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Comparison of the Gleason scores from prostate biopsy and from radical prostatectomy

PhD thesis

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Abbreviations used in the text:

PCa- prostate cancer

GS- Gleason score

TURP - transurethral resection of the prostate

BPH - benign prostatic hyperplasia

PSA - prostate-specific antigen

RP - radical prostatectomy

mpMRI - multiparametric magnetic resonance imaging

PB - prostate biopsy

EPE - extraprostatic extension

SVI - seminal vesicles involvement

PET-CT-positron emission tomography with computed tomography

PSAD- PSA density

ISUP- International Society of Urological Pathology, in the text-classification of prostate cancer according to the system of this organization

EAU - European Association of Urology

BPFS- biochemical progression-free survival

Contents

1. Introduction

Since 1984, prostate cancer (PCa) has been the most common non-cutaneous malignant disease in men in the United States. The situation is similar in Europe. PCa does not give typical complaints that lead us to the diagnosis - so usually PCa is suspected after digital rectal examination and / or elevated PSA. The final diagnosis is made after histological examination of material taken after prostate biopsy, less common after examination of material obtained by transurethral resection of the prostate (TURP) or adenomectomy for BPH. There are many therapeutic options, ranging from active surveillance, radical prostatectomy (RP), radiation therapy, hormonal therapy, chemotherapy - to a combination of these methods.

The main task of the urologist is to categorize the tumor as high, moderate or low risk in order to maximize the effectiveness of the treatment with minimizing its side effects. The purpose of any classification system (including TNM, Gleason score) is to try to predict the future behavior of the tumor and, on this basis, to choose the most appropriate treatment method. The treatment method must be aggressive enough to eliminate the tumor, but also not too aggressive –in order to minimize the side effects of treatment on the patient.

Histological examination has a central place in the classification of PCa. However, unlike many other tumors, histological examination in case of PCa is often performed twice- after prostate biopsy and after RP. This rule naturally applies only to patients undergoing radical prostatectomy. It does not apply to those treated by other methods.

It is generally accepted that the pathological result from the prostate biopsy is to some extend preliminary and not as accurate as the pathological result of the specimen taken after the radical prostatectomy. This is because PCa often has a mosaic spread in the prostate, and a biopsy may not always target all areas of cancer. After RP, the pathologist has the entire prostate and can examine it extensively, finding even the smallest loci with tumor cells. Despite their small size, they could contain very poorly differentiated cells, which is why the latest version of the GS includes the lowest differentiated gland model, regardless of its volume. In practice, however, the pathologist examining the gland after prostatectomy may be "lost" in the large volume of material and may not always accurately reflect all of Gleason's degrees. The prostate biopsy itself has also undergone serious development in recent years, which has significantly increased its accuracy. This has led some researchers to accept GS from prostate biopsy as a stand-alone prognostic sign that can guide us in the patient's expected survival.

The discrepancy between GS from prostate biopsy and RP is not uncommon. It is important for the patient's therapy in two aspects. First, some patients nowadays prefer active surveillance rather than active treatment of PCa — at least in the early stages. If the GS from the biopsy gives an unrealistically low value (well-differentiated tumor), this will lead to an underestimation of the malignant potential of PCa with a possible delay in the more aggressive treatment required for this type of cancer. It is therefore necessary to know the factors that suggest such a false underrating of the GS.

Second, there are cases in which the GS of the prostatectomy is lower than that of the prostate biopsy. We will study patient survival to see if this lower GS actually means well-differentiated carcinoma or is a false sedation masking a poorly differentiated tumor.

The present study was conducted on the basis of the patients operated at “St. Anna Hospital” in Varna and reflects our experience in the diagnosis and treatment of PCa.

2. Literature review with critical analysis of literature data

MEDLINE database is used to search for publications on the topic (via PubMed) and the keywords “discrepancy, Gleason score, biopsy, prostatectomy” were used in the search.

29 articles were found that meet the required criteria in English.

A monograph dedicated to the PCa was found in Bulgarian, in which the problem is partially addressed, 3 articles in “Uronet” and 1 article in “Endourology and Minimally Invasive Surgery”, commenting on the histological finding after prostate biopsy and RP.

The above articles take into account the presence sometimes of inconsistencies between the GS from prostate biopsy and after prostatectomy. In 18 articles (two of them in Bulgarian) an attempt was made to connect the changes in GS with other characteristics of the tumor (patient).

In 3 articles in English (to some extent in one of the articles in Bulgarian) a causal link is found between the GS from the biopsy (respectively from the RP) and patient survival.

For example, in January 1986, Cancer published an article entitled “Gleason histologic grading of prostatic carcinoma. Correlations between biopsy and prostatectomy

specimens". It examines the relatively small number of 53 patients, noting that in 51% of cases the GS from the biopsy is the same as that of the RP, in 4% it is higher and in 45% it is lower. Two factors have been identified leading to a mismatch between the two GSs-insufficient material taken from the prostate biopsy, as well as the low GS found from the biopsy. The authors recommend repeating the biopsy in the presence of these two factors if the change in GS will affect therapeutic behavior.

In the following years, other factors related to the discrepancy between the two GSs were discovered –namely elevated PSA, analysis of the biopsy in a non-academic center, low baseline (from the biopsy) GS.

Interesting is the article published in European Urology, issue 53 from 2008 – "Prognostic Significance of Gleason Score Discrepancies between Needle Biopsy and Radical Prostatectomy". Authors of the article are Michael Muntener, Jonathan I. Epstein, David J. Hernandez, Mark L. Gonzalgo, Leslie Mangold, Elizabeth Humphreys, Patrick C. Walsh, Alan W. Partin, Matthew E. Nielsen from the Department of Urology at the Johns Hopkins Medical Institute in Baltimore, USA. 6625 patients treated with radical prostatectomy were analyzed. A comparison was made between the GS from the prostate biopsy (GS- PB) and the GS from the radical prostatectomy (GS- RP).

Differences in survival without biochemical progression (biochemical progression-free survival -BPFS) were determined by Kaplan - Meier analysis in patients with GS-mismatch. Patients undergoing hormonal therapy, pre- or postoperative radiotherapy were excluded from the analysis.

Patients with low GS-PB have a better prognosis (BPFS) than patients with high GS-PB, even in those whose GS decreases after surgery. This is true for every value of the GS (3 + 4, 7, 8, 8-10). On this basis, the authors of the article conclude that GS from the prostate biopsy is an independent prognostic factor along with GS from the radical prostatectomy. As possible hypotheses explaining this fact, the authors propose: errors of the pathologist in determining GS, borderline cases that can be attributed to one stage or another, errors in sampling - when a very small tumor was examined in the biopsy sample, but was omitted in the sample after the RP.

In more recent articles (after 2012) the impact of the changes in the pathological nomenclature is discussed, and also the description in the post prostatectomy pathological report of the tertiary gland model of Gleason. The importance of PSAD and the prostate volume is also established, to predict a possible increase in GS after RP.

In February 2020, the article “Concordance Between Biopsy and Radical Prostatectomy Pathology in the Era of Targeted Biopsy: A Systematic Review and Meta-analysis” was published in European Urology Oncology. It contains a meta-analysis of studies comparing GS from prostate biopsy to GS from prostatectomy. The studies were divided into two groups - those who underwent systematic prostate biopsy and those with targeted biopsy (under MRI guidance). It was found that undergoing systematic PB is more likely to lead to underestimation of GS compared to targeted biopsy, which more accurately identifies the final GS (determined by prostatectomy).

In Bulgarian, the problem of the discrepancy of GS from prostate biopsy and after RP is briefly addressed in the monograph (from 2015) of Plamen Dimitrov Dimitrov, MD from the Medical University of Sofia **Radical Treatment of Prostate Cancer - Possibilities of The Retropubic Radical Prostatectomy** for awarding the educational and scientific degree "DOCTOR"**.** The emphasis in it is the surgical treatment of PCa. The author describes the preoperative characteristics of the patients, including GS. After a detailed description of the operative technique follows a description of the postoperative characteristics of the patients - the final TNM, GS, etc.

Based on the analysis of the data, it was concluded that there is a statistically significant difference between pre- and postoperative GS ($p = 0.001$). This difference is lowest at values up to 6 and highest at values above 7 of the GS from the biopsy.

However, the conclusion is in complete contradiction with another study (by urologists from Istanbul University). They find that the biggest discrepancy is in well-differentiated tumors, where there is a significant risk of underestimation of the malignant potential of the tumor only on the basis of GS from prostate biopsy.

Another three articles in Bulgarian on the topic have been published in Uronet after 2015. In addition, in 2016 there was one publication in “Endourology and Minimally Invasive Surgery”. They present in what percentage of cases the GS from the biopsy is identical (or different) from that from the RP. The average PSA is also indicated and in one of the articles the authors try to establish a connection between the change in GS and some characteristics of the patients, namely age and PSA. No such link has been found, which contradicts other studies proving similar relationships. Another article reported a difference in the survival (Kaplan-Meier test) of patients with different degrees of malignancy (G 1, G 2 and G 3) of PCa.

The above analysis of the literature shows that at the beginning (the earliest article is from January 1986) the authors are content to find that there is a discrepancy between

the GS from the biopsy and that from the RP. The frequency of this discrepancy is determined. Later, the characteristics of the patients are described and associated with GS. Thus, factors are sought to predict possible discrepancies in the two GSs. However, the analysis of the articles shows that there is occasionally a controversy (sometimes drastic) when the GSs (from biopsy and prostatectomy) differ - for example, whether the discrepancy is in well- or poorly-differentiated PCa. In addition, only two articles have raised the question of whether this discrepancy has prognostic significance for patient survival. Some authors assume that GS from the biopsy is an independent prognostic factor. This assertion is not confirmed in other articles. The purpose of this study is to analyze our clinical experience and to seek evidence to support (or disprove) these controversial statements.

3. Aim and tasks

Aim of the study:

To make a comparative analysis of the Gleason scores from prostate biopsy and radical prostatectomy and to analyze its dependence on the main features of prostate cancer.

Research tasks:

- To analyze the values of Gleason score of the patients with prostate cancer who underwent radical prostatectomy in the Clinic of Urology of MHAT "St. Anna-Varna" for the period after 2013 until now in order to establish a difference between the values of the biopsy and the radical prostatectomy.
- To analyze the patients with well-differentiated prostate cancer (Gleason score up to 6 from the biopsy) to identify statistically significant prognostic factors for a possible increase in Gleason score after radical prostatectomy.
- To analyze the relationship between Gleason score change and the perioperative characteristics of the patients - age, preoperative PSA, prostate volume, PSA density, digital rectal examination, data from the pathological reports after radical prostatectomy.
- In the analysis of the main perioperative characteristics of patients to identify indicators suggesting of high-risk prostate cancer.

- In the analysis of patients, the new ISUP classification (modified Gleason system) should also be applied, and an attempt should be made to evaluate its effectiveness.
- To analyze patient survival - overall, survival without biochemical progression, survival without metastases.
- To investigate the effect of Gleason score on patient survival.

Research hypotheses:

- Patients with well-differentiated prostate cancer (Gleason score ≤ 6 from biopsy) have a higher risk of increasing Gleason score after radical prostatectomy. Certain preoperative patient characteristics (age, PSA, PSA density, prostate volume, and palpable nodule) could predict a possible increase in Gleason score.
- There is a correlation between Gleason score change after radical prostatectomy (compared to that of prostate biopsy) and some pathological features such as seminal vesicle involvement, extraprostatic tumor extension, and the presence of lymph node metastases.
- There is a relationship between Gleason score change after radical prostatectomy (compared to that of prostate biopsy) and the time to biochemical progression.
- Longer survival without biochemical progression can be expected in patients with Gleason score ≤ 6 compared with patients with Gleason score ≥ 7, irrespective which Gleason score is used (from the biopsy or from the prostatectomy).
- Patients with lower PSA can expect longer survival without biochemical progression.
- In patients with extraprostatic tumor extension (pT3a), seminal vesicle involvement (pT3b) and lymph node metastases (pN1), shorter survival without biochemical progression can be expected.

4. Materials and methods

4.1 Research materials

A single-center, non-interventional study was conducted. The study is performed on the basis of analysis of patients undergoing radical prostatectomy at the Clinic of

Urology of St. Anna Hospital in Varna. 468 prostatectomies were performed in the clinic for the period from January 2013 to May 2021.

During the preliminary processing of the data it was found that in a large number of cases until 2018 there is no information about GS from prostate biopsy and / or prostatectomy. As a result, the final number of patients available for analysis was 203.

Data about overall survival, survival without biochemical progression, as well as the time to occurrence of metastases of patients in our sample were obtained from the registers of the oncology center "Marko Markov", Varna.

4.2 Research methods

4.2.1. Clinical methods

All patients underwent a transrectal biopsy of the prostate - in some cases, however, the biopsy was performed in another medical institution, not in MHAT "St. Anna-Varna".

Some patients, especially in recent years, underwent fusion biopsy of the prostate under MRI guidance. In our clinic we have the opportunity to perform only cognitive fusion biopsy and the results, according to literature data, are close to the results of classical fusion biopsy.

All patients included in the study underwent radical prostatectomy at the clinic - either open retropubic or laparoscopic.

4.2.2. Data organization and statistical methods

Given the wide range of GS, we developed an exemplary algorithm that takes into account the presence (absence) of a change in GS from biopsy and after radical prostatectomy. Through it, the data are summarized in three groups without losing the meaning and significance of GS, simplifying their analysis. The following three main groups were used:

- First group - no change in GS after RP,
- Second group - with an increase in GS after RP (compared to biopsy) and
- Third group - with decrease of GS after RP (compared to biopsy).

The data were analyzed with IBM SPSS version 23. The normality of the distribution of continuous variables was tested with Shapiro-Wilk and Kolmogorov-Smirnov test for one sample.

Continuous variables that follow a normal distribution are represented by mean and standard deviation (SD). Variables that do not follow a normal distribution and / or include very remote and extreme values are represented by median and interquartile range (IQR).

The averages of the normally distributed variables were compared by Student's t-test (for two independent samples or for two correlated samples) and ANOVA (for more than two samples). Post Hoc tests were used to determine differences when comparing the averages of more than two normally distributed variables.

The frequencies of the category variables were compared by nonparametric tests (Pearson's X^2 or Fisher's exact test).

Nonparametric tests of Mann-Whitney U and Kruskal-Wallis H were used to compare, respectively, two and more than two independent variables that did not follow the normal distribution and or category / rank variables. Wikoxon Sign Rank Test (for two variables) and Friedman test (for more than two variables) were used for dependent (correlated) variables.

Correlation analysis (Pearson correlation coefficient for linearly dependent variables, Phi correlation coefficient for nominal variables and Spearman's Rho correlation coefficient for rank variables) was applied to determine the strength and direction of the dependencies. Goodman and Kruskal's Gamma correlation coefficient was used to determine the strength and direction of the relationship between two ordinal variables with more than two attribute levels.

Preoperative values of PSA, PSA Density, GS, ISUP and EAU-risk groups and postoperative values of "Main group", GS and ISUP were tested as predictors of pT3b, pN1 and pT3a by ROC curves.

Regression analysis (linear regression for quantitative variables and logistic or rank regression for qualitative variables) was applied to establish the relationship between two or more variables.

Kaplan-Meier test and Cox regression - was used to study the survival of patients after surgery and the survival of patients without biochemical progression.

The tests were performed at a significance level $\alpha = 0.05$ or $p < 0.05$.

The figures are designed with MS Excel.

5. Results

5.1. General characteristics of the survey sample

The number of patients operated for the period from 2013 to 2021 and suitable for analysis, is 203 (these are the patients for whom we know both biopsy and postoperative GS). From the initial analysis of the data, it was found that there is a coincidence of the two GSs in 70 patients - in the other GSs after RP either increases or decreases (Figure 5.1).

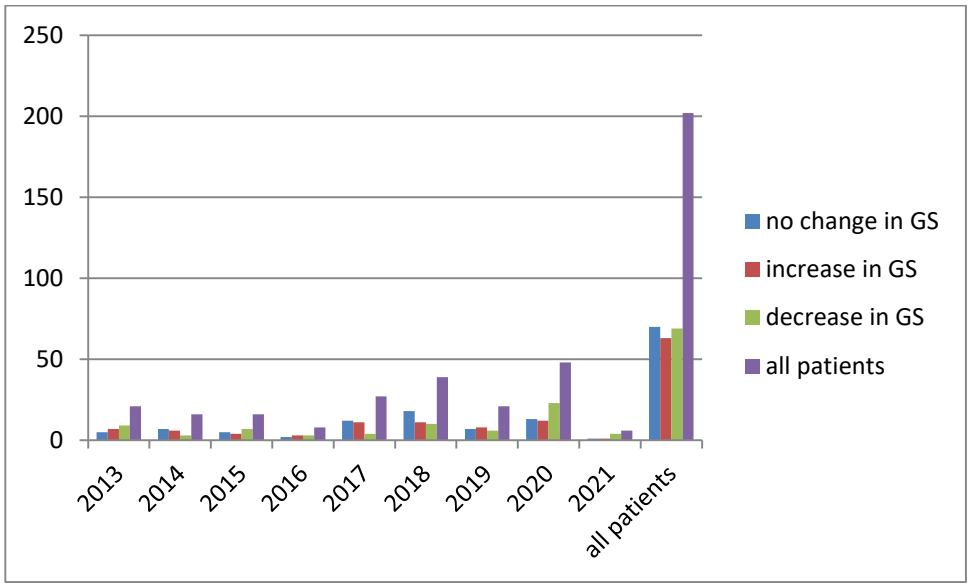


Figure 5.1 Distribution by years of operated patients suitable for analysis.

The distribution of all cases by years, as well as their trend is shown in Figure 5.2. The data for 2021 are excluded from the analysis, as they do not cover the whole year. As can be seen, the trend is to increase the total number of cases per year.

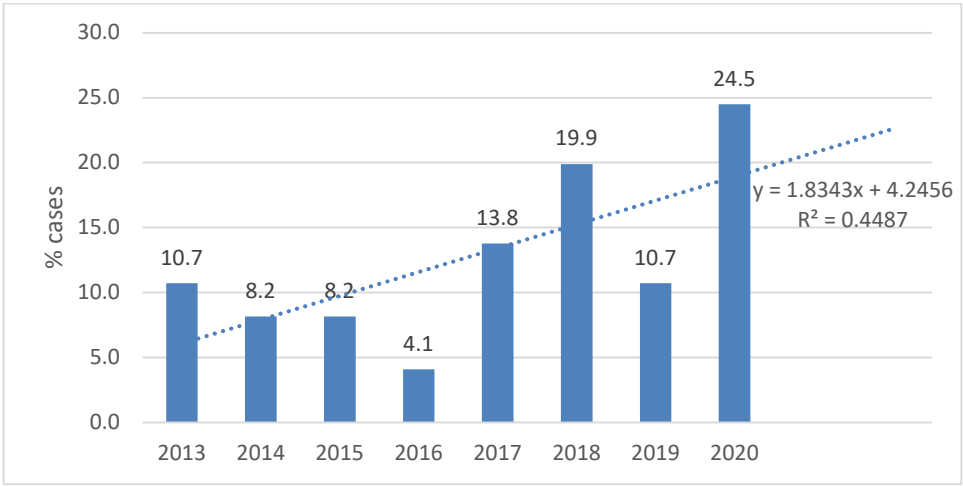


Figure 5.2. Dynamics of the cases with PCa for the period 2013 – 2020

The trend is similar for pT3b, pT3a and pN1. Although different numbers were found for different tumor variants over the years, statistical significance of the difference was found only for extraprostatic growth (pT3a), (X^2 = 31,927; p =, 000), with a weak and positive correlation (r =, 307; p =, 000), i.e. cases of extraprostatic extension are increasing (Figure 5.3). No statistically significant difference or correlation was found for pT3b and pN1 (p>, 05). For 2015, no cases with pT3b, pT3a and pN1 were identified (registered).

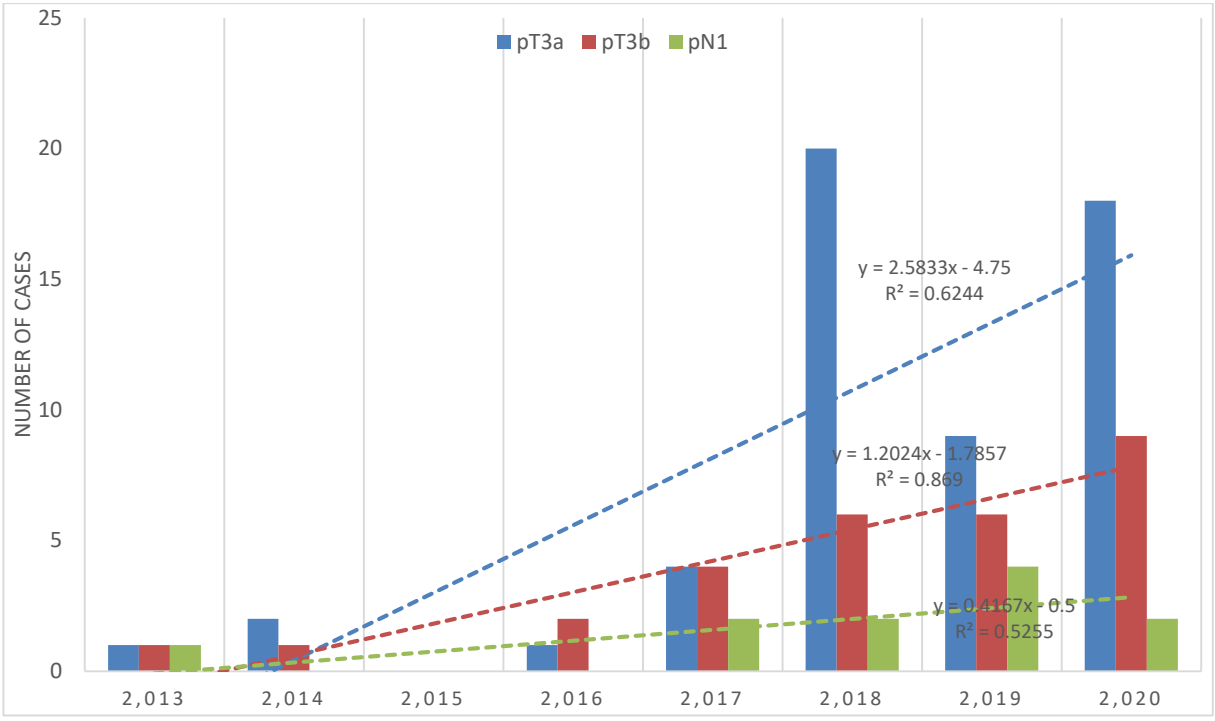


Figure 5.3 Dynamics of the cases of pT3a, pT3b and pN1 for the period 2013 - 2020

The highest average age of patients was in 2015 (69.9; SD = 4,582), and the lowest - in 2014 (65; SD = 5,259). No statistically significant difference was found in the mean age of the patients for the individual years (F = 1.022; p = .417) - Figure 5.4.

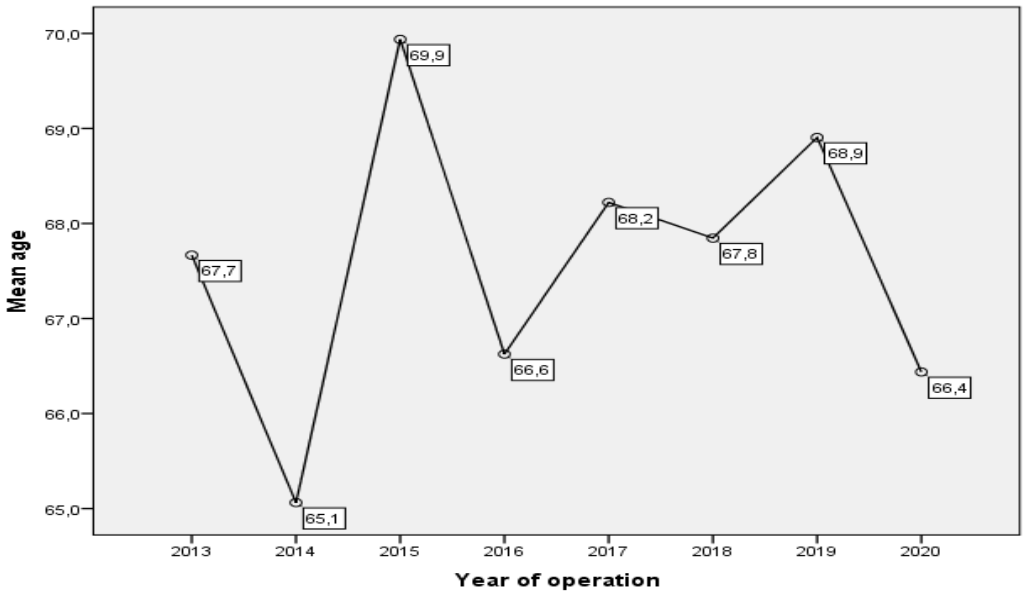


Figure 5 .4 Average age of patients in the period 2013 - 2020 (by years)

In the following table we present how the GS changes after RP (compared to the one from the prostate biopsy) - it can be seen that most often it is a change with one unit:

increase in GS					Decrease in GS				
with 1 unit	with 2 units	3 units	4 units	5 units	1 unit	2 units	3 units	4 units	5 units
30	17	3	2	1	31	11	6	2	5

The following table shows that when the GS changes (regardless of up or down), both the primary and secondary GS are affected:

increase in GS			decrease in GS		
primary GS	secondary GS	both	primary GS	secondary GS	both
12	18	29	11	22	28

In case of change of only the primary or only of the secondary GS (both increase and decrease) it is a variation of GS with only one unit in 93% of the cases.

Cases of change 4 + 5 to 5 + 4 and vice versa are reported as no change in GS, because according to the classification of ISUP both options are highly malignant poorly-differentiated tumors. However, the shift from 3 + 4 to 4 + 3 is reported as an increase

in GS, because according to the same classification we have an increase from second to third degree with proven deterioration in survival.

Particularly interesting, but also difficult to interpret, are the cases in which the biopsy (or RP) shows that there is no tumor, but from the other pathological analysis turns out to have. They are included in the general analysis, but as a separate group the cases are very few, so that conclusions to be made only for them.

The 203 patients available for analysis were divided into 3 main groups - the first group - no change in GS after RP, the second group - with an increase in GS from RP (relative to biopsy) and the third group – decrease in GS after RP. 70 (34.48 %) patients were classified in the first group. In this group the largest (26 or 37.1%) is the share of patients with GS = 6 (GS = 3-3), followed by those with GS = 7 (22 or 31.4%), of which 18 (25.7%) are with GS = 3 + 4 and 4 (5.7%) are with GS = 4 + 3. (Figure 5.5).

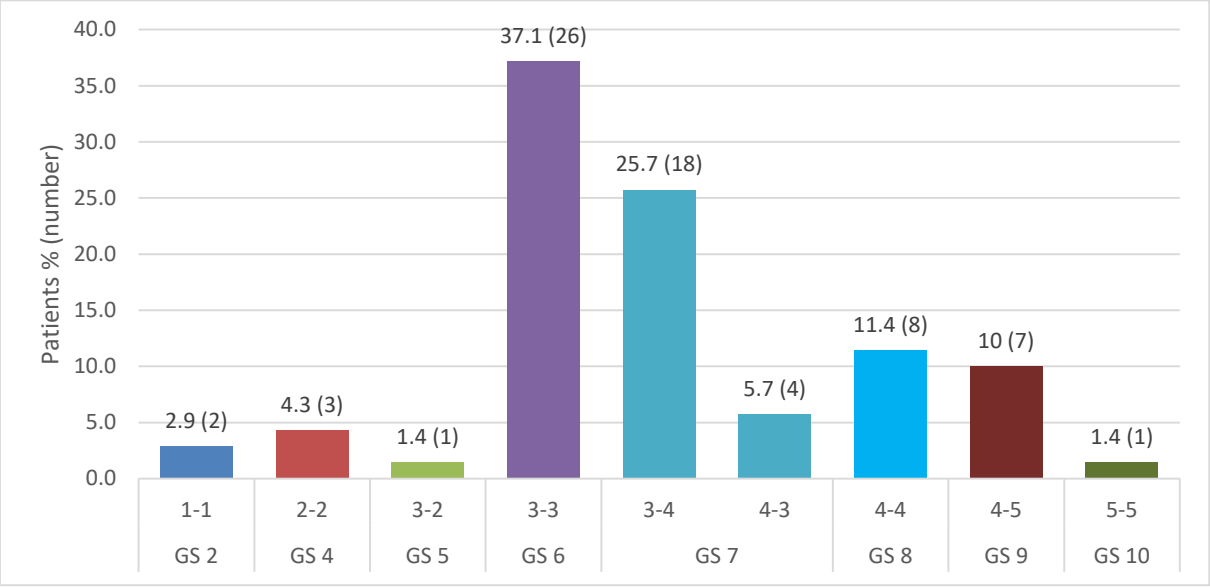


Figure 5.5. Distribution of patients in the first group according to the values of GS after surgery.

The second group includes 63 (31.03 %) patients. In this group the largest (27 or 42.9 %) is the share of patients with GS = 7, of which 19 (30.2%) are with GS = 3 + 4, and 8 (12.7%) are with GS = 4 + 3. This is followed by the share of patients with GS = 8 (14 or 22.2%), of which 12 (19%) have GS = 4 + 4 and 2 (3.2%) have GS = 3 + 5. The share of patients with GS = 9 (11 or 17.5%) is also significant, of which 9 (14.3%) have GS = 4 + 5, and 2 (3.2%) have GS = 5 +4. (Figure 5.6).

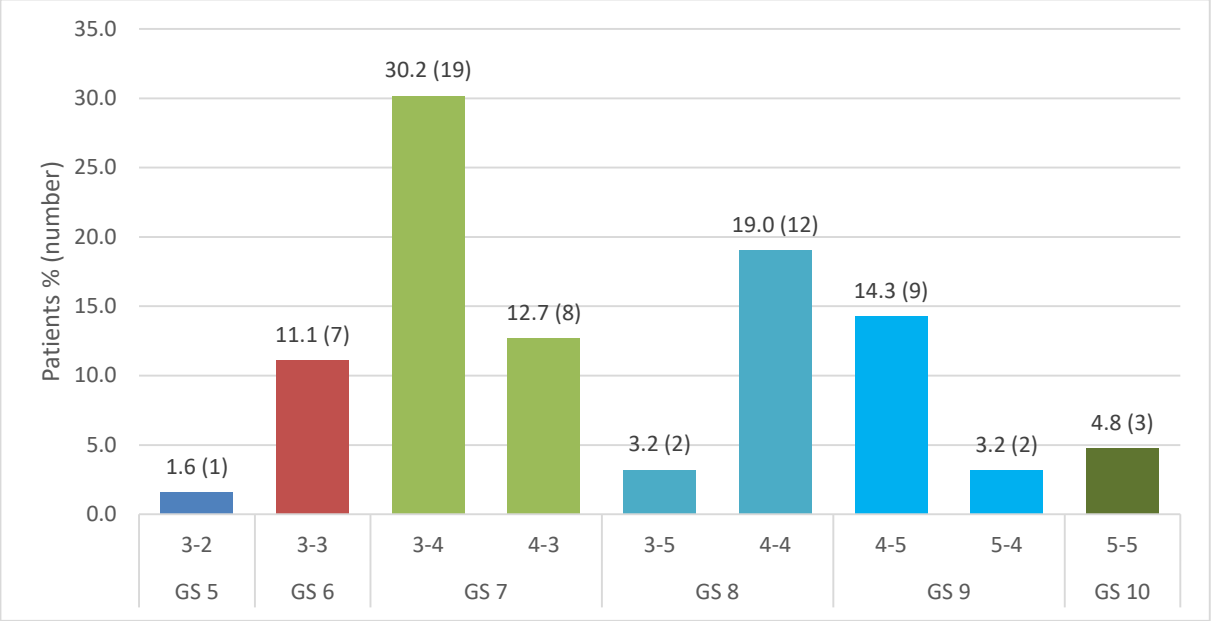


Figure 5.6. Distribution of patients in the second group according to the values of GS after surgery.

70 (34.48 %) patients were classified in the third group. In this group the largest (26 or 42.6 %) is the share of patients with GS = 6, followed by the share of patients with GS = 7 (14 or 23%), of which 12 (19.7%) are with GS = 3 + 4 and 2 (3.3%) are with GS = 4 + 3. (Figure 5.7).

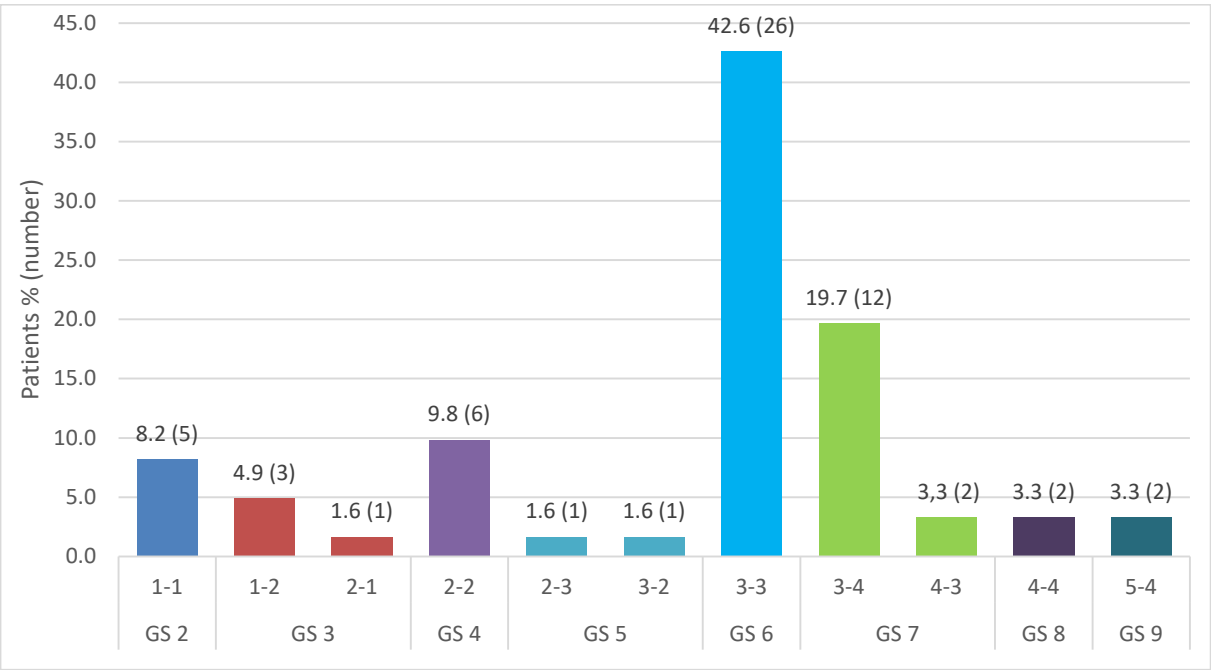


Figure 5.7. Distribution of patients in the third group according to the values of GS after surgery.

The total distribution of GS values for the individual groups is presented in Figure 5.8.

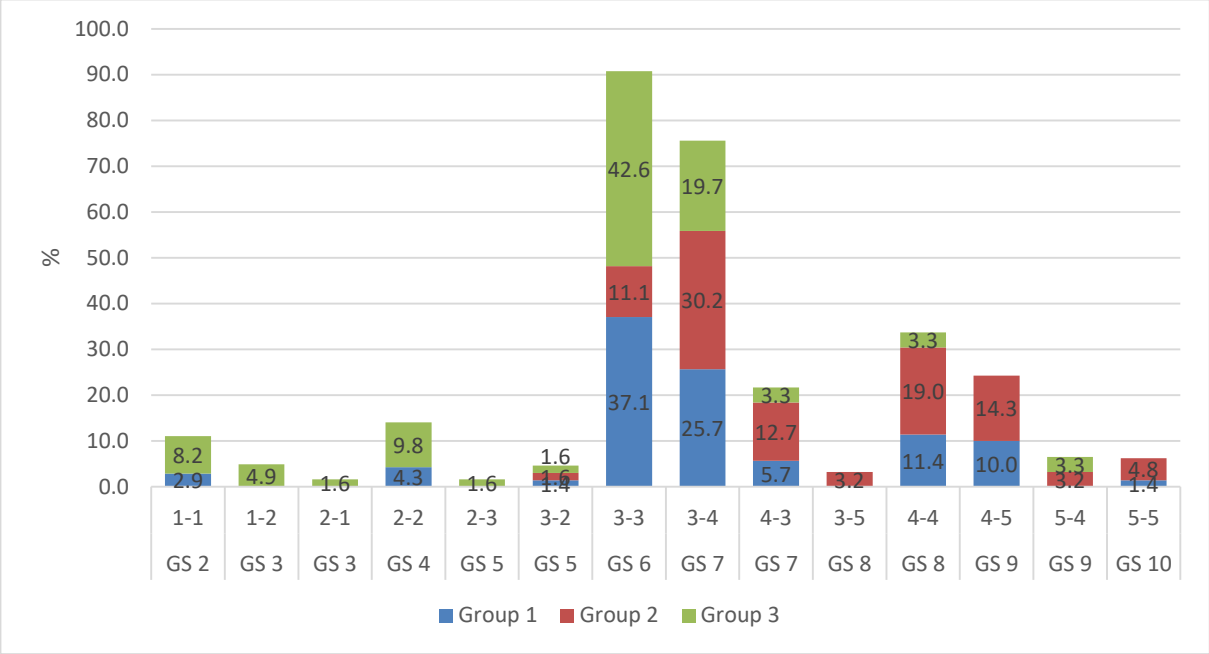


Figure 5.8. Distribution of patients according to GS values after the operation in the groups.

5.2. Patients with well-differentiated PCa from the biopsy (GS ≤ 6) -factors pointing to possible increase in GS after RP.

With GS ≤ 6 after biopsy are 78 (38.6%) of all patients. The average value of GS in this group is 5.46 (SD = 1.00); the median is 6 (IQR = 5 - 6).

Of these patients, 32 (41%) are in the first group, 34 (43.6%) are in the second group and 12 (15.4%) are in the third group.

a. Age

The age of patients in the first group varied between 55 and 76 years with a mean age of 65.6 (SD = 6.28) years; the age of patients in the second group varied between 53 and 78 years with a mean age of 66 (SD = 6.57) years, and in the third group the age ranged from 53 to 78 years, with a mean age of 69.5 (SD = 7.21) years. There is no statistically significant difference in the age of the patients in the three main groups (ANOVA, F = 1,648; p =, 199). Our hypothesis that the patient's age is associated with a possible increase in GS after RP is rejected.

b. PSA

Data about PSA are available for 77 (96.3%) of patients with GS <7, of which 32 are from the first group, 33 - from the second group and 12 - from the third group. PSA

values in the first group ranged from 3.09 to 67.87 with a mean of 13.98 (SD = 13.25); the median is 10 (IQR = 8.4 - 13.8). For the second group, the PSA values varied between 4.57 and 30.99 with an average value of 12.76 (SD = 7.31); the median is 9.6 (IQR = 7.6 - 1 4.5). For the third group, the PSA values varied between 0.9 and 20 with an average value of 11.24 (SD = 5.54); the median is 9.75 (IQR = 8,1 - 16). No statistically significant difference was found in PSA values of patients in the three main groups (Kruskal Wallis test; X^2 =, 012; p =, 994). No statistically significant difference was found in the PSA values of the patients in the three main groups and when using 3 PSA levels (below 10, 10 - 20 and over 20) (Kruskal Wallis test; X^2 =, 943; p =, 624).

When dividing the PSA values in three levels (below 10; 10-20 and above 20) again no statistically significant difference was found in the PSA values in the three main groups (X^2 = 5,853; p =, 210).

Our hypothesis that the PSA value is related to a possible increase in GS after the RP is rejected.

c. PSA Density (PSAD)

PSAD data are available for 63 (80.8 %) patients with GS <7, of whom 30 are from the first group, 25 from the second group and 8 from the third group. The PSAD values of the patients in the first group varied between 0.3 and 1.72 with an average value of 0.27 (SD = 0.367); the median is 0.13 (IQR = 0.1 - 0.34). For the second group, PSAD values ranged between 0.06 and 0.81 with a mean value of 0.27 (SD = 0.195); the median is 0.19 (IQR = 0.06 - 0.81). For the third group, PSAD values ranged between 0.04 and 0.30 with a mean value of 0.11 (SD = 0.08); the median is 0.095 (IQR = 0.07 - 0.012). A statistically significant difference was found in PSAD values of patients in the three main groups (Kruskal Wallis test; X^2 = 10,656 ; p = .005). The difference is statistically significant between the first and second main groups (MWU = 257,000; p = .046) - PSAD values are higher in patients in the second group than in the first group. The difference in PSAD between the first and third main groups (MUW = 64,000; p = .045) and the second and third main groups (MWU = 29,000; p = .002) is also statistically significant. PSAD in patients in the third group is lower than in patients in the first and second main groups. Median values were used for graphical representation (Figure 5.9).

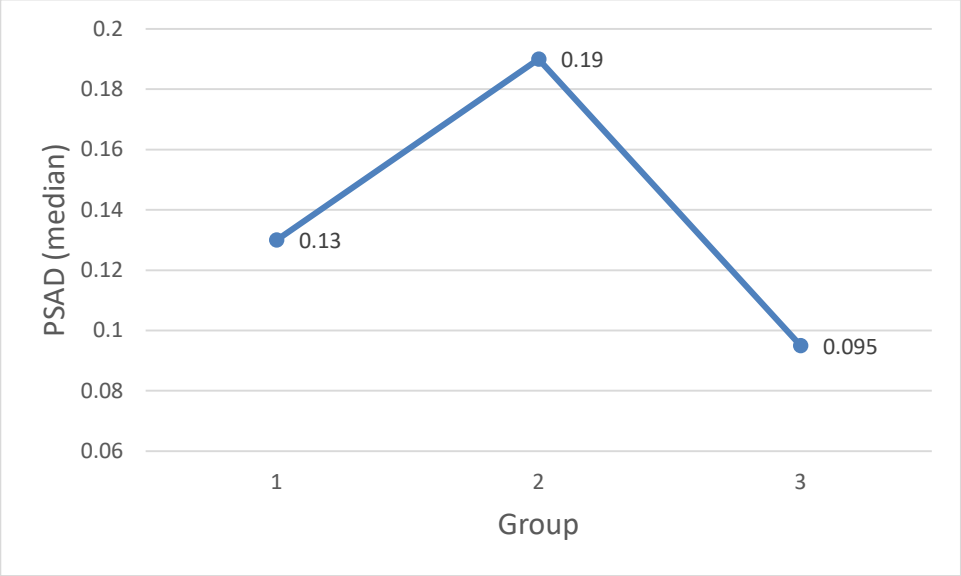


Figure 5.9. Median values of PSAD in the groups for patients with GS <7

Dividing PSAD into three levels (below 0.1; 0.1 - 0.15 and above 0.15) also showed a statistically significant difference in PSAD values in the three main groups (X^2 = 13,710; p = .008). Here the difference applies to the first and second groups (MWU = 244.5; p = .016) and the second and third group (MWU = 31.5; p = .003). The PSAD values of the patients in the second group are higher than those of the patients in the first and third main groups. Our hypothesis that the value of PSAD (higher) is related to a possible increase in GS after the RP is accepted.

d. Prostate volume

Data about the prostate volume are available for 64 (80.8 %) of patients with GS <7, of whom 30 are from the first group, 26 from the second group and 8 from the third group. Prostate volume values of patients in the first group vary between 32.5 and 145.2 with an average value of 73.51 (SD = 30.135); the median is 85.72 (IQR = 42.3 - 88.5). For the second group, the values of prostate volume vary between 21 and 181.1 with an average value of 57.38 (SD = 36.08); the median is 53.65 (IQR = 34.6 - 64.3). For the third group, prostate volume values vary between 22.6 and 164.8 with an average value of 83.675 (SD = 42.433); the median is 76.35 (IQR = 60.95 - 103.7). A statistically significant difference was found in the values of prostate volume of patients in the three main groups (Kruskal Wallis test; X^2 = 8,213; p =, 0 16). The difference is statistically significant only between the first and second main groups (MWU = 229,000; p = .008) - the values of prostate volume are lower in the patients from the second group compared to those from the first main group. The other

comparisons did not show a statistically significant difference. Median values are used for graphical representation (Figure 5.10)

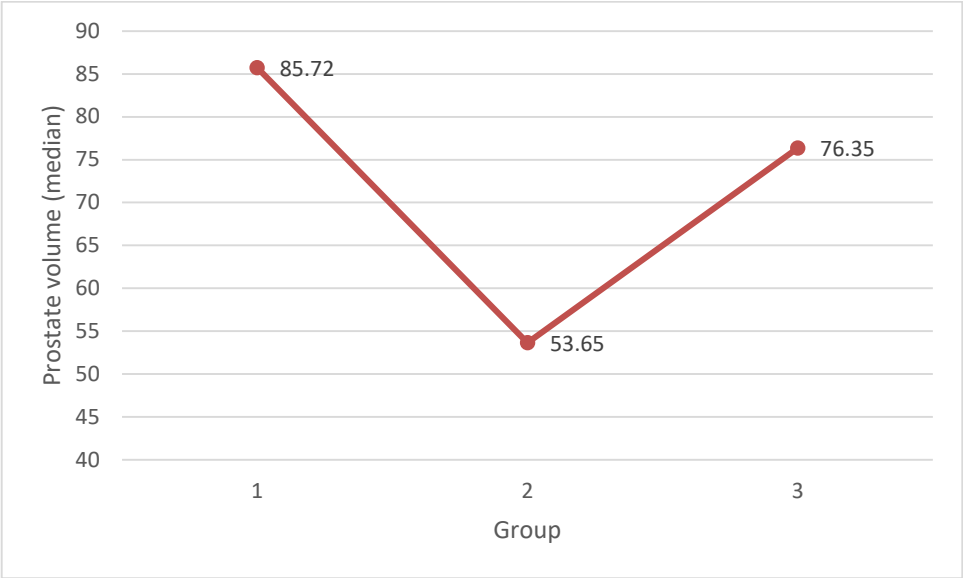


Figure 5.10. Median values of prostate volume in the groups for patients with GS <7

When dividing the volume of the prostate in three levels (less than 40 ml; 40 - 80 and over 80 ml.) there was also a statistically significant difference in prostate volume values in the three main groups ($X^2 = 13,100$; p = .001). This difference also applies only to the first and second group (MWU = 190,000; p =, 000). The prostate volume of the second group of patients is lower than those of the first main group of patients. Our hypothesis that prostate volume (smaller) is associated with a possible increase in GS after RP is proven.

e. Palpation of a nodule in the prostate during the digital rectal examination (stage T2)

In the study group of 78 patients with GS <7, a nodule is palpated in the prostate in 11 (14.1%) of them- this corresponds to stage T2. 3 patients are from the first main group, 7 patients from the second main group and 1 patient - from the third main group. With T1 are 29 patients from the first group, 27 patients from the second group and 11 patients from the third group. No statistically significant association was found between patients belonging to the three main groups and the two groups (T1 and T2) of the rectal examination ($X^2 = 2,101$; p =, 350). Our hypothesis that the presence of a palpable nodule in the prostate is associated with a possible increase in GS after RP is rejected.

5. 3. Preoperative characteristics of the patients and their relation with the GS change after RP.

a. Change in GS and age of the patient

The first group (no change in GS after surgery) includes 70 (34.6%) patients, with ages ranging from 55 to 78 years and a mean age of 67.8 (SD = 6.1) years. The second group (with an increase in GS after surgery) includes 63 (31.2%) patients, ranging in age from 52 to 78 years, and their mean age is 67.3 (SD = 5.9) years. The third group (with a decrease in GS after surgery) includes 69 (34.2%) patients, with ages ranging from 51 to 80 years and a mean age of 67.7 (SD = 7.6) years.

The age of the patients from the second group is the lowest, and the highest is the age of the patients from the first group, but no statistically significant difference was found in the age of the patients from the three main groups (ANOVA, F = . 109; p =, 897). (Figure 5.11).

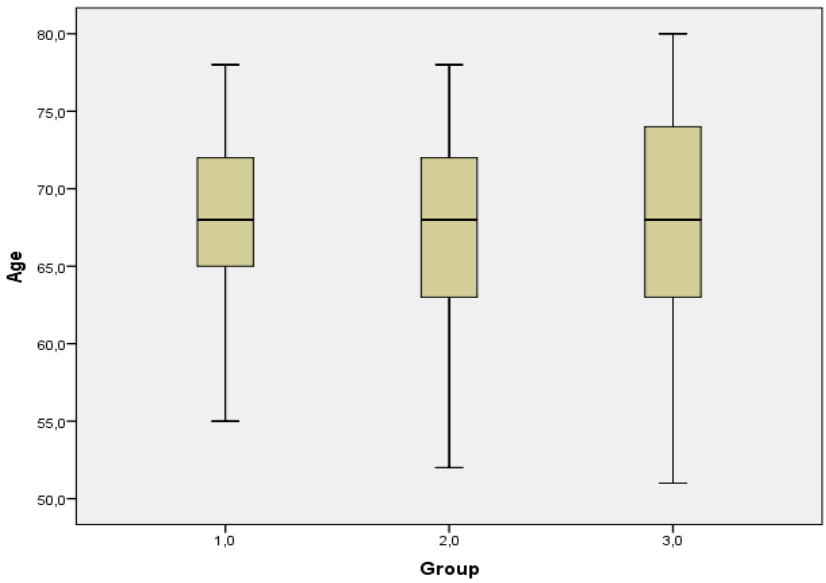


Figure 5.11. Age of the patients in the main groups

b. Change in GS and PSA

PSA values do not follow a normal distribution. Non-parametric tests are applied. PSA data are available for 199 (98.5%) of patients. For all patients, they ranged from 0.9 to 71.84 with a mean of 15.52. The median is 12.00 (IQR = 7.9 - 18.8).

The values of the patients from the first group vary from 3.09 to 71.84 with an average value of 16.65 (median = 12.00; IQR = 8.43 - 19.24). The values of the patients from the second group vary from 4.57 to 49.76 with a mean value of 14.07 (median = 10.7; IQR = 7.67 - 15.14). The values of the patients from the third group vary from 0.9 to

56.61 with an average value of 15.68 (median = 12.26; IQR = 7.95 - 19.67). The values of the means are used for graphical representation (Figure 5.12).
No statistically significant difference in PSA values was found between the different main groups (Kruskal Wallis test, X 2 = , 658 ; p =, 720) - that is, the PSA level is not related to a possible increase / decrease in GS after the RP.

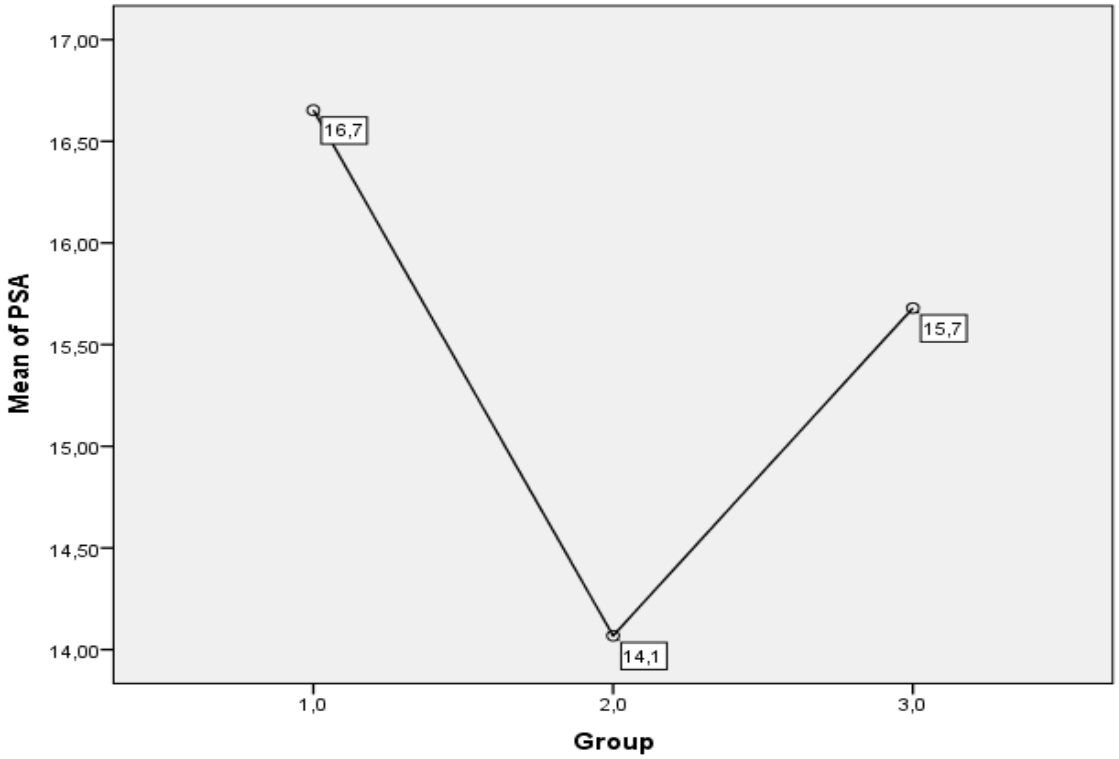


Figure 5.12. Average PSA in the main groups

It is interesting to compare the PSA and the GS data after prostate biopsy. For a more detailed analysis, the PSA values are grouped into 3 stages: below 10, from 10 to 20 and above 20 ng / ml.
A statistically significant relationship was found between the GS values and PSA (Kruskal Wallis test, X ^{2} = 17,082; p =, 000). GS values for PSA between 10 and 20 are statistically significantly higher than those for PSA below 10 (Mann-Whitney U test, NWU = 2017,500; p =, 001). GS values for PSA above 20 are statistically significantly higher than those for PSA below 10 (Mann-Whitney U test, MWU = 923,000; p = .001). No statistically significant difference was found between the GS values for PSA between 10 and 20 and over 20 (Mann-Whitney U test, MWU = 1486,000 p =, 230). The values of the means are used for the graphical representation (Figure 5. 13).

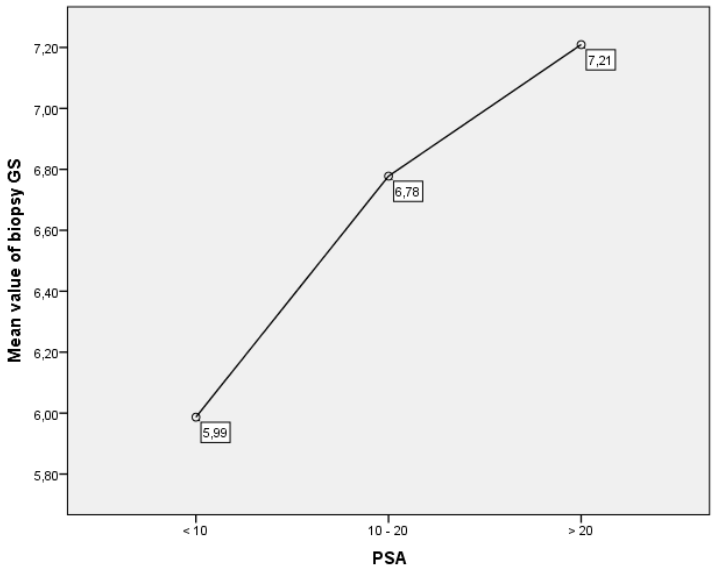
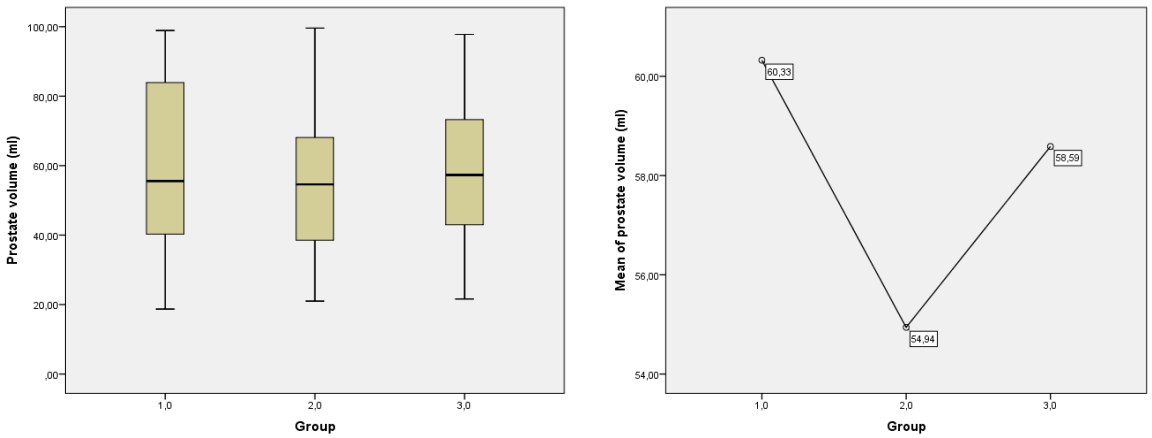


Figure 5.13. Mean values of biopsy-GS at different levels of PSA

These data are confirmed when comparing the PSA with the GS after the RP.

c. Change in GS and prostate volume

Data about prostate volume in milliliters are available for 147 (72.8%) patients. In the first group there are 52 (35.4%) patients and their prostate volume varies from 18.7 to 98.9 ml., and the mean value is 60.33 ml. (SD = 23.076). There are 44 (29.9%) patients in the second group and their prostate volume varies from 21 to 99.6 ml. with an average prostate volume of 54.94 ml. (SD = 19.091). In the third group there are 51 (34.7%) patients and their prostate volume varies from 21.6 to 97.8 ml. with a mean prostate volume of 58.59 ml. (SD = 20.274). (Figure 5.14a).



(a) Prostate volume (ml) in the main groups

(b) Mean values of prostate volume in the main groups

Figure 5.14. Prostate volume in the main groups.

Despite the differences in the values of the means in the main groups, no statistically significant relationship was found between prostate volume and the distribution of patients to group 1, 2 or 3. (ANOVA, F =, 805; p =, 449). The values of the means is used for graphical representation (Figure 5.14b). This indicates that there is no relationship between prostate volume and possible increase / decrease in post-operative GS.

d. Change of GS and PSAD.

Data about PSAD are available for 158 (78.2%) patients. PSAD ranges from 0.03 to 1.72; the mean value is 0.29 7 (SD = .298); the median is 0.19 (IQR = 0.12 - 0.35). Data do not follow a normal distribution (Kolmogorov-Smirnov and Shapiro-Wilk test, p <, 05).

The first main group includes 56 (35.4%) patients. PSAD values range between 0.03 and 1.72, the mean is 0.33; the median is 0.19 (IQR = 0.12 - 0.43). The second group includes 46 (29.2%) patients with PSAD data. PSAD values range between 0.06 and 0.98, the average is 0.27; the median is 0.19 (IQR = 0.1 4 - 0.37). The third group includes 56 (35.4%) patients with PSAD data. PSAD values range between 0.04 and 1.58 with an average PSAD value of 0.28; the median is 0.195 (IQR = 0.12-0.295). The values of the averages is used for graphical representation (Figure 5.15).

No statistically significant relationship is found between PSAD values and the three main groups (X^2 =, 644; p =, 725) - i.e. PSAD is not related to possible increase / decrease in GS after surgery.

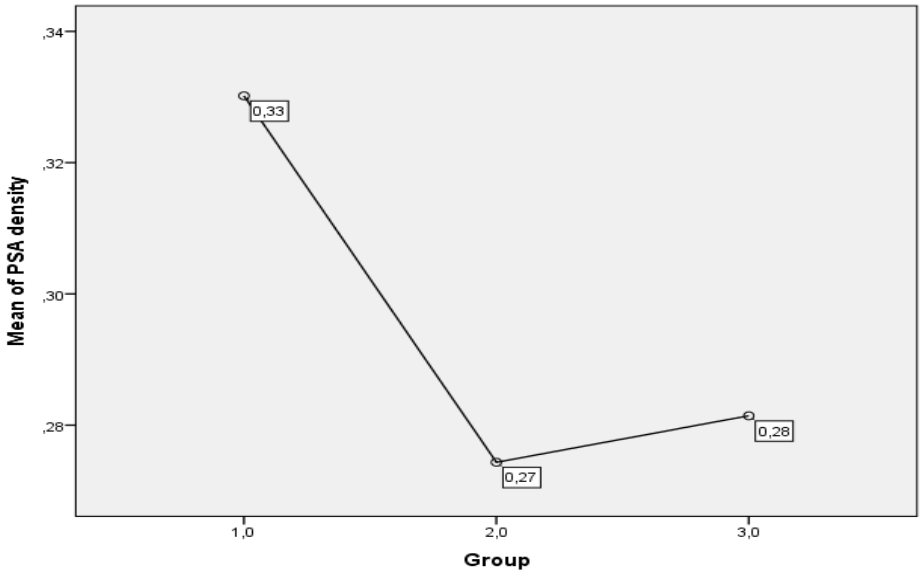


Figure 5.15. Chart of PSAD averages of the main groups

Using the original PSAD values, a statistically significant relationship is found between the PSAD values and the GS levels after biopsy (Kruskal Wallis test, $X^2 = 16,488$; p = .0 21) -for the analysis we divided the PSAD values into three levels (*below 0.1; between 0.1 and 0.15 and above 0, 15*). The subgroup with PSAD below 0.1 includes 27 (17.2%) patients with mean GS = 6.07. The subgroup with PSAD 0.1 and 0.15 included 36 (22.9%) patients with mean GS = 6.16. The subgroup with PSAD above 0.15 included 94 (59.9%) patients with mean GS = 7.03. A statistically significant relationship was found between the GS and PSAD (Kruskal-Wallis test, $X^2 = 12,553$; p = .002). The values of the means are used for graphical representation (Figure 5.16). A statistically significant difference is found in the GS values after biopsy between subgroups with PSAD values below 0.1 and above 0.15 (Mann-Whitney U test, MWU = 864,000; p = .007). GS values are statistically significantly higher in the subgroup with PSAD values above 0.15. A statistically significant difference is found in the GS values after biopsy between the subgroups with PSAD values 0.1 - 0.15 and above 0.15 (Mann-Whitney U test, MWU = 1177.5 00; p = .004). GS values are statistically significantly higher in the subgroup with PSAD values above 0.15. No statistically significant difference is found between the subgroups with PSAD values below 0.1 and 0.1 - 0.15 (Mann-Whitney U test, MWU = 470,000; p =, 817).

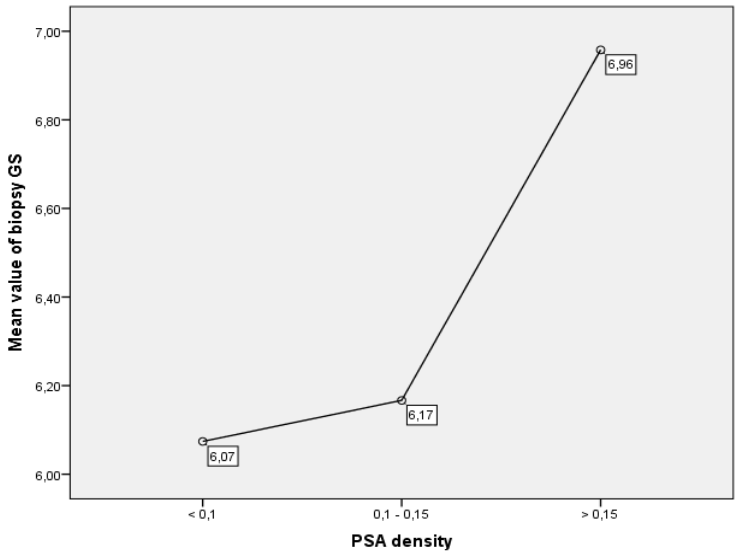


Figure 5.16. Mean GS values after biopsy at different PSAD levels

When using GS categorization in two groups (≥ 7 and <7), a statistically significant relationship is also found between GS and PSAD ($X^2 = 8,956$; p =, 011). The chance of a patient with PSAD above 0.15 to have GS ≥ 7 is 1.3 times higher compared to a

patient whose PSAD is between 0.1 and 0.15 (OR = 1.291; 95% CI = 1.006 - 1.656; p = .228). The chance of a patient with PSAD above 0.15 to have GS ≥ 7 is 1.3 times higher than in a patient with a PSAD below 0.1 (OR = 1, 308 ; 95% CI = 1.0 39 - 1.6 48 ; p = .010) .
The results are similar when comparing PSAD and GS levels after surgery (Kruskal Wallis test, X^2 = 17,223; p =, 0 28).

e. Change in GS and presence of a palpable nodule in the prostate (stage T2)

With stage T2 are 35 (17.3%) patients, distributed as follows: T2a - 8 (4.0%); T2 b - 17 (8.4%); T2c - 10 (5%). The remaining 167 (82.7%) patients correspond to stage T1. No statistically significant relationship is found between the three main groups and the presence (respectively the absence) of a palpable nodule in the prostate (X^2 = 4,698; p =, 583). That is, the presence of a palpable nodule in the prostate is not associated with a possible increase / decrease in GS after surgery.

f. Change in GS after surgery and relationship to the degree of GS from the biopsy

GS data from the biopsy are available for 198 (98.2%) patients. 70 (35.4%) patients are classified in the first main group. In this group the largest (26 or 37.1%) is the share of patients with GS = 6 (GS = 3 + 3), followed by those with GS = 7 (22 or 31.4%), of which 18 (25.7%) are with GS = 3 + 4 and 4 (5.7%) are with GS = 4 + 3 (Figure 5.17).

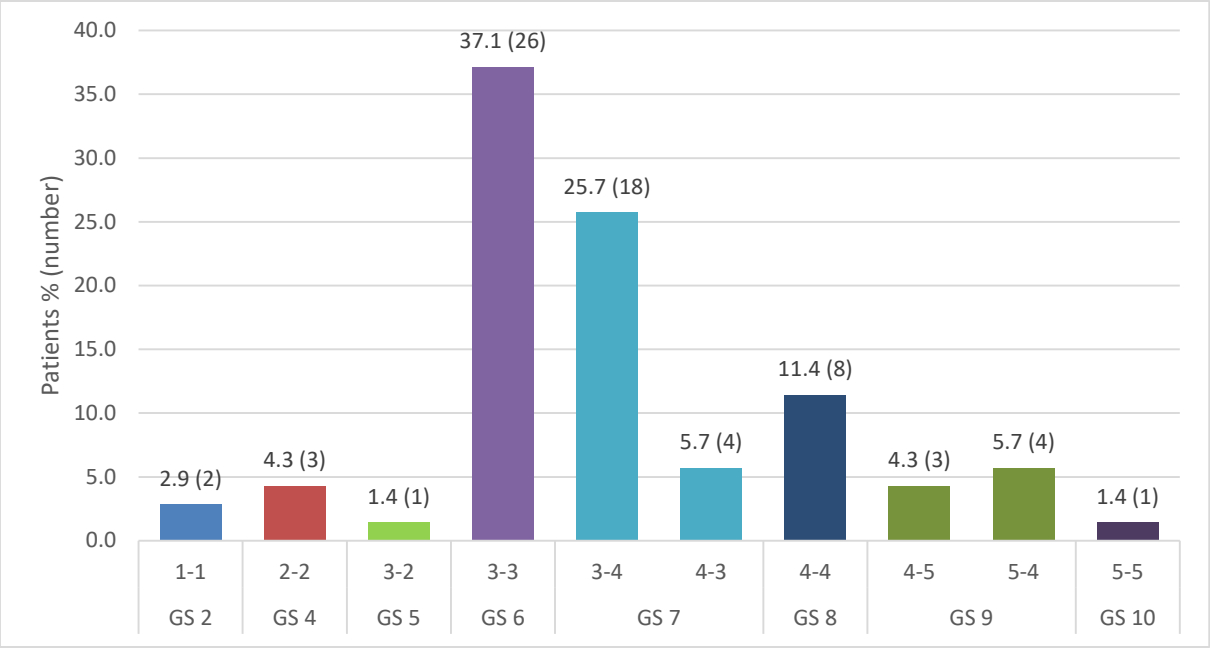


Figure 5.17. Distribution of patients in the first main group according to the values of GS from the biopsy

59 (29.8%) patients are classified in the second main group. In this group the largest (23 or 39%) is the share of patients with GS = 6 (GS = 3 + 3), followed by those with GS = 7 (19 or 32.2%), all with GS = 3+ 4 (Figure 5. 18).

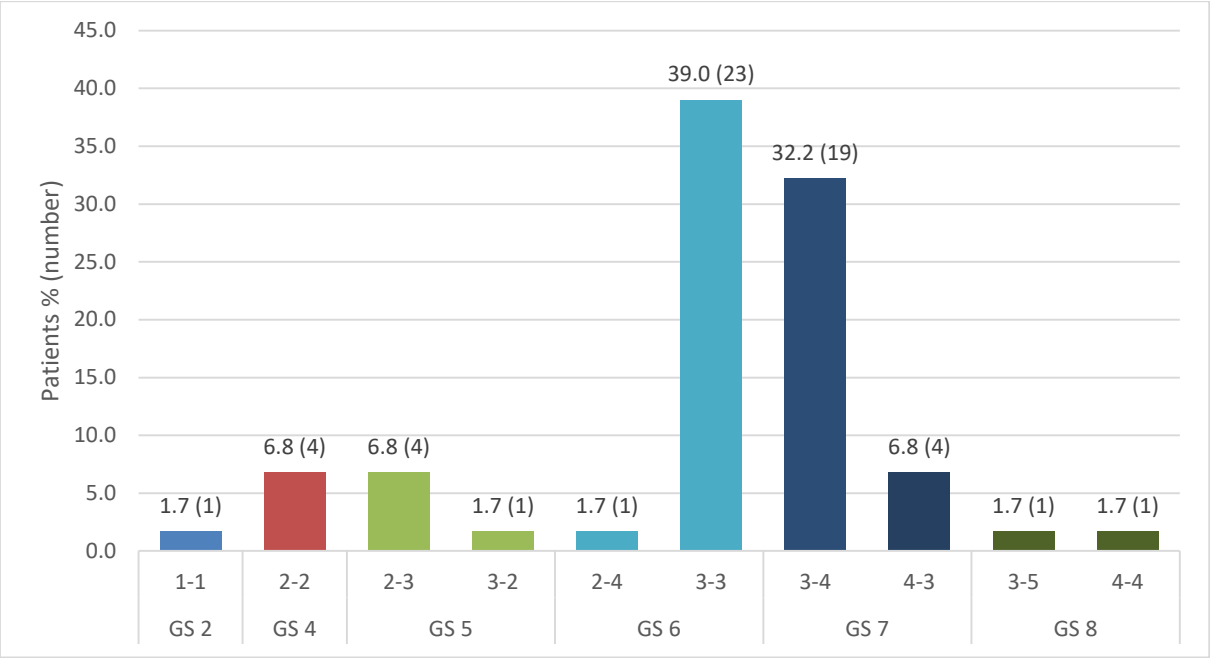


Figure 5.18. Distribution of patients in the second main group according to the values of GS from the biopsy.

In the third group are classified 69 (34, 8%) patients. In this group the largest (33 or 47.8 %) is the share of patients with GS = 7, of which 25 (36.2 %) are with GS = 3 + 4 and 8 (11.6 %) are with GS = 4 + 3, followed by those with GS = 8 (15 or 21.7%), all with GS = 4 + 4 (Figure 5.19).

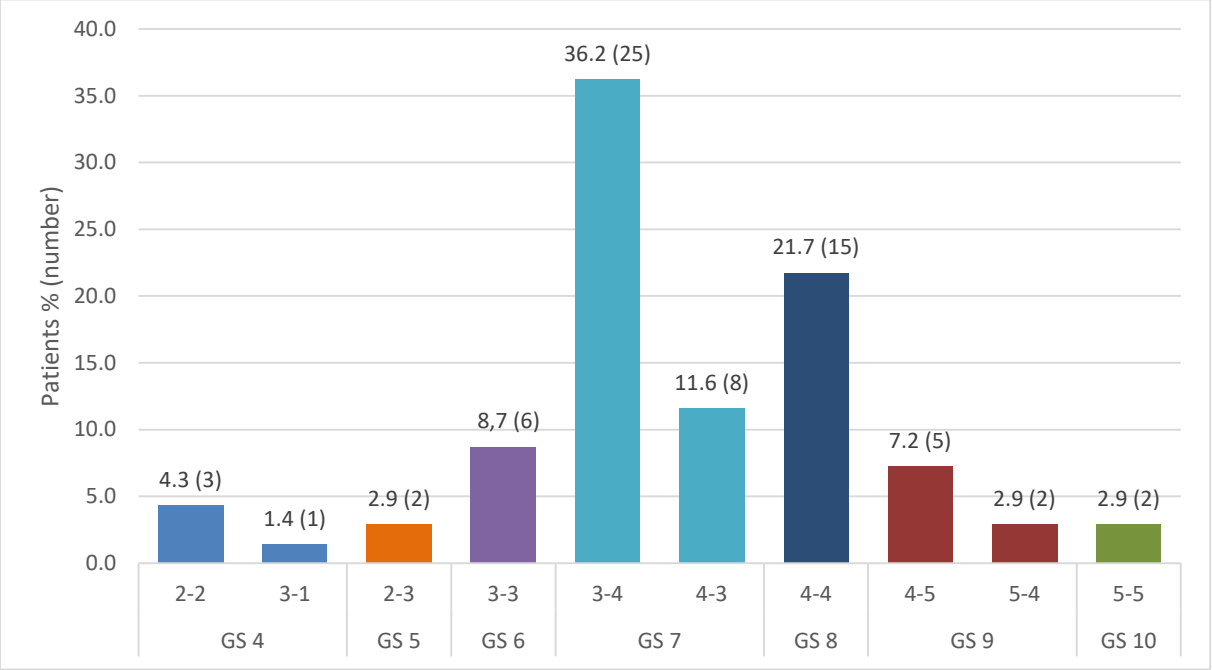


Figure 5.19. Distribution of patients in the third main group according to GS values after biopsy.

The total distribution of GS values in the main groups is presented in Figure 5. 20.

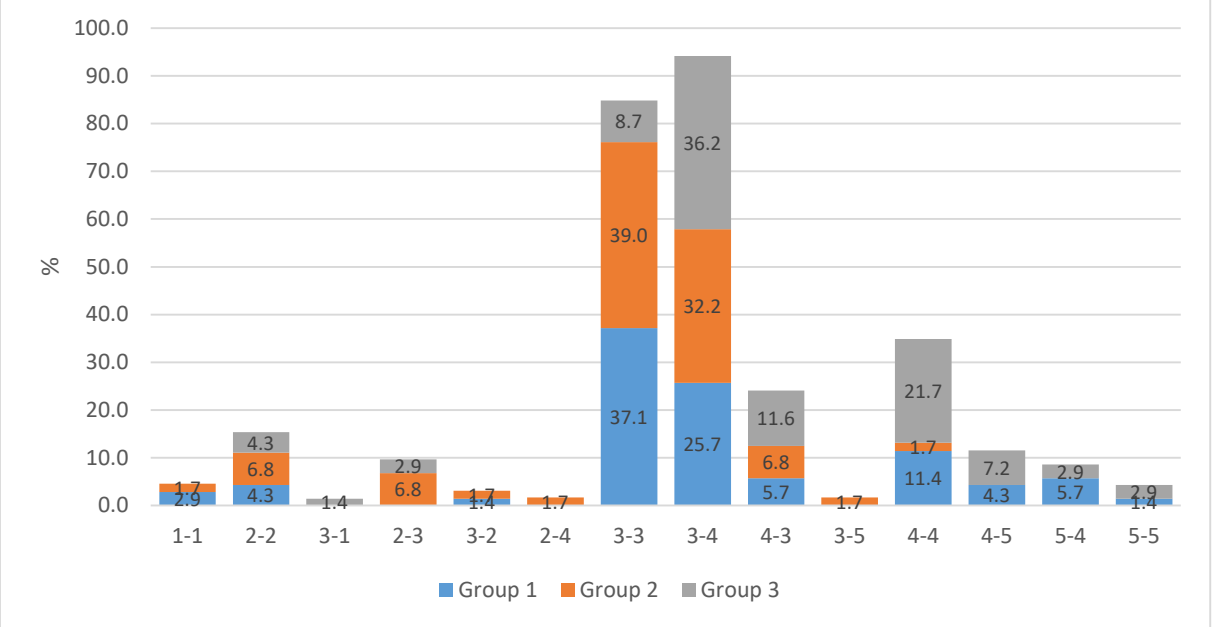


Figure 5.20. Distribution of patients according to the values of GS from the biopsy by main groups.

There is a statistically significant difference in the values of GS in the main groups (Kruskal-Wallis test, $X^2 = 25,545$; p =, 000). (Figure 5.21).

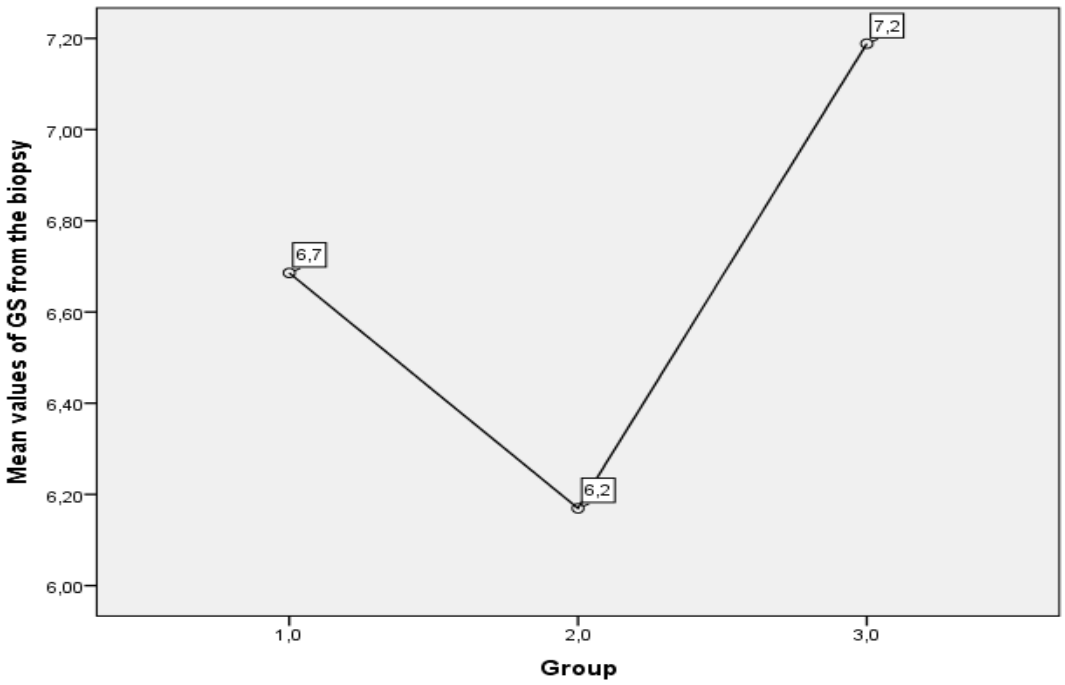


Figure 5.21. Mean values of GS from the biopsy in the three main groups

The values of GS after biopsy of the patients in the first group are statistically significantly higher than those of the patients in the second group (Mann-Whitney U test, MWU = 1603,500; p =, 021) and statistically significantly lower than those of patients in the third group (MWU = 1804,500; p = .007). The GS values of the patients in the second group are statistically significantly lower than those of the patients in the third group (Mann-Whitney U test, MWU = 1009,500; p = .00 00). That is, there is a relationship between the level of GS from the biopsy and the possible increase / decrease in GS after surgery.

g. Analysis of the cases when the same pathologist examines the material from the biopsy and from the operation.

For 190 patients data are available about the pathologists performing the analysis - in only eight of the patients the material from the biopsy and that after the operation is examined by the same pathologist. Of these, 1 is from the first main group, 6 are from the second main group and 1 is from the third main group.

A statistically significant relationship is found between the examination of the specimen by the same pathologist and the inclusion of patients in the three main groups (X^2 = 7,450; p =, 024). The chance of a patient whose materials (from the biopsy and the operation) are examined by the same pathologist to be from the second group (with GS increase) is 8 times higher than the chance the same patient to be from the first

group (unchanged GS) (OR = 7.811; 95% CI = .913 - 66.862; p = .229) and about 4 times higher than that patient to be in the third group (with GS decrease) (OR = 3.717 ; 95% CI =, 601 - 23,005 ; p =, 0 46). It should be noted however that this result is based on a very small sample. The other comparisons are not statistically significant.

h. Analysis of the cases in which the biopsy and the operation are performed in the same hospital.

Data about the location of the biopsy and the operation are available for 190 patients. 69 (35.4%) patients are biopsied and operated in the same hospital (in St. Anna Hospital in Varna). Of these, 24 (34.8%) are from the first main group, 29 (42%) are from the second main group and 16 (23.2%) are from the third main group. A statistically significant association was found between the biopsy and surgery site and the allocation of the patients in the main groups (X^2 = 6,975; p = 031). The chance of a patient, whose biopsy and surgery are performed at MHAT "St. Anna", to be from the second group is 1.7 times higher than the same patient to be from the third group (OR = 1,687 ; 95% CI = 1,093 - 2,606 ; p = .10 10). The other comparisons are not statistically significant.

i. Change in GS and allocation of patients to the EAU- risk groups

Data about the EAU-risk groups from biopsy are available for 201 (99,5%) patients. Their distribution is as follows: the first main group includes 38 patients (18.9 %), the second - 89 (44.3%), and the third - 74 (36.8%).

No statistically significant relationship was found between the main groups and the distribution of patients in the risk groups by EAU (Kriskal Wallis test, X^2 = 3,654; p =, 161).

However, in our study, EAU-risk groups showed a correlation with some postoperative pathological characteristics. A statistically significant relationship is found between the risk groups and the presence of pT3b - seminal vesicles involvement (X^2 = 16,961; p =, 000) – with a weak positive correlation ($r_{(Sp)}$ =, 2 68; p =, 000). That is, patients with pT3b are more often from the high-risk group.

No statistically significant association is found between the EAU-risk groups and the presence of lymph node metastases (**X^2** = 4,164; p =, 1 25).

A statistically significant relationship is found between the risk groups and the presence of pT3a -extraprostatic tumor extension (X^2 = 9, 224; p =, 0 10) - with weak positive correlation (r (Spearman) =, 211; p =, 003). That is, patients with pT3a are more often from the high-risk group.

5.4. Initial experience with ISUP grade system (modified Gleason system).

The attached table shows the first grade of the ISUP grade system from the biopsy and how it changes (or remains the same) after the RP. First grade covers GS from 2 to 6 including. There is a coincidence with the result after RP in 46 (58.97%) of the patients. In 27 (34.62%) patients there is an increase in the grade after surgery:

ISUP grade from biopsy		ISUP grade from radical prostatectomy	
Grade	***Number of patients***	**Grade**	***Number of patients***
No tumor from the biopsy	4	1	1
		2	3
Grade	***Number of patients***	**Grade**	***Number of patients***
1	78	No tumor	5
		1	46
		2	16
		3	2
		4	6
		5	3
Total	**82**		**82**

Second grade includes GS = 3 + 4. Here, there is a match between biopsy and surgery in 18 (29.03%) patients. In 23 (37.1%) patients there is a decrease in the grade, and in 19 (30.65%) - an increase. Third grade includes GS = 4 + 3. There is a match between biopsy and surgery in 4 (25%) patients. In 8 (50%) patients there is a decrease in the grade, and in 4 (25%) - an increase. The fourth grade is heterogeneous and includes GS = 8, including 4 + 4, 3 + 5 and 5 + 3. Here, there is a match between biopsy and surgery in 8 (32%) patients. In 15 (60%) patients there is a decrease in the grade, and in 2 (8%) - an increase. The fifth grade includes GS = 9-10, including 4 + 5, 5 + 5 and 5 + 4. There is a coincidence between biopsy and surgery in 10 (58.82%) patients, and in 7 (41.18%) patients there is a decrease in the grade.

We then examined the relationship between ISUP grades and some perioperative characteristics of the patients. A connection was established between the value of the PSA and the ISUP grades - as PSA data do not follow a normal distribution and ISUP grades data are ranked, non-parametric tests have been applied. For PSA, the distribution of values in three groups was used - below 10, between 10 and 20 and above 20 ng / ml.

The total number of patients with valid PSA values and ISUP grades of the biopsy is 195. In the group with PSA values less than 10 there are 73 (37.4%) patients with a

mean ISUP of 1,753 (SD = 1.09) and median = 1 (IQR = 1 - 2) ; in the group with PSA values between 10 and 20 there are 79 (40.5%) patients with a mean ISUP of 2.38 (SD = 1.38) and a median of 2 (IQR = 1 - 4) , and in the PSA group over 20 there are 43 (22.1%) patients with a mean ISUP of 2.63 (SD = 1.36) and a median = 2 (IQR = 2 - 4).

The total number of patients with valid PSA values and ISUP grades after surgery is 191. In the group with PSA values under 10 there are 69 (36.1%) patients with a mean ISUP of 1, 942 (SD = 1, 3) and median = 1 (IQR = 1 - 2); in the group with PSA values between 10 and 20 there are 79 (41.4%) patients with a mean ISUP of 2.29 (SD = 1.43) and median = 2 (IQR = 1 - 4); in the PSA group over 20 - 43 (22.5%) patients with a mean ISUP 2,74 (SD = 1.54) and median = 2 (IQR = 1 - 4). The mean values of the ISUP grades (Figure 5.22) are used for graphical representation.

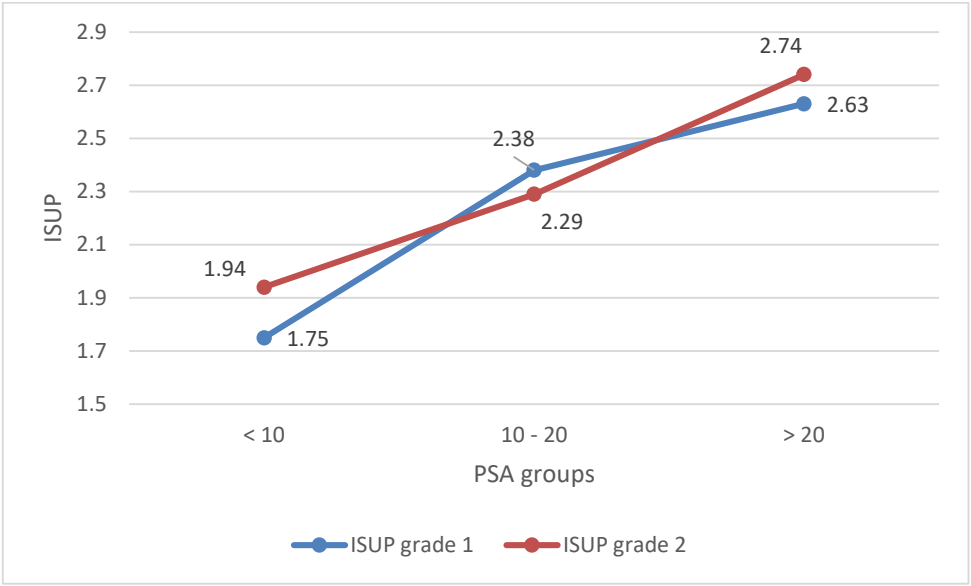


Figure 5.22. ISUP grades from biopsy (in blue) and after surgery (in red) referred to the levels of PSA.

There is a statistically significant difference in ISUP grades between the PSA-groups- concerning both the biopsy ISUP grades (Kruskal Wallis test, $X^2 = 15,628$; p =, 000) and post-prostatectomy ISUP grades ($X^2 = 9,595$; p =, 008). After comparing the ISUP grades by pairs of PSA groups, it was found that:

- ISUP grades from biopsy are statistically significantly lower at PSA below 10 ng / ml than those at PSA between 10 and 20 ng / ml (Mann-Whitney U test ;

MWU = 1872.5; p = .003) - however, there is no difference if the ISUP grades from the operation are used.

- ISUP grades (both from the biopsy and from the operation) are statistically significantly lower at PSA below 10 ng /ml than those at PSA above 20 ng /ml (Mann-Whitney U test, MWU = 864,000 p = .000).
- ISUP grades (both from the biopsy and from the operation) do not differ statistically significantly at PSA between 10 and 20 ng / ml from those at PSA above 20ng / ml (p> .05).

In addition, a link was found between biopsy-ISUP grades and some post-RP pathological characteristics. The seminal vesicles (pT3b stage) are involved in 32 (16.2%) patients. According to the ISUP grades from the biopsy, they are distributed as follows:

			ISUP grades from biopsy					Total
			1.0	2.0	3.0	4.0	5.0	
seminal vesicle involvement pT3b	no	Number of patients	72	51	13	21	9	166
		%	36.4%	25.8%	6.6%	10.6%	4.5%	83.8%
	Yes	Number of patients	6	11	3	4	8	32
		%	3.0%	5.6%	1.5%	2.0%	4.0%	16.2%
Total		Number of patients	78	62	16	25	17	198
		%	39.4%	31.3%	8.1%	12.6%	8.6%	100.0%

A statistically significant association was found between the ISUP grades of the biopsy and the presence of stage pT3b (X^2 = 16,300; p = .003) – with a weak positive correlation ($r_{(Sp) = .227; p = .001}$). This means that patients with pT3b have higher ISUP grades from the biopsy.

There are 11 (5.6%) patients with lymph node metastases (pN1 stage). According to the ISUP grades from the biopsy, they are distributed as follows:

			ISUP grades from biopsy					Total
			1.0	2.0	3.0	4.0	5.0	
Lymph node metastases pN1	no	Number of patients	75	59	16	23	14	187
		%	37.9%	29.8%	8.1%	11.6%	7.1%	94.4%

	Yes	Number of patients	3	3	0	2	3	11
		%	1.5%	1.5%	0.0%	1.0%	1.5%	5.6%
Total		Number of patients	78	62	16	25	17	198
		%	39.4%	31.3%	8.1%	12.6%	8.6%	100.0%

No statistically significant association was found between ISUP grades and the presence of lymph node metastases ($X^2 = 6,458$; p =, 167).
There are 57 (28.8%) patients with extraprostatic extension (stage pT3a). According to the ISUP grades from the biopsy, they are distributed as follows:

			ISUP grades from biopsy					Total
			1.0	2.0	3.0	4.0	5.0	
extraprostatic extension pT3a	no	Number of patients	66	36	12	18	9	141
		%	33.3%	18.2%	6.1%	9.1%	4.5%	71.2%
	Yes	Number of patients	12	26	4	7	8	57
		%	6.1%	13.1%	2.0%	3.5%	4.0%	28.8%
Total		Number of patients	78	62	16	25	17	198
		%	39.4%	31.3%	8.1%	12.6%	8.6%	100.0%

A statistically significant association was found between ISUP grades from biopsy and the presence of stage pT3a ($X^2 = 14,951$; p = .005) –with a weak positive correlation ($r_{Sp} = .194$; p = .244). This means that patients with pT3a have a higher ISUP grade from the biopsy.

5.5. Changes in GS and their connection with some pathological features found after RP.

32 (16.5%) patients have seminal vesicle involvement. 11 (5.4%) patients have lymph node metastases. 58 (28.7%) patients have extraprostatic tumor extension. In some cases, prostate biopsy revealed PCa in one lobe, and after RP, cancer was detected in

both lobes – such are 28 (13.9%) patients. The distribution of patients according to postoperative characteristics by main groups is presented in Figure 5.23. From the above data is evident that with the largest share for each of the considered unfavorable postoperative characteristics are the patients in the second group, that is in patients in whom the GS increases after RP compared to that of the biopsy. However, differences are not statistically significant. Thus, our hypothesis that there is a link between the change in GS after RP (compared to that of prostate biopsy) and some pathological characteristics such as seminal vesicle involvement, extraprostatic tumor extension, the presence of lymph node metastases, is not accepted.

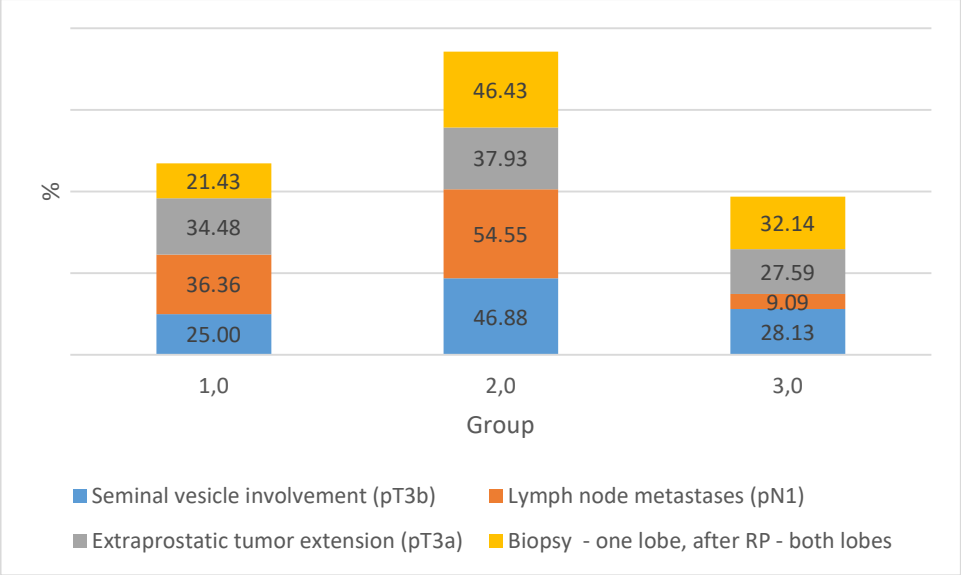


Figure 5.23. The distribution of patients according to postoperative pathological characteristics by main groups.

5.6. Changes in Gleason score and their implication on biochemical progression-free survival

Data about the time to biochemical progression are available for 111 (55%) patients. Only patients who did not receive neoadjuvant or adjuvant therapy were used in the BPFS analysis. Of all patients monitored, 42 (37.8 %) were in the first group; 40 (36 %) are in the second group and 29 (26,2%) are in the third group. 16 (38.1%) patients from the first group are with biochemical progression. The time to onset of biochemical progression varies from 1 to 51 months, the average duration is 18.9 months, the median is 15.5 (IQR = 4.0 - 31.0) months. From the second group with biochemical progression are 26 (65%) patients. The time to onset of biochemical progression varies from 1 to 22 months, the average duration is 5,6 months, the median is 2.5 (IQR = 1 , 0 - 1 0 , 0) months. From the third group with biochemical progression are 10 (34.5 %)

patients. The time to onset of biochemical progression varies from 1 to 24 months, the average duration is 7.0 months, the median is 2.5 (IQR = 1,0 - 12.0) months. The values of the means (Figure 5.24) were used for graphical representation.

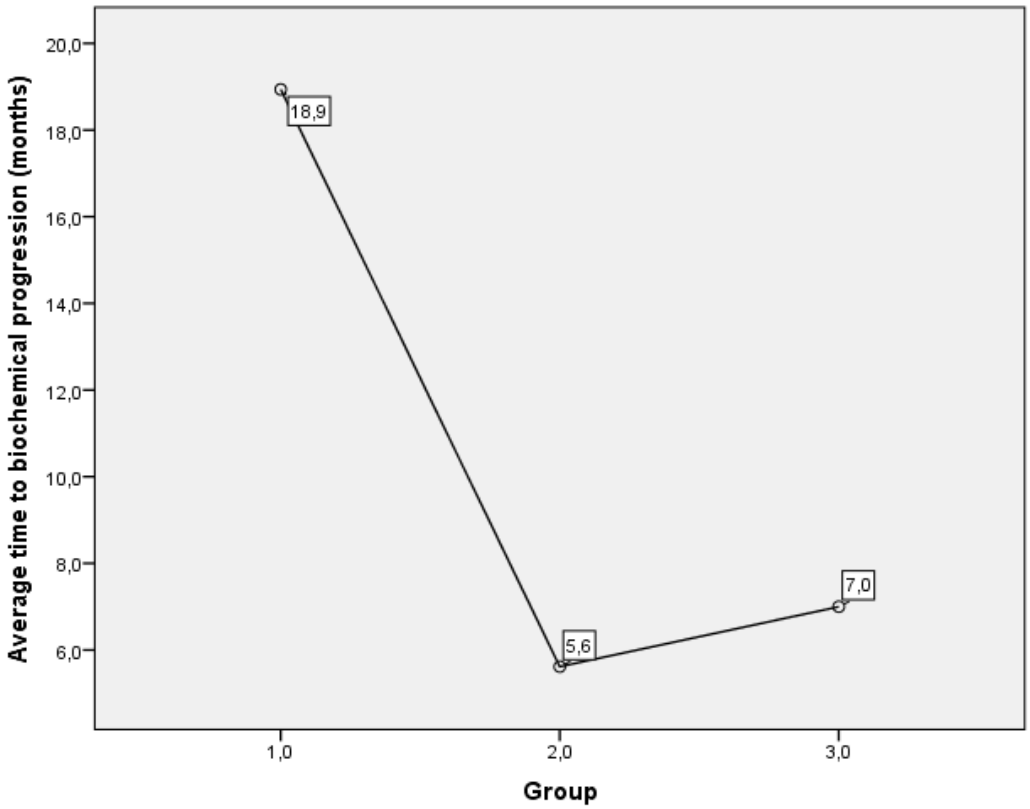


Figure 5.24. Average time to biochemical progression.

A statistically significant relationship was found between the distribution of patients to the three main groups and the time to onset of biochemical progression (X^2 = 7,938; p =, 019). This applies to the first and second main groups, as well as to the first and third main groups. The time to onset of biochemical progression in patients in the second group (mean 5.6 months) was statistically significantly shorter than in patients in the first group (mean 18.9 months), (Mann-Whitney U test, MWU = 105,000 ; p = .0 07). Time to onset of biochemical progression of patients in the third group (average 7.0 months) is statistically significantly shorter than that of patients in the first group (MWU = 42,500; p = .047). The time to onset of biochemical progression in patients in the second group did not differ statistically significantly from that in patients in the third group (MWU = 129,500; p = .986).

The analysis of survival in order to assess the prognostic significance of the main groups also showed that they have prognostic significance in terms of survival without biochemical progression. Shorter survival without biochemical progression can be

expected in patients in the second and third groups than in patients in the first group (Kaplan-Meier - *Log Rank test (Mantel-Cox); X^2 = 10,785; p = , 0 05*) (Figure 5.25).

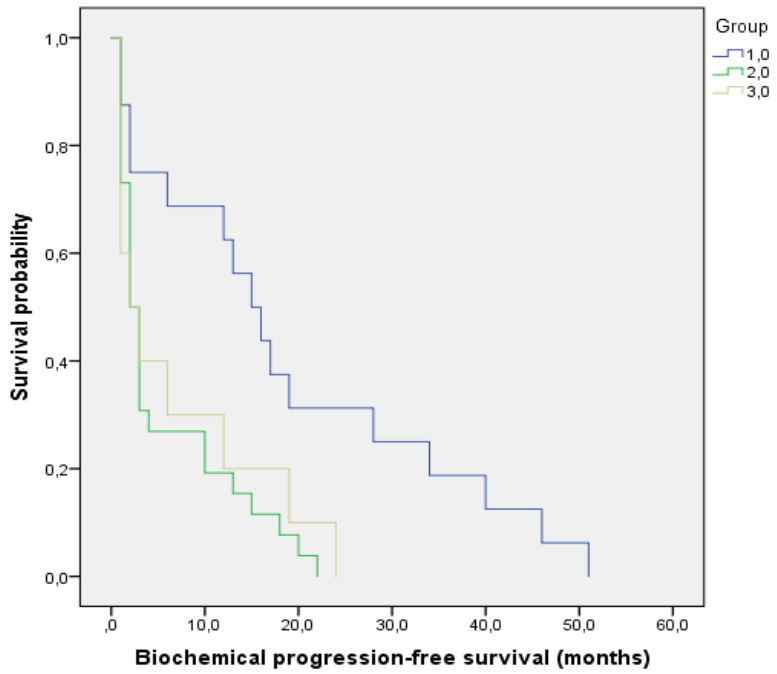


Figure 5.25. Kaplan-Meier survival analysis of the patients from the groups.

A statistically significant relationship was found between the distribution of patients to the main groups and the occurrence of biochemical progression (X^2 = 8,366; p =, 015). The chance of a patient from the second group to develop biochemical progression is 3 times higher than that of a patient from the first group (OR = 3.018 ; 95% CI = 1.227 - 7.423 ; p = .105), and the chance of a patient from the third group to have biochemical progression is 2 times greater than that of the patient from the first group (OR = 2, 073; 95% CI = 1, 135 - 3,787; p =, 012). Our hypothesis about the relationship between the change in GS after radical prostatectomy (compared to that of prostate biopsy) and the occurrence of biochemical progression is proven.

In addition to patients' belonging to the three main groups, BPFS was found to depend on:

a. PSA

The mean preoperative PSA of patients with biochemical progression was 21.75 (17.03 excluding extreme values) and the median was 14.2 (IQR = 10.0 to 25.0). The mean PSA of the remaining patients was 9.86; the median is 9.8 (IQR = 7.5 to 12.00) (Figure 5 .26).

There is a statistically significant difference in PSA values for patients with and without biochemical progression (Mann-Whitney U test, MWU = 1163,000 p = .041). After exclusion from the analysis of extreme values, PSA values followed by a normal distribution (Kolmogorov-Smirnov and Shapiro-Wilk test, p>, 05). PSA values of patients with biochemical progression were on average 7.17 higher than those without biochemical progression (t = -4.054; p = .0 34). Our hypothesis that PSA may have prognostic significance in terms of survival without biochemical progression is proven.

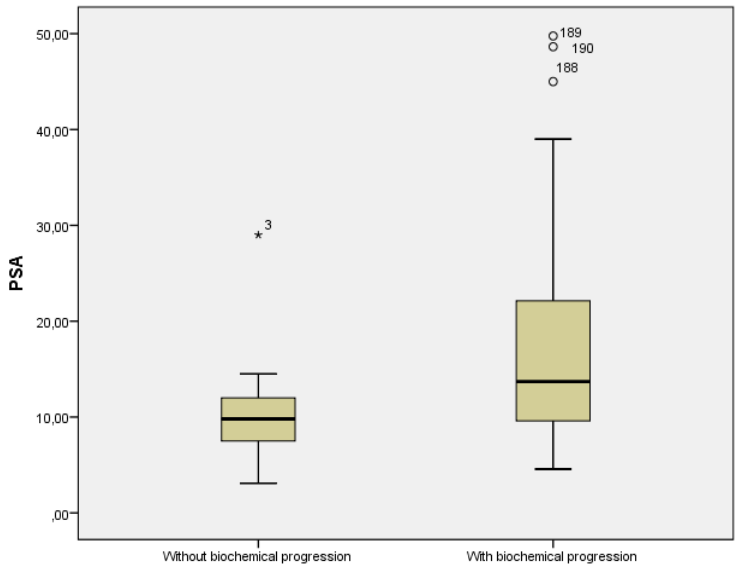


Figure 5 .26. PSA values in patients with and without biochemical progression

b. Gleason Score (GS)

The mean value of GS after biopsy and its values after surgery in patients with biochemical progression is 6.54 (SD = 2.01) and 7.02 (SD = 1.87), respectively. The mean value of GS after biopsy and its values after surgery of the other monitored patients is 6.31 (SD = 1.14) and 5.62 (SD = 2.2), respectively.

There is no statistically significant difference in post-biopsy GS values in patients with biochemical progression and patients without biochemical progression (X^2 = 13,362; p =, 064).

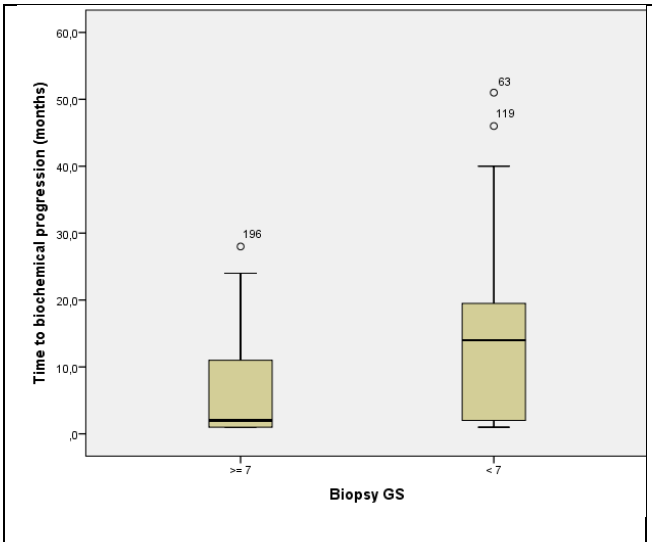
There is a statistically significant difference in the values of the postoperative GS in the two groups. The values of the postoperative GS of patients with biochemical progression are statistically significantly higher than those of patients without biochemical progression (X^2 = 16,266; p = .39).

To clarify the differences, we classified patients into two groups - with postoperative GS ≥ 7 and GS <7.

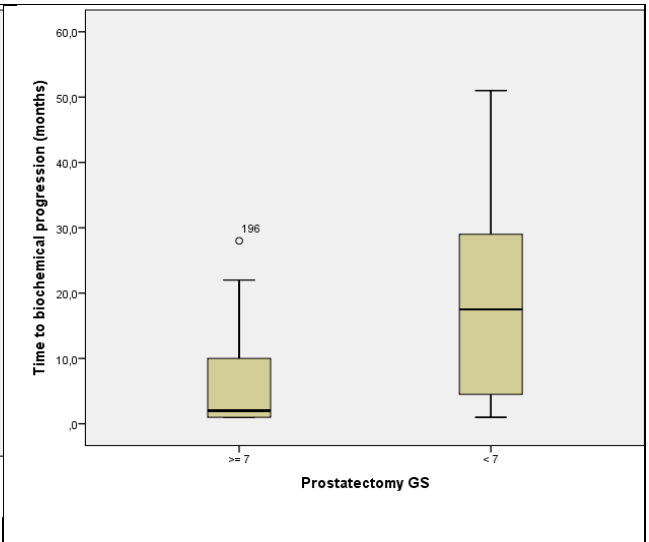
The lack of a difference in the GS values after biopsy was confirmed in patients with and without biochemical progression (X^2 = 1,493; p =, 222), as well as a statistically significant difference in postoperative GS values in patients with and without biochemical progression (X^2 = 8,678; p =, 003). Patients with postoperative GS ≥ 7 are 1.9 times more likely to develop biochemical progression than patients with GS <7 (OR = 1, 862, 95% CI = 1, 190 - 2,914).

The time to onset of biochemical progression in patients with biopsy GS ≥ 7 varies from 1 to 28 months with a mean time of 6.5 months and a median of 2 (IQR = 1.0 - 1 2 , 0) months. The time to onset of biochemical progression in patients with biopsy GS <7 ranged from 1 to 51 months with a mean time of 15.7 months and a median of 1 4 (IQR = 2 - 19.5) months. There was a statistically significant difference in the time to biochemical progression in the groups with biopsy GS <7 and GS ≥ 7. It is shorter in patients with GS ≥ 7 on average by about 9 months (t = -2.420; p = .223). (Figure 5.27a).

The time to onset of biochemical progression in patients with postoperative GS ≥ 7 ranged from 1 to 28 months with a mean time of 5.9 (SD = 2.23) months and a median of 2 (IQR = 1.0 - 10.0) months. The time to onset of biochemical progression in patients with postoperative GS <7 ranged from 1 to 51 months with a mean time of 19.3 (SD = 5.27) months and a median of 17.5 (IQR = 4.5 - 29.0) months. There is a statistically significant difference in time to biochemical progression in the groups with postoperative GS <7 and GS ≥ 7. It is shorter in patients with GS ≥ 7 by an average of about 13 months (t = -3.213; p = .005). (Figure 5.27b):



a). GS from the biopsy



b). GS from the RP

Figure 5 .27. Time to biochemical progression in GS <7 and GS ≥ 7 (from biopsy and after RP)

The analysis of survival in order to assess the prognostic value of biopsy GS showed that it has prognostic value in terms of survival without biochemical progression. Patients with biopsy GS <7 may have a longer survival without biochemical progression than patients with GS ≥ 7 (Kaplan-Meier - Log Rank test (Mantel-Cox); X^2 = 7,057; p = , 008) (Figure 5 .28).

Patients with biopsy GS <7 were 2 times more likely to survive without biochemical progression than patients with GS ≥ 7 (Exp (B) = 2,147; 95% CI = 1,153 - 3,996; p =, 013).

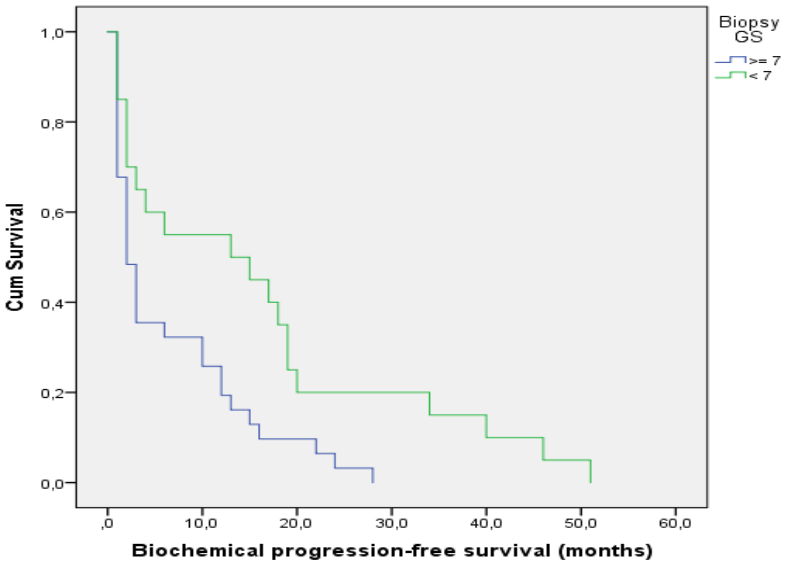


Figure 5.28. Biochemical progression-free survival for biopsy GS < 7 and GS ≥ 7

The analysis of survival in order to assess the prognostic significance of postoperative GS showed that it has prognostic significance in terms of survival without biochemical progression. Longer survival without biochemical progression can be expected in patients with postoperative GS <7 than in patients with GS ≥ 7 (Kaplan-Meier - Log Rank test (Mantel-Cox); X^2 = 12,836; p = , 00 0) (Figure 5.29).

Patients with postoperative GS <7 are 3 times more likely to survive without biochemical progression than patients with GS ≥ 7 (Exp (B) = 3.046; 95% CI = 1.358 - 6.036; p =, 0 01).

Our hypothesis that patients with GS <7 may expect longer survival without biochemical progression than patients with GS ≥ 7, regardless of whether GS is determined by biopsy or after surgery, is proven.

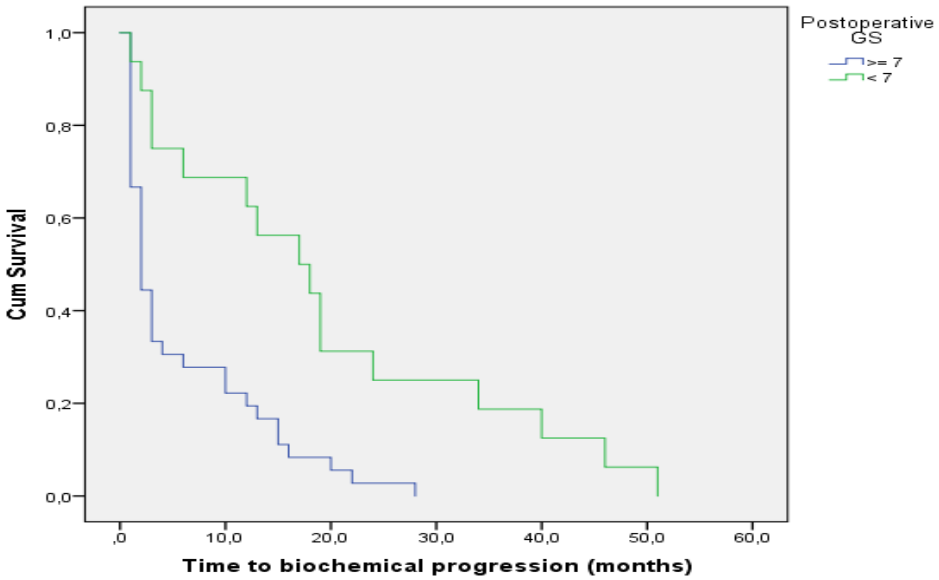


Figure 5.29. Survival without biochemical progression in postoperative GS <7 and GS ≥ 7.

c. Distribution of the patient to the three risk groups (low, moderate and high risk), defined by the EAU.

No statistically significant association was found between patients' belonging to the EAU groups and the time to biochemical progression (X^2 = 2,880; p =, 237).

d. Postoperative pathological features - the presence of extraprostatic tumor extension (pT3a), seminal vesicle involvement (pT3b) and lymph node metastases (pN1).

Of the 111 patients followed, 17 (15.3%) had pT3b, of which 14 (82.3%) developed biochemical progression. Eight (7.2%) patients had pN1, of which 5 (62.5%) had biochemical progression. Of 35 (31.5%) patients with pT3a, 17 (48.6%) experienced biochemical progression. No statistically significant association was found between the presence of pN1 and p T3a and the occurrence of biochemical progression. (p>, 05 for all comparisons)**.** This was found only in patients with pT3b (X^2 = 10,163; p =, 0 01). The chance of a biochemical progression in a patient with pT3b is about 1.3 times higher than in a patient without pT3b (OR = 1, 299; 95% CI = 1,090 - 1,548).

The analysis of survival in order to assess the prognostic value of pT3b, pN1 and pT3a showed that they have prognostic value in terms of survival without biochemical

progression. In patients with pT3b, pN1 and pT3a, a shorter period without biochemical progression can be expected than in those without pT3b (Kaplan-Meier - *Log Rank test (Mantel-Cox); $X^2 = 22,308$; p = , 001*), pN1 ($X^2 = 11,892$; p = .001) and pT3a ($X^2 = 26,543$; p =, 000). (Figures 5.30, 5.31 and 5.32).

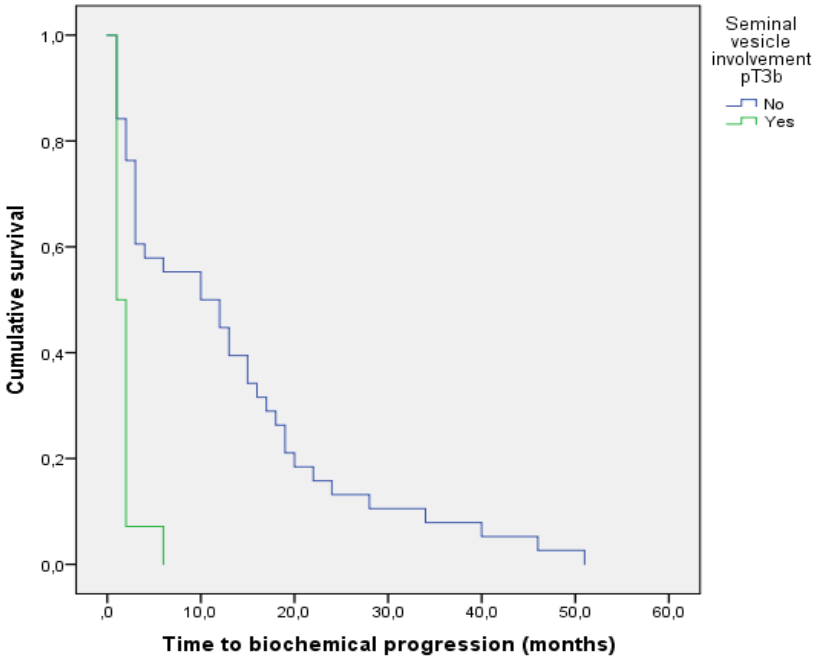


Figure 5.30. Survival without biochemical progression in the presence / absence of pT3b

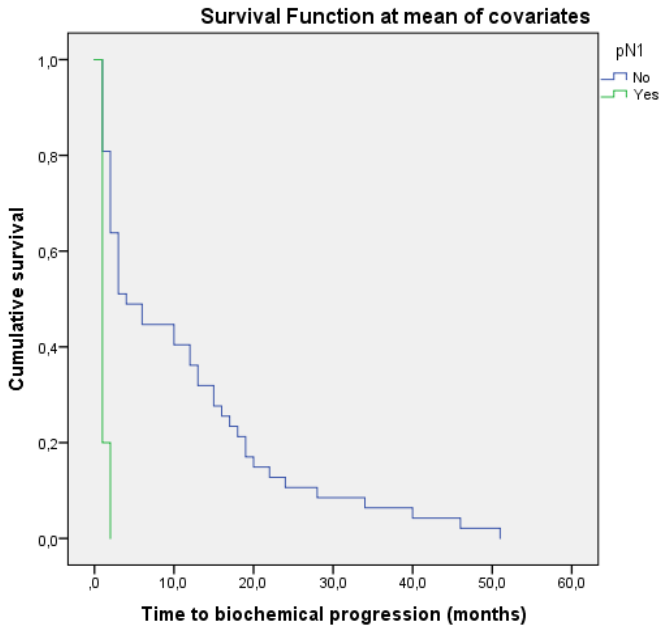


Figure 5.31. Survival without biochemical progression in the presence / absence of pN1

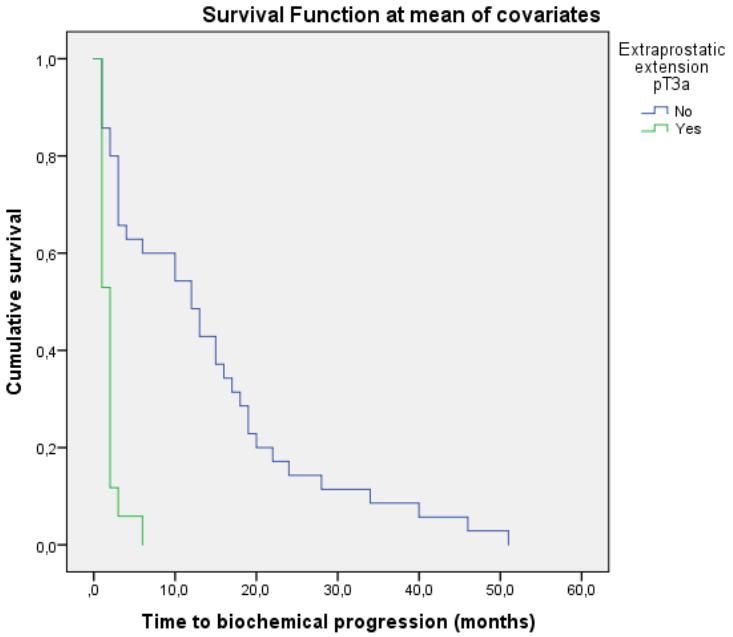


Figure 5.32. Survival without biochemical progression in the presence / absence of pT3a

Our hypothesis that in patients with extraprostatic tumor extension (pT3a), seminal vesicle involvement (pT3b) and lymph node metastases (pN1), shorter survival without biochemical progression can be expected, is proven.

5.7. Changes in GS and their relation with the overall survival after RP.

Follow-up data are available for 130 (64.4%) patients. For the remaining 72 (35.6%) there are no data about the follow-up.

Of all patients monitored, 44 (33.8%) are in the first group; 47 (36.2%) are in the second group and 39 (30.0%) are in the third group. From the first group 1 patient (2.3%) died 71 months after the operation. From the second group, 9 (19.1%) patients died, with survival ranging from 1 to 85 months with a median survival after surgery of 37.1 months and a median of 32 (IQR = 17.5 - 58.5) months. From the third group, 5 (12.8%) patients died, with survival ranging from 0.5 to 85.5 months with a median survival of 32.7 months and a median of 18 (IQR = 3.25 - 69.5) months – see Figure 5.33.

According to the available data, the survival of patients in the first group is the longest, followed by those in the second group, and the survival of patients in the third group

is the lowest. A statistically significant relationship was found between the survival of the patients and their belonging to the main groups (X2 = 6.431; p = .040).
After comparisons of the groups by two, a statistically significant relationship was found between patient survival and belonging to the first and second main groups (X2 = 6.617; p = .01), as well as their belonging to the first and third main groups (X2 = 4.175; p =, 041).

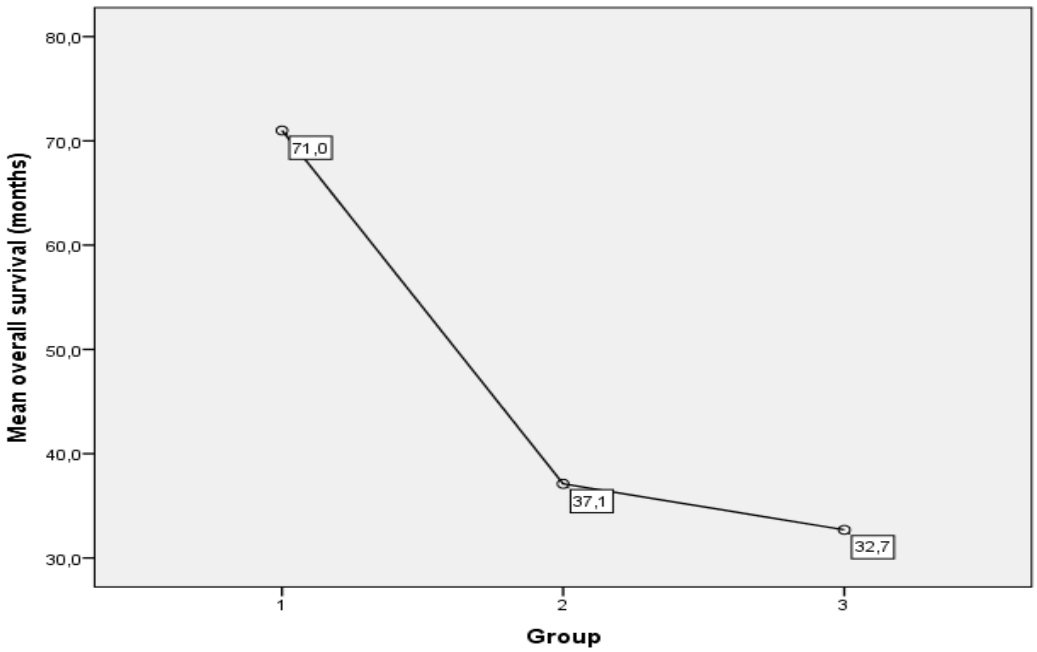


Figure 5.33. Overall survival (months) of patients by main groups

The chance of a patient from the second group to die is 7 times higher than that of a patient from the first group (OR = 10,184; 95% CI =, 818 - 34,465). The chance of a patient from the third group to die is 10 times higher than that of a first group patient (OR = 7.414; 95% CI = .8 23 - 66.781).
Nevertheless, the analysis of survival in order to assess the prognostic significance of the patient's belonging to the main groups shows that belonging to the main groups do not have statistically significant prognostic significance in terms of overall survival (Log Rank test (Mantel-Cox); X^2 = .487; p = .784) - due to the small number of cases.

5.8. Occurrence of metastases after RP.

Data on metastases after RP are available for 9 patients - one in the first group, six in the second and two in the third main group. The mean time to occurrence of metastasis is:

19 months - in the first main group (without change in GS)

16.5 months - in the second main group (with decrease in GS after surgery, compared to biopsy)

18.7 months - in the third main group (with increase in GS after surgery), but only 12.8 months in 5 out of the 6 cases (there is one case with very long survival).

Statistical analysis cannot be done due to the small number of patients; the results are discussed in the next section.

5.9. Patients with fusion biopsy of the prostate.

Data are available for 16 patients. In 80% of cases (8 out of 10 patients) the side of the tumor is correctly determined. However, there is a coincidence in GS in only 7 of 16 patients (43.75%) - they fall into the first main group, five are in the second group (31. 25 %) and four - in the third (25 %):

		Biopsy		
		Systematic	Target	Total
Group	1	63	7	70
	2	58	5	63
	3	66	4	70
Total		187	16	203

There is a tendency, when targeted biopsy is performed, the patients in the first group to be more than the patients in the other two groups. The differences are not statistically significant ($X^2 = 1{,}047$; $p =, 592$).

6. Discussion of the results

The analysis shows that there is a trend toward increase in the number of prostatectomies performed each year. There is also a trend toward increase in the

incidence of extraprostatic tumor extension after RP, probably related to the rise of the indications for RP.

Of the total number of patients available for analysis (203 patients), in 70 of the cases there is a coincidence of GS from the biopsy and from the RP - this is equal to about one third of the patients (34.48%). For the rest, there is either an increase in GS (63 patients) or a decrease (70 patients). Comparing these data with the information from the literature review, it can be seen that they are in the middle. Pathologists from the prominent American Johns Hopkins Hospital indicate a 58% complete match in the two GSs. Urologists from a tertiary urology center in Canada reported a 29.2% match. However, in the newer articles there is a tendency for the coincidence to be at least 50%, including for the hospitals in Bulgaria.

For the purpose of the study, the patients available for analysis were divided into three main groups:

a. First group, in which the GS from the biopsy is the same as that from the RP.

b. Second group, in which the GS from the RP increases compared to that from the biopsy.

c. Third group, in which the GS after RP decreases compared to that of the biopsy.

As a first step in the present study we analyzed patients with well-differentiated PCa (GS $\leq$ 6) to identify possible factors that predict its increase after RP (i.e. belonging to the second main group). According to literature data, it is in these patients that the two GSs are most likely to diverge. In addition, patients with well-differentiated PCa are sometimes referred to less radical treatments (active surveillance) due to the presumed benign nature of their tumor and hence the practical importance of finding factors that predicts possible increase in the GS. We analyzed the following parameters**:**

a. Age - no statistically significant difference was found in the age of the patients in the three main groups. Here our results differ from the data published in English, according to which old age predicts a possible increase in GS after the FP. However, the result is in line with that of the Bulgarian publication from 2016 in "Endourology and Minimally Invasive Surgery".

b. PSA - no statistically significant difference was found in PSA values of patients in the three main groups. This result also differs from other publications according to which higher PSA increases the risk of GS-upgrade after the RP. Again, however, there is a coincidence with the above-mentioned publication in Bulgarian.

c. PSA Density-PSAD – A statistically significant difference was found in PSAD values of patients in the three main groups. PSAD values were higher in patients in the second group than in the first group. PSAD values in patients in the third group were lower than in patients in the first and second main groups. The result is consistent with data from other studies, which show that elevated PSAD is an important prognostic sign for a possible increase in GS after surgery. However, this conclusion applies only to well-differentiated carcinomas, probably due to lower PSA production by high-grade PCa.

d. Prostate volume - A statistically significant difference was found in the values of the prostate volume of patients in the three main groups. The difference is statistically significant only between the first and second main groups - the values of prostate volume are lower in patients of the second group compared to those of the first main group. This result is consistent with data from other studies showing that patients with a small prostate are more likely to have an increase in GS after RP.

e. Palpation of a nodule in the prostate - after the introduction of PSA in practice, the majority of patients are in stage T1c. However, some of them are in stage T2-with a palpable nodule in the prostate. No statistically significant association was found between patients' distribution to the three main groups and the presence of a palpable nodule. That is, the presence of a nodule in the prostate is not a likely sign of worse histology after RP. However, a possible reason for this result is the insufficient number of patients in stage T2 preoperatively.

In summary, we can say that two indicators predict a possible increase in postoperative GS (relative to biopsy) in patients with well-differentiated PCa - high PSAD and small prostate volume.

We then analyzed different preoperative characteristics of the whole group of patients (not only those with well-differentiated PCa) to find a possible connection with the patients' distribution into one of the three main groups. Here are the following characteristics:

a. Age of the patient - no statistically significant relationship was found between the age of the patients and their belonging to the main groups.

b. PSA - No statistically significant difference in PSA values was found between the different main groups. A statistically significant relationship was found between the GS values and PSA (both from biopsy and after RP). GS values for PSA between 10 and 20 are statistically significantly higher than GS values for PSA below 10. GS

values for PSA above 20 are statistically significantly higher than those for PSA below 10. That is, with increasing PSA, the degree of tumor differentiation decreases accordingly. This fact is reflected in the EAU classification, which divides PCa into high-, intermediate- and low-risk for PSA above 20, 10 to 20 and below 10 ng / ml, respectively.

c. Prostate volume - no statistically significant relationship was found between the prostate volume of patients and their inclusion in group 1, 2 or 3. As noted above, there is such a relationship, but only in well- differentiated tumors (GS ≤ 6).

d. PSAD Density - No statistically significant relationship was found between PSAD values and the three main groups. As noted above, such a relationship exists in more differentiated tumors (GS ≤ 6). A statistically significant relationship was found between PSAD values and GS levels after biopsy - GS values are statistically significantly higher in the group with PSAD values above 0.15. The same correlation is present between the PSAD values and the post- RP GS levels. These facts correspond to the previously stated result that a higher PSA correlates with a lower GS. PSAD has this advantage over PSA in that it eliminates prostate volume, which also affects PSA levels — as PSAD provides the amount of PSA produced by a gram of tissue. According to the recommendations of the European Urological Society PSAD is one of the main indicators determining whether a patient is suitable for active surveillance - in the presence of low-risk PCa based on the EAU risk groups. The threshold indicated there is also 0.15 ng / ml / cm ^{3}, above which the risk of having a poorly differentiated PCa increases. On the other hand, PSAD is not a commonly used indicator in the clinical practice, because it is more difficult to calculate - we must first know the volume of the prostate. The volume itself is usually calculated after an ultrasound of the prostate (CT is probably a more accurate method, but less commonly used) and is also subject to errors and deviations.

e. Presence of a palpable nodule in the prostate (stage T2) - No statistically significant relationship was found between the main groups and the presence of a palpable nodule in the prostate.

f. GS after biopsy - the values of GS after biopsy of the patients in the first group are statistically significantly higher than those of the patients in the second group and statistically significantly lower than those of the patients in the third group. The GS values of the patients in the second group were statistically significantly lower than those of the patients in the third group. This result is one of the most common in the publications on the change of GS after RP (compared to that of the biopsy). The authors

traditionally point out that low baseline GS (from biopsy) is in itself a risk factor for subsequent increase in GS after RP- the exceptions are the two publications in Bulgarian mentioned in the literature review. They state that in biopsy-GS 7, 8 and 9, there is more often a subsequent increase in GS after RP-results, which is not supported by our analysis. There is a particularly high risk of an increase in very low baseline GS (2 to 4) - there only in 15% of patients there is a match between GS from the biopsy and from the RP. As a result, some authors draw attention to the tendency less and less to be reported after a biopsy GS from 2 to 4 - in 1991 24% of pathological reports contained GS 2-4; in 2001 - only 2.4%. Finally, in 2005 and 2014, The International Society of Urological Pathology (ISUP) recommended that Gleason Grade 1 and 2 not to be reported after biopsy, and the GS itself is modified in the ISUP classification. Thus, by eliminating the low levels, an attempt was made to solve the problem of the increase in GS after the biopsy. All this is a consequence of the discrepancy between the GSs from the biopsy and from the operation, and the unclear reasons why this is happening.

g. Analysis of the cases when the same pathologist examines the material from the biopsy and from the operation - A statistically significant relation was found between the examination of the specimens by the same pathologist and the patients' distribution in one of the three main groups. The chance of a patient whose materials (from the biopsy and from the operation) are analyzed by the same pathologist to be from the second group is 8 times higher than that the patient to be from the first and about 4 times higher than that the patient to be from the third group. The reason for this result is unclear - the logic suggests that if the same pathologist examines the biopsy-specimen and the material from the operation, he/she will probably be inclined to confirm the previous examination and the patient will fall into the first main group (with the same GS from the biopsy and from the RP). This is also shown by the data from the literature. In our practice, however, it turns out that pathologists often increase the rate of GS after RP. It should be noted that this result is based on an insufficiently large sample.

The result is similar when the patient is biopsied and operated in the same hospital (in this case MHAT "St. Anna-Varna"). The chance of such a patient to be in the second group is 1.7 times higher than the chance to be in the third group.

h. Classification of the patients in risk groups according to EAU (high, intermediate and low risk) - no statistically significant relationship was found between the different main groups and the risk groups of EAU. However, there is a

correlation between EAU risk groups and some postoperative pathological features. Patients with seminal vesicle involvement and extraprostatic tumor extension are from the high-risk group according to EAU.

We can summarize that of the preoperative characteristics of the patients, a possible link with a change in GS after surgery shows only the low GS from the biopsy-it is a prerequisite for GS-upgrade after the RP. As mentioned above, an attempt has been made to solve this problem by summarizing the low Gleason scores in the ISUP grade system (a modification of the traditional Gleason system). It is relatively unknown in Bulgaria. In the analysis of patients according to it, we paid attention to the following issues:

a. Is there a discrepancy between the GSs from the biopsy and from the operation in this classification as well, despite the serious aggregation of the degrees? The well-differentiated PCa falls into the first grade in the ISUP system (GS from 2 to 6 inclusive). We found a coincidence with the result after RP in 46 (58.97%) patients, and in 27 (34.62%) patients there was an increase in the grade after surgery. In the classical Gleason system, the corresponding values are 32 (41%) and 34 (43.6%). That is, there is a significant improvement in accuracy. However, even in this system, despite the pronounced reduction in the number of degrees, there is a risk of underestimating the malignant potential of PCa from the biopsy result.

b. How much is the correlation between the ISUP grades and the clinical and pathological characteristics of the patients – i.e. how valid is the system so that at low levels the patient has favorable histology, and at high levels - unfavorable. Here, a relationship was found between PSA values and ISUP grades (both before and after surgery) - ISUP grades for PSA below 10 are statistically significantly lower than those for PSA above 20

There is also a correlation between ISUP grades and some post-RP pathological features - in particular seminal vesicle involvement and extraprostatic tumor extension. A statistically significant association was found between the ISUP grades of the biopsy and the presence of stage pT3a and pT3b, respectively, with a weak positive correlation. That is, patients with pT3a and pT3b have higher ISUP grades. This means that higher ISUP grades actually correspond to PCa with less favorable pathological characteristics.

In conclusion, we can say that our initial experience with the ISUP classification shows a better correspondence between the histological result of the biopsy and that after the

RP. Even here, however, there are often discrepancies, including in well- differentiated PCa, where there are many therapeutic options. Thus, the question of the factors predicting a possible increase in GS after the RP remains relevant. Also, the new classification shows a good correspondence between its degrees and the postoperative pathological characteristics of the tumor. However, an analysis of many more patients is needed to fully validate the system, including in terms of postoperative patient survival.

An analysis was also made of the pathological characteristics of the RP material, in particular the involvement of seminal vesicles, the presence of lymph node metastases, extraprostatic tumor extension and the spread of the tumor from one prostate lobe (found on biopsy) to both lobes (found after RP). The patients with the largest share for each of the considered unfavorable postoperative characteristics are in the second group (with an increase in GS after the operation). However, differences are not statistically significant.

In tumor diseases, one of the most important indicators to be analyzed is patient survival. The most accurate, of course, is OS-overall survival. It reflects both the mortality caused by the tumor and also mortality, caused by the therapeutic methods themselves. It is known, for example, that hormonal therapy for PCa increases mortality from cardiovascular disease, although it slows the development of the tumor itself. Thus, OS is not always beneficial in hormonal therapy. But with PCa, treated by surgery (RP), there is a pre-selection of patients-operated are mainly low- and moderate-risk patients, with no evidence of metastases. Thus, the OS is quite long and the analysis is difficult unless a study of very long duration is done (at least 5 years, and even better 10 years). Therefore, in the present study, we analyzed the so -called biochemical progression-free survival (BPFS), that is the time from surgery to the onset of PSA progression.

In our study, BPFS data are available in 111 (55%) patients. Only patients who underwent systematic prostate biopsy (not targeted fusion biopsy) are included in the analysis. Patients who have undergone pre- or postoperative hormonal or radiation therapy are also excluded. 16 (38.1%) patients from the first group are with biochemical progression. The time to onset of biochemical progression varies from 1 to 51 months, the average duration is 18.9 months, the median is 15,5 months. From the second group with biochemical progression are 26 (65%) patients. The time to onset of biochemical progression varies from 1 to 2 2 months, the average duration is 5,6 months, the median is 2.5 months. From the third group with biochemical progression

are 10 (34.5 %) patients. The time to onset of biochemical progression varies from 1 to 24 months, the average duration is 7.0 months, and the median is 2.5 months.

The analysis of the three main groups of patients revealed a statistically significant relationship between the distribution of patients to the main groups and the occurrence of biochemical progression. The chance of a patient in the second group to develop biochemical progression is 3 times higher than that of a patient in the first group, and the chance of a patient in the third group to develop biochemical progression is 2 times higher than that of patient from the first group - despite the fact that there is a decrease in GS from RP compared to that from the biopsy! Survival analysis with the Kaplan-Meier test shows that shorter survival without biochemical progression can be expected in patients in the second and third groups than in patients in the first group. The time to onset of biochemical progression of patients in the second group did not differ statistically significantly from that of patients in the third group. This result is consistent with other studies that show that patients with improved GS from surgery (compared to biopsy) ultimately have no improvement in survival — it is worse than that in patients whose GS is the same both from biopsy and RP. This shows that the result of a prostate biopsy also has prognostic value - although it is traditionally accepted that the result of the operation is the one that is more accurate. We can assume that the reason for this is the establishment of less differentiated PCa by biopsy, which was then omitted in the postoperative pathological analysis. Possible explanations for this are: pathologist's error, borderline cases, very small tumor. The fact that we have only analyzed patients with systematic prostate biopsy is not a disadvantage in our opinion; in fact it may even be an advantage. This is because a systematic biopsy is considered more inaccurate than a targeted one. If this "inaccurate" biopsy shows predictive possibilities, then the results of the fusion biopsy would probably be even better. This is yet to be proven in future research.

In addition to patients' belonging to the three main groups, BPFS was found to depend also on:

a. PSA - There was a statistically significant difference in PSA values for patients with and without biochemical progression. PSA values of patients_with biochemical progression are on average 7.17 higher than those without biochemical progression.

b. Gleason score - The earlier claim that biopsy GS also has prognostic value for survival (and not just the post-prostatectomy GS) was further investigated. There was a statistically significant difference in the time to onset of biochemical progression in the groups with GS from biopsy <7 and GS ≥ 7. BPFS is shorter in patients with biopsy

GS ≥ 7 by an average of about 9 months. The results are similar in the analysis of postoperative GS - a statistically significant difference in time to biochemical progression was found in the groups with postoperative GS <7 and GS ≥ 7. It is shorter in patients with postoperative GS ≥ 7 by an average of about 13 months. Longer survival without biochemical progression can also be expected in patients with biopsy GS <7 than in patients with GS ≥ 7 - Kaplan-Meier test. Patients with biopsy GS <7 are 2 times more likely to survive without biochemical progression than patients with GS ≥ 7. Similarly, patients with postoperative GS <7 can expect longer survival without biochemical progression than patients with GS ≥ 7.

c. Postoperative pathological characteristics - presence of extraprostatic tumor extension (pT3a), seminal vesicle involvement (pT3b) and lymph node metastases (pN1). The chance of a patient with pT3b to develop biochemical progression is about 1.3 times higher than that of a patient without pT3b. The time to biochemical progression was then analyzed and statistically significant time differences were found between patients with and without pN1, pT3a and pT3b. These indicators are also prognostic in terms of survival without biochemical progression. In patients with pT3b, pN1 and pT3a, a shorter period to the onset of biochemical progression can be expected than in patients without pT3b, pN1 and pT3a-Kaplan-Meier test.

In summary, BPFS depends on both biopsy and postoperative GS. An interesting question is whether we should always consider GS from the biopsy when assessing the risks of disease progression in a particular patient. According to the literature, if the doctor has a detailed pathological report from the RP and the corresponding prognostic algorithm (nomogram), it is probably not necessary to take into account the GS from the biopsy. Adding it to this algorithm does not significantly increase its prognostic accuracy. Otherwise, however, GS from prostate biopsy is an easily accessible and important indicator for assessing the risk of disease progression in a particular patient.

An analysis of the overall survival (OS) of the patients in the three main groups was also performed. According to the available data, the survival of patients in the first group is the longest, followed by those in the second group, and the survival of patients in the third group is the lowest. The differences are statistically significant - The chance of a patient from the second group to die is 7 times higher than that of a patient from the first group. The chance of a third group patient to die is 10 times higher than that of a first group patient. Nevertheless, the analysis of survival in order to assess the prognostic value of the three main groups showed that belonging to the main group has no statistically significant prognostic value in terms of overall survival - probably due

to the small number of cases. As already explained, this is related to the pre-selection of patients undergoing RP - this should be patients with an expected survival of at least 10-15 years. That is, to obtain a sufficient number of patients suitable for analysis of overall survival we must conduct a study lasting at least 15 years. However, it is interesting and indicative that the patients in the third group (with a decrease in GS after RP) do not actually have improved OS, although their GS improves compared to the biopsy. This corresponds to the previously mentioned data that BPFS is lower in the second and third main groups and highest in the first group (where there is no change in GS from the biopsy and from the RP).

In the analysis of the patients with metastases after RP it was found that out of 203 operated patients in 9 cases there are metastases - 6 of them are in the second main group, one in the first and two in the third. The small number of patients with metastases (as well as in the analysis of overall survival) is due to the pre-selection of patients. Actually, the fact that we have few patients with metastases in the course of follow-up is in itself indicative of a good initial selection. The mean time to metastasis is:

19 months - in the group without change in GS

16.5 months - in the group with decrease in GS

18.7 months in the group with an increase in GS, but only 12.8 months in 5 of the 6 cases (there is one case with a very long survival).

The number is too small to draw statistically significant conclusions. However, there is a tendency for more metastases in the second major group. Survival without metastases is highest in the first major group. Again, a third major group has no clear advantage, despite the improvement in post-operative GS.

An analysis was also made of the patients who underwent fusion biopsy of the prostate - a total of sixteen (out of 203 patients). In 80% of cases (8 out of 10 patients) the side of the tumor is correctly determined. There is a coincidence in GS (from the biopsy and from the RP) in 7 of 16 patients (43.75%) - they fall into the first main group, five are in the second group (31.25%) and four - in the third (25%). When performing the standard systematic biopsy, 34.48% of the patients fall into the first group, 31.03% into the second group, and 34.48% into the third group. Obviously, there is a tendency, when targeted biopsy is performed, the patients in the first main group to be more than the patients in the other two groups– i.e. for greater coincidence of the GS from the biopsy with that of the RP. However, the number of patients is extremely insufficient

to obtain statistically significant differences. With the increasing use of MRI and targeted biopsy, we hope to gather enough material for future research on this topic. According to the literature, systemic biopsy is more likely to lead to underestimation of GS than the targeted biopsy, which more accurately identifies the final GS (determined by prostatectomy).

7. Implications

From the analysis made so far we can draw the following conclusions:

1. There is a trend to increase the number of prostatectomies performed each year. Only in 34.48% of cases there is a coincidence of the Gleason score from biopsy and radical prostatectomy. In the remaining patients there is either an increase in Gleason score (31.03%) or a decrease (34.48%).

2. In the analysis of patients with well-differentiated prostate cancer (Gleason score up to 6 inclusive from the biopsy) it was found that increased PSA density and small prostate volume are statistically significant prognostic factors for possible increase in Gleason after radical prostatectomy.

3. Low Gleason score from biopsy is a major risk factor for its subsequent increase after radical prostatectomy.

4. The analysis of the preoperative characteristics of the operated patients revealed the following relations indicative of high-risk prostate cancer:

• A statistically significant association is found between PSA and the Gleason scores (both from biopsy and after radical prostatectomy) - higher PSA is a risk factor for the presence of poorly-differentiated prostate cancer.

• Gleason score from the biopsy is statistically significantly higher in patients with a PSA density more than 0.15 ng/ml/cm^3.

• Patients in the high-risk group according to the EAU classification have more frequent seminal vesicle involvement and/or extraprostatic tumor extension postoperatively.

5. In the analysis of patients with the new classification according to ISUP (modified Gleason system) a better correspondence was found between the result of the biopsy and that of the operation (58.97% according to ISUP compared to 41% according to

the old system - in well-differentiated carcinomas). However, even here in 34.62% of the patients there is an increase in the degree after surgery. There is also a statistically significant association between the ISUP grades of the biopsy and the presence of pT3a and pT3b stages after surgery with a positive correlation.

6. Survival without biochemical progression is highest in patients without Gleason score change after radical prostatectomy. The time to onset of biochemical progression in patients with increased Gleason score postoperatively did not differ statistically significantly from that in patients with decrease of the Gleason score. This shows that the Gleason score from the biopsy also has prognostic significance - not just the Gleason score from the radical prostatectomy.

7. An analysis of patient survival revealed the following relations:

• Patients with Gleason score ≤ 6 have a longer survival without biochemical progression than patients with Gleason score ≥ 7, regardless of whether Gleason score is determined by biopsy or after surgery.

• Patients with lower PSA have a longer survival without biochemical progression.

• Patients with extraprostatic tumor extension (pT3a), seminal vesicle involvement (pT3b) and lymph node metastases (pN1) have a shorter survival without biochemical progression.

8. Conclusion

Prostate cancer is the most common non-malignant disease in men. Its frequency has increased especially since the introduction of PSA screening in the 1990s. This determines the great social significance of this cancer. Along with the increased number, the structure of newly registered patients is gradually changing - in the past these were people with complaints of urination and/or symptoms of tumor metastases. Currently, the majority of patients are asymptomatic, with a small tumor limited to the prostate-found only on the basis of elevated PSA. Diagnosis must include histological examination (after prostate biopsy) to determine the degree of malignancy (i.e. the degree of differentiation) of the tumor according to the Gleason system. Henceforth, the course (and thus the possible treatment) of PCa is very different in individual patients. Observing the natural course of the disease, it has long been established that it can be presented in two very different variants. Either as a relatively harmless tumor that is successfully treated and the man dies after years **with** prostate cancer, but not **of**

prostate cancer. Or as a severe malignant disease, which, despite timely treatment, progresses rapidly, leading to bone metastases, pathological fractures with disability of the patient and intolerable pain with subsequent death of the patient. The main goal of doctors is to determine which variant of the tumor the patient is facing. Accordingly, today there are many treatment options - starting with active surveillance (in order to reduce the side effects of the treatment itself) and leading to multimodal treatment with a combination of radical prostatectomy, radiation and hormone therapy (for the most aggressive tumors).

As noted, the main indicator for determining the degree of malignancy of PCa is GS. It is examined after prostate biopsy and, in patients undergoing RP, it is examined a second time after surgery. Often the two GSs diverge, which is the subject of this study. It was found that the coincidence of the two GSs occurs in only 34.48% of cases. In the others there is either an increase in GS after the operation or a decrease. The increase in GS after RP is obviously very worrying, because it shows that in the biopsy we have underestimated the malignant potential of the tumor. Which raises the difficult question - among the patients we have allocated to active surveillance, are there any patients with underestimated GS? Accordingly, we attempted to identify the factors that may lead to a possible increase in GS after RP - namely the increased density of PSA, small prostate volume and low baseline GS.

The reduction of GS after the RP is at first sight a favorable sign, because it is traditionally accepted that the postoperative GS is the final and most accurate. However, the analysis of the survival of the patients in the group with improved GS showed that they had a statistically significantly worse survival compared to those with unchanged GS. This means that the GS from the biopsy is an independent prognostic factor and its improvement after the RP should not reassure us. Therefore, the GS from the biopsy should also be taken into account when determining the postoperative treatment after the RP.

In general, PCa is a heterogeneous disease, the assessment of which must take into account many factors. In the analysis we looked at how the level of PSA, density of PSA, GS, age of the patient, prostate volume, baseline GS (from prostate biopsy) affect the pathological characteristics of PCa (invasion of seminal vesicles, extraprostatic extension of tumor, lymph node metastases). We also studied the influence of some factors on patient survival. Thus, we tried to convey a more accurate and complete picture of this disease in order to optimize its treatment.

9. Contributions

1. The cases with change of Gleason score were studied on the basis of our own clinical material, the preoperative characteristics of the patients and the pathological features after radical prostatectomy were examined. The survival of patients was studied, both overall and without biochemical progression, as well as the time to occurrence of metastasis. With the help of uni- and multivariate statistical analysis we studied the relationship between patient characteristics and Gleason score change after radical prostatectomy.

2. The fact that the Gleason score from the biopsy has also prognostic value, and not only the Gleason score from the radical prostatectomy, is considered a confirmatory contribution. This was established by analyzing the survival of patients with Gleason score change after radical prostatectomy.

3. It was also confirmed that the low baseline Gleason score (from biopsy) is a major risk factor for subsequent increase in Gleason score after radical prostatectomy.

4. The new ISUP grade system (modified Gleason system), which is still little known in Bulgaria, was used in the analysis of patients. We found a better coincidence between the histological result of the biopsy and that from the operation compared to the older system.

5. As a practical contribution is considered that in patients with well- differentiated prostate cancer (Gleason score up to 6 inclusive) increased PSA density (above 0.15 ng/ml/cm ^{3}) and small prostate volume are statistically significant prognostic factors for a possible increase in Gleason score after radical prostatectomy.

Publications related to the topic of the dissertation:

Lazarov BI (2022) The application of the ISUP-classification in the analysis of patients with prostate cancer. Clinical urology, 2(1), 5-9.

Lazarov B, Ganev T, Statelov T, Kosev P (2020) Comparison of Gleason score of prostate biopsy and radical prostatectomy. Uronet, 2(2020), 19-20.

Summary

Prostate cancer is the second most common cancer in men. After the introduction of PSA most of the tumors are discovered in an early stage and hence there are many different treatment options. The treatment depends on the disease stage and on the risk assessment of the cancer. The pathological characteristics of the tumor are one of the most important elements of this risk assessment — it is done according to the Gleason grading system. In patients who have undergone radical prostatectomy the Gleason score (GS) is examined twice-after the biopsy and after the operation. A well-known fact is that the two GSs very often do not coincide. Many investigators are concerned with the fact that after radical prostatectomy the GS can increase. This is of course important because some patients are treated with deferred treatment — either active surveillance or watchful waiting. If their GS is underestimated during the biopsy this may have a negative impact on the survival of the patients. That is why we have tried to find factors which may suggest a possible increase of the GS after the operation.

When the GS decreases after the radical prostatectomy the prognosis seems quite favorable because the postoperative pathological report is considered more accurate. Nevertheless there are data that patients without changes in GS after the operation actually have the best prognosis — not the patients with decrease. Analyzing the operated patients in our hospital we tried to check this hypothesis which, if proven correct, would mean that the GS of the biopsy also has prognostic significance.

All the patients had prostate cancer proven with transrectal biopsy of the prostate, performed between 01Jan.2013 and 31Dec.2021. The GS of the biopsy was collected. The patients underwent radical prostatectomy (either open or laparoscopic). The second GS (from the operation) was also collected and compared with the first one. Different perioperative characteristics of the patients (e.g. PSA, PSA-density, prostate volume etc.) were also collected and analyzed.

The research is based on patients who have undergone radical prostatectomy at MHAT Sveta Anna-Varna and presents our experience in the diagnosis and treatment of prostate cancer.