 24.2277 | 98.50 | 50.49 | 99.70 |

The differences were even more pronounced when the PC patients were divided into two main groups: those without metastases and those with metastases (**Table 26 and Fig. 41**). With a mean PSA level of 12.0632±7.8575 ng/ml for the PC patients without metastases, the 4 patients with metastases had a mean PSA level of 86.8225±24.2277 ng/ml. The significance of these differences was extremely high (Mann-Whitney U=861.00; Z=3.271; p=0.000).

***Table 26. Mean PSA values in patients with PC without metastases and with metastases***

| Groups compared | Number | Mean ± SD | Median | Min | Max |
|---|---|---|---|---|---|
| **PC without metastases** | 41 | 12,0632 ± 7,8575 | 9.10 | 0.14 | 40.70 |
| **PC with metastases** | 4 | 86.8225 ± 24.2277 | 98.55 | 50.49 | 99.70 |

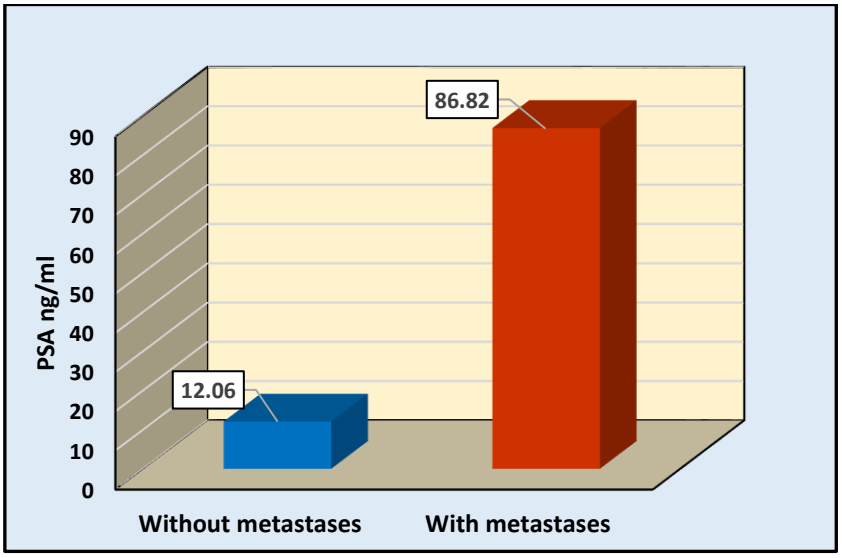



***Figure 41. Mean PSA values in PC without and with metastases***

The differences were statistically significant for RARP (Wilcoxson Z=2.651; p=0.008) and for the whole group of PC patients (Wilcoxson Z=3.053; p=0.002), but the differences were non-significant for LEPG (Wilcoxson Z=1.456; p=0.145).

The individual PSA values in 3 of the 4 patients with metastases reached extremely high values. close to 100 ng/l (**Fig. 42**).

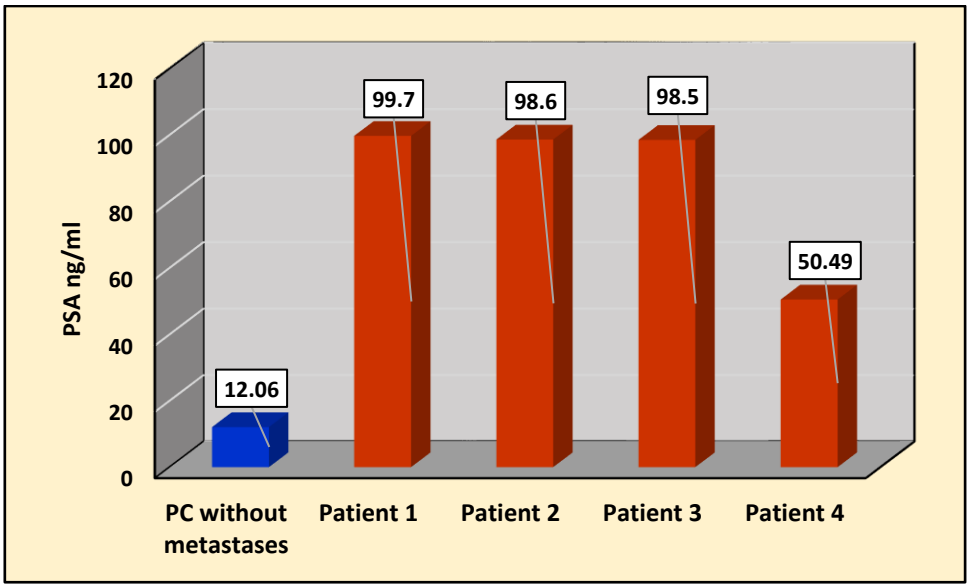



***Figure 42. Mean PSA value in PC without metastases and individual PSA values in the 4 patients with metastases***

**Task 8. To determine whether there is a change in PSA values depending on the PC stage and the presence of metastases.**

The data in **Table 27** show that the mean TAS levels at admission for patients with stage I-II and stage III PC are very close to each other (1.7060±0.884 mmol/l for stage I-II patients and 1.7077±0.0978 mmol/l for stage III patients). However, the mean TAS level in the 4 patients with PC metastases was significantly lower at 1.5500±0.2035 mmol/l.

***Table 27. Mean TAS levels in patients with PC at admission and at discharge according to disease stage and significance of differences***

| Groups compared | Number of patients | At admission | | At discharge | | p-value |
|---|---|---|---|---|---|---|
| | | Mean ± SD | Median | Mean ± SD | Median | |
| **1st - 2nd stage** | 15 | 1.7060 ± 0.8838 | 1.70 | 1.7533 ± 0.7678 | 1.750 | 0.005 |
| **3rd stage** | 26 | 1.7077 ± 0.0979 | 1.72 | 1.7388 ± 0.0858 | 1.735 | 0.014 |
| **With metastases** | 4 | 1.5500 ± 0.2035 | 1.56 | 1.6950 ± 0.2319 | 1.705 | 0.046 |

At all stages of the disease, there was an increase in TAS values at discharge, reflecting the positive effect of tumour removal. The most substantial increase was observed in stage I-II patients (from 1.7060±0.8838 to 1.7533±0.7678), followed by the stage III patients (from 1.7077±0.0979 to 1.7388±0.0858). The increase in TAS values was significantly less in 4 patients with metastasis (from 1.5500±0.2035 to 1.6950±0.2319).

The comparison of the mean TAS values at admission and at discharge in the three groups by Friedman's test (used for more than two dependent samples) confirmed the presence of significant differences, with a decrease of p-value from 0.005 for stage I-II to p=0.014 for stage III and p=0.046 for patients with metastases.

The differences in mean TAS values at admission and discharge for PC patients, according to disease stage, are shown in **Fig. 43.**

The positive effect of tumour removal becomes even more pronounced when comparing the two main groups. PC without metastases and PC with metastases (**Table 28 and Fig. 44)**. TAS values increased in both groups compared, but the trend for significantly lower TAS values in PC with metastases persisted.

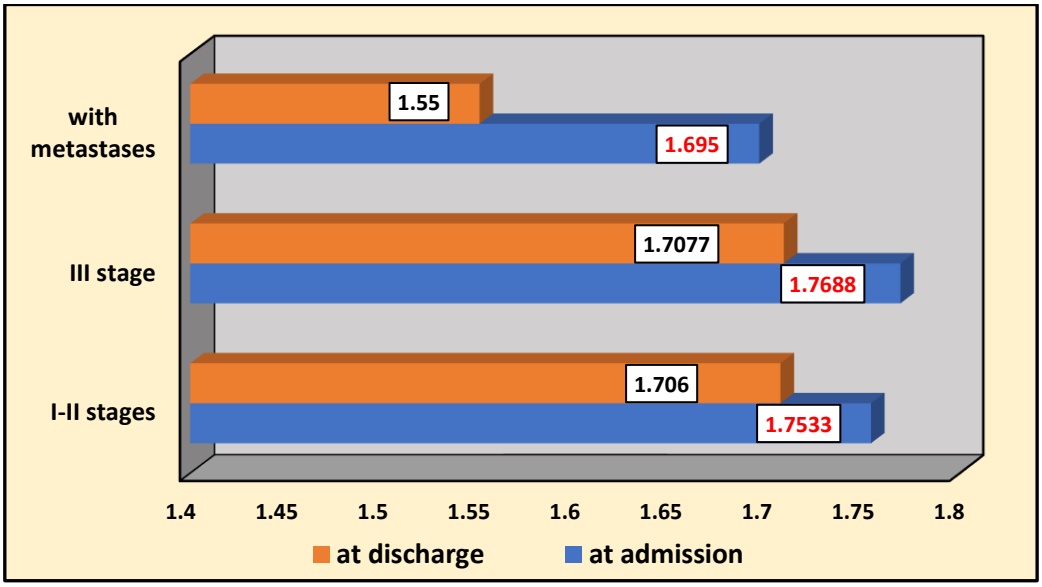



*Figure 43. Mean TAS values at admission and discharge according to PC stage and presence of metastases*

*Table 28. Mean TAS values in patients with PC without metastases and with metastases at admission and discharge*

| Groups | Comparisons | Mean ± SD | Median | Min | Max |
|---|---|---|---|---|---|
| PC without metasatses (41 patients) | At admission | 1.7071 ± 0.0933 | 1.700 | 1.53 | 1.87 |
| | **At discharge** | **1.7441 ± 0.0819** | **1.740** | **1.57** | **1.91** |
| PC with metastases (4 patients) | At admission | 1.5500 ± 0.2034 | 1.555 | 1.35 | 1.74 |
| | **At discharge** | **1.6950 ± 0.2318** | **1.705** | **1.47** | **1.90** |

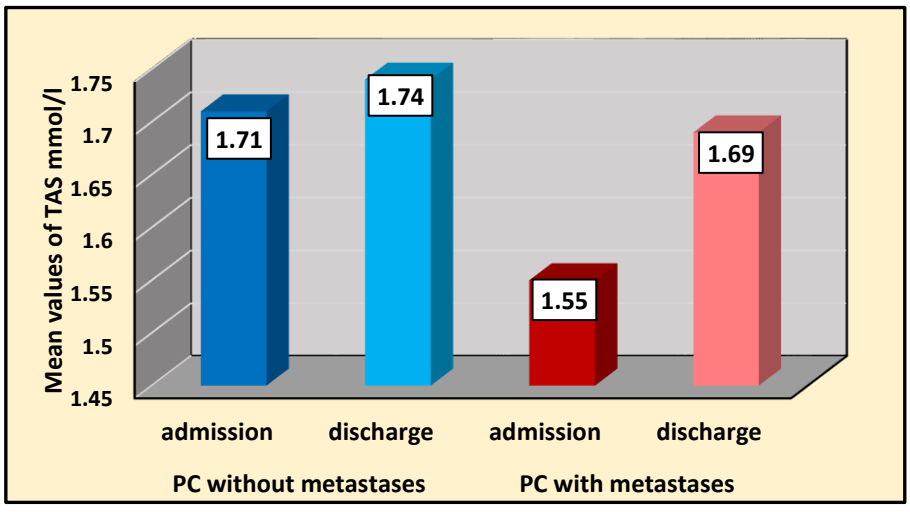



*Figure 44. Mean TAS values in patients with PC without metastases and with metastases before and after surgery*

According to a study carried out by Wood S. and Brown J. (2020), bone metastasis is one of the most severe complications arising from PC and other cancers. Bone metastases significantly impair the quality of life of affected patients and reduce life expectancy. Thanks to improvements in treatment, 84% of men with PC have a 10-year or longer survival rate. Skeletal metastases are seen in up to 80% of patients with advanced PC.

There is relatively little up-to-date data in the literature from studies of TAS levels and the presence of metastases in PC. Still, our results for TAS clearly show that after treatment in the urology clinic, despite the small length of stay of patients, there is an increase in antioxidant status at each stage, with a significant increase in the group of patients with metastases (Bassey IE et al. (2020).

Determination of TAS may help assess tumour burden in patients with different stages of PC and be an important prognostic factor. Despite the small number of patients with metastases, it is obvious that TAS values were significantly lower in these patients compared to patients without metastases. This fact supports the contention that the advancing disease stage and the presence of metastases have a negative impact on the antioxidant status scores of these patients.

In conclusion, we can say that the antioxidant status among the selected groups of patients should be further investigated in the future, as it is a significant indicator of the overall condition of the body to counteract the increasing OS. This may prevent prophylaxis more promptly, properly diagnose early, and successfully treat patients with BPH and PC.

Investigating, the intake of supplements with antioxidant function would also be useful to assess whether they would positively affect the assessment of antioxidant status and whether their use could prevent the development of PC and BPH. There are studies on antioxidant intake in the literature, but opinions are still controversial and inconclusive.

The study of TAS as a newer and more relevant indicator, as well as of other markers with antioxidant significance among at-risk patient groups should be continued. and this should serve for their timely prevention and early diagnosis, as well as for improving the health status of already ill patients.

## V. CONCLUSIONS:

1. From the analysis conducted on the role of some lifestyle factors in patients with PC and BPH. we have shown that:

- in both groups studied. the highest proportion of individuals aged 70-79 years.

- systematic moderate consumption of red wine can be considered a protective factor against the development of PC and BPH;

- pre-obesity and obesity are significantly more common in patients with PC and BPH and can be considered a significant risk factor for the development of the disease;

- the presence of diabetes increases the likelihood of developing PC.

2. We demonstrated that mean TAS values at admission were significantly lower in patients with PC and BPH than in healthy subjects. TAS values were lowest in the PC group and highest in healthy subjects.

3. Our results show that of the other parameters studied with antioxidant significance, the uric acid level plays the most significant role in changes in TAS. A reliable moderate straight correlation between TAS and total bilirubin was observed only for the healthy subjects.

4. Significantly higher mean preoperative CRP values were found in PC patients than in healthy subjects. The differences between the two groups were generally at a high level of significance. Differences in mean preoperative leukocyte values between the two groups were also statistically significant.

5. The study demonstrated that removal of the tumour mass contributed to an increase in the TAS level after surgery in two-thirds of the patients with PC and BPH (in 63 out of 90 patients). Mean TAS levels at admission and discharge were higher in patients with BPH compared with patients with PC.

Postoperatively, a significant correlation was found between TAS and the three investigated parameters of antioxidant significance (uric acid. albumin and total bilirubin).

Only in PC patients it was found that after surgery, with increasing TAS, there was a tendency to decrease the value of CRP as a marker of inflammation.

6. The type of surgical technique used did not significantly affect the TAS results.

7. Our data demonstrated that tumour marker values increase significantly in the presence of metastases. A significant inverse correlation existed between PSA level and TAS values in patients with PC. The correlation between PSA and TAS was extremely weak in patients with BPH. There were significant differences in PSA and TAS values in patients with and without metastases.

8. We demonstrated that comparing the mean TAS values at admission and discharge in patients with PC (stage I-II. stage III) and those with metastases) confirmed the presence of significant differences, with a trend towards lower TAS values in the group with metastases.

## VI. CONTRIBUTIONS

### 1. Original contributions:

1.1. 1.

1.2. For the first time in Bulgaria, the relationships between TAS levels with other markers of antioxidant significance, with PSA and markers of inflammation before and after surgery in patients with BPH and PC, were investigated.

1.3. For the first time in Bulgaria, a study and analysis of changes in antioxidant status before and after surgical treatment, depending on the type of surgical technique used in patients with BPH and PC, was performed.

1.4. For the first time in Bulgaria, the importance of TAS determination was proved as a newer and up-to-date indicator, showing a significant change, especially in patients with PC and those with metastases.

### 2. Contributions of scientific and confirmatory nature

2.1. A significant body of literature on the topic has been analysed, confirming its relevance and demonstrating the need for effective prevention and early detection methods of BPH and PC.

2.2. The data confirmed that with the removal of the tumour mass, TAS levels increase, which is associated with a decrease in OS in the body with the removal of the disease-causing agent.

2.3. Our results can inform the prevention and management of patients with BPH and PC and can be used to evaluate the importance of TAS in the early diagnosis and successful treatment of these patient groups.

2.4. Our study may also serve to further research on the use of antioxidants as dietary supplements and their influence on TAS levels, as well as to make a comprehensive assessment regarding their possible protective role.

## VII. SCIENTIFIC WORKS RELATED TO THE THESIS

### 1. Original articles:

**Velkovska T**. M.. Ruseva A. L.. Antioxidant status in patients with hyperplasia and prostate cancer. Journal of Biomedical and Clinical Research 17(2): 167-176

doi: 10.3897/jbcr.e125424

**Velkovska T..** Ruseva A.. Study of total antioxidant status levels in patients with benign hyperplasia and prostate cancer. General Medicine. 2024. 26(3). 3-8. ISSN: 1311-1817. EMBASE/ExcerptaMedicaDatabase. **Scopus** and EBSCO

**Velkovska T..** Dunev V.. Ruseva A.. Is there a relationship between PSA levels and Total Antioxidant Status in patients with benign prostatic hyperplasia and prostate cancer? . Clinical Urology vol. 4. №1/2024. ISSN: 2738-778X

### 2. Participation in a research project at Medical University - Pleven. related to the thesis:

Project D8/ 2022.

“Markers of oxidative stress and inflammation in patients with some oncological diseases”.

### 3. Participation in scientific forums in Bulgaria:

B. V. **A.** Racheva2**. T. Velkovska1**. Changes in the concentrations of five plasma proteins in women with ovarian tumours. Poster XIV National Conference on Clinical Laboratory 14- 16 October 2022. Plovdiv

**T. Velkovska**. V. V. V. Ruseva; Study of the levels of total antioxidant status in patients with benign hyperplasia and carcinoma of the prostate gland. 29th National Symposium on Urology and Endourology with International Participation 06-08 June 2024 Sandanski. Coll. p. 53

**T. Velkovska**. A. Ruseva; Comparison of the results of measurement of total antioxidant status in patients with benign hyperplasia and prostate cancer. Days of Science 2024. Sofia. Plovdiv