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Research Article

Significance of Tumour Biological Markers for Overall Survival and Clinical Parameters for Disease-Free Survival in Patients with Prostate Cancer after Radical Prostatectomy

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ABSTRACT

Objective: The aim of the study was to assess the prognostic significance of pretreatment serum PSA level, immunohistochemical expression (labelling index, LI) of Ki-67, prostate-specific membrane antigen (PSMA), glucose-1 transporter (GLUT-1), vascular endothelial growth factor (VEGF), human telomerase reverse transcriptase (hTERT), as well as micro vessel density (MVD) for overall survival (OS), local recurrence-free survival (LRFS), metastatic-free survival (MFS) and disease-free survival (DFS) in a group of 130 prostate cancer (PCa) patients treated with radical prostatectomy (RP) between 2007 and 2011.

Methods: In order to investigate the prognostic value of the analyzed variables in univariate and multivariate Cox analysis, the patients were divided into two subgroups based on the marker cut-off points selected by the receiver operating characteristic curves.

Results: There were 83 (63.8%) cases staged pT1-2 and 47 (36.1%) staged pT3-4. The majority of tumours (53.1%) were well-differentiated (grade group G1), 49 (37.7%) moderately differentiated (G2-3) and 12 (9.2%) poorly differentiated (G4-5). In 85 patients (65.4%) the surgery was radical, 59 (45.4%) had positive surgical margins (PSM), and in 23 (17.7%) seminal vesicle(s) involvement was diagnosed. Median follow-up was 79 (1-148) months, during which 55 (42.3%) men died, in 12 (9.2%) local relapse and in 13 (10%) distant metastases occurred. In multivariate Cox analysis, independent negative prognostic factors for OS were: Ki-67LI >6.7% (p=0.010), PSMALI ≤51.7% (p=0.009), hTERTLI ≤20.5% (p=0.004) and lack of adjuvant treatment (AT) (p=0.013), while for MFS: seminal vesicle(s) involvement (p=0.007) and hTERTLI ≤43.8% (p=0.011). However, non-radical surgery (p=0.007), PSM (p=0.005) and AT (p=0.037) were negatively associated with DFS.

Conclusion: Tumour biological markers are significant for OS and clinical parameters for DFS in PCa patients' after RP.

Introduction

Prostate cancer (PCa), the most common malignancy in men, is characterized by intratumoural heterogeneity and variable clinical course. Traditionally, for the purpose of patient management, pretreatment serum prostate-specific antigen (PSA) level, clinical stage, Gleason score, and histological subtype are established. However, there are remarkable differences in the biological behavior of cancers of the same grade and stage, as clinical prognostic grouping for localized PCa is imprecise. Therefore, reliable distinction between indolent and aggressive PCa prior to treatment implementation is not achievable [1]. In early-stage PCa, both radiotherapy (RT) and radical prostatectomy (RP) are approved primary treatment modalities. However, RP remains the gold standard for curative treatment of PCa, because it significantly

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reduces mortality, local progression risk and the onset of distant metastasis [2]. We do not currently have accurate, non-invasive biomarkers to help determine the aggressiveness of PCa and select cases requiring adjuvant intervention.

After the analyses of prognostic significance of particular biomarkers assessed immunohistochemically, for example prostate-specific membrane antigen (PSMA) (which is a type II transmembrane glycoprotein), and their prediction for disease recurrence following curative therapy, gene expression profiles have been widely scrutinized in order to develop expression signatures for the prediction of biochemical recurrence or clinical outcome [3]. Very few clinically applicable expression signatures have been developed, including Prolaris (46-gene test) from Myriad Genetics Inc., 22-gene test from Decipher Inc., and Genomic Prostate Score (17-gene test) from Oncotype DX, however, all of them still leave great room for improvement. The difficulty in establishing prognostic markers based on PCa tissue could be due to the heterogeneity of the tumours and the offsetting expression of signature genes in the tumour vs. its microenvironment, which makes it difficult to utilize these gene markers in a mixture of neoplastic and stromal cells [4].

It has been assumed that proteins (products of gene transcription) directly reflect functions of the genes which play critical roles in biological processes *in vivo*. However, there is not always a correlation between protein and mRNA expression. Therefore, the quantification of proteins might be better correlated with the phenotypes of interest, including cancer aggressiveness, which was confirmed by recent analysis of bone metastases in PCa patients [5]. Based on gene expression profiles and immunohistochemical (IHC) analysis of selected proteins expression, as well as histological and clinical parameters, three molecular subtypes of bone metastases (MetA-C) with different morphology, biology, and clinical outcome were identified [5]. Therefore, predictive models based on the progression-associated antigens (protein markers and IHC) may potentially have an increased prognostic power compared to the models that are solely based upon gene expression profiles [4].

In PCa patients, the prognostic significance of serum PSA and other biomarkers, including Ki-67, PSMA, glucose-1 transporter (GLUT-1), vascular endothelial growth factor (VEGF), human telomerase reverse transcriptase (hTERT) expression and microvessel density (MVD) has not been clinically confirmed. Therefore, the purpose of the study was to assess the prognostic value of the analysed biomarkers for overall survival (OS), local recurrence-free survival (LRFS), metastatic-free survival (MFS) and disease-free survival (DFS) in PCa patients subjected to RP and identify markers that may be used as surrogate end points for clinical progression.

Material and Methods

I Patients

The studied population consisted of a retrospective cohort of 130 PCa patients who underwent RP between 2007 and 2011, and in whom both pretreatment serum PSA level and archival tissue sections were available. In all cases clinical (cTNM) and pathological (pTNM) stage was assessed, as well as Gleason score, according to the current American Joint Committee on Cancer (AJCC) guidelines. The study protocol was approved by the Ethical Committee of the Centre of Oncology and each patient submitted written consent. Adjuvant treatment (AT) was delivered to 53 (40.8%) patients; 26 (20.0%) patients received RT, 11 (8.5%) – hormonotherapy (HT), 12 (9.2%) – both RT and HT, while 4 (3.1 %) patients – RT, HT and chemotherapy (CHT) (Table 1).

Table 1: Clinicopathological characteristics of 130 PCa patients treated with radical prostatectomy.

Variables	Total N = 130 (%)
Median age, years (range)	(130) 62.0 (49-77)
cT stage	
T1	11 (8.5)
T2A	14 (10.8)
T2B	57 (43.8)
T3	37 (28.5)
T4	10 (7.7)
Tx	1 (0.8)
pT stage	
pT1	12 (9.2)
pT2	71 (54.6)
pT3	38 (29.2)
pT4	9 (6.9)
Histological grade AJCC	
1	69 (53.1)
2-3	49 (37.7)
4-5	12 (9.2)
Gleason Score	
≤ 6	67 (51.5)
7	50 (38.5)
≥ 8	13 (10.0)
Radicality of surgery	
Yes	85 (65.4)
No	45 (34.6)
Hist-pat surgical margins	
Negative	71 (54.6)
Positive	59 (45.4)
Seminal vesicle(s) invasion	
Negative	107 (82.3)
Positive	23 (17.7)
Adjuvant treatment	
None	77 (59.2)
Radiotherapy (RT)	26 (20.0)
Hormontherapy (H)	11 (8.5)
RT + H	12 (9.2)
RT + H + chemotherapy (CHT)	2 (1.5)
antiLH-RH[a]	1 (0.8)
LH-RH + RT	1 (0.8)
Follow-up time (months)	
Median (range)	79.0 (1 – 148)
Local recurrences	12 (9.2)
Median time, months (range)	29 (5-92)
Metastases	13 (10.0)
Median time (range)	46 (11-88)

[a]Luteinizing hormone-releasing hormone.

II Immunohistochemical Analysis and Scoring

Protein expression was evaluated immunohistochemically on histological specimens, using the suitable antibody and BrightVision visualization system (ImmunoLogic). For Ki-67 visualization we used a mouse anti-Ki-67 monoclonal antibody (clone MIB-1, DAKO, 1:75), for GLUT-1 a rabbit monoclonal antibody (Millipore, 1:300), for CD34 a mouse anti-human monoclonal antibody (DAKO, 1:200), for VEGF a mouse anti-VEGF monoclonal antibody (DAKO, 1:25), for hTERT a rabbit polyclonal antibody to human telomerase (Novus Lab. Biologicals, Littleton, USA, 1:300) and, finally, for PSMA a mouse anti-human antibody (Novocastra, Newcastle, United Kingdom) (1:200), diluted in TRIS-buffered saline (pH=7.4) as described earlier [6, 7]. Proteins expression was presented as a number of positively staining cells (labelling index, LI), with nuclear staining for Ki-67, nuclear and cytoplasmic for hTERT, membranous for PSMA, CD34, membranous/cytoplasmic for GLUT-1, and cytoplasmic staining for VEGF considered positive. Micro vessel density was measured as a mean CD34-positive vessel count per 1 mm^2 of tumour volume. The slides were evaluated by two investigators unaware of the clinicopathological variables.

III Evaluation of Follow-Up

Overall survival (OS) was analyzed from the date of surgery until death from any cause. Local recurrence-free survival (LRFS) was defined as survival free of locoregional relapse occurring at any time before systemic recurrence. Metastatic-free survival (MFS) – survival free of systemic recurrences identified using radiographic studies (computed tomography, CT; magnetic resonance imaging) and/or nuclear imaging (bone scans), and/or visceral (liver, lung, brain) or extra-pelvic nodal metastases visualized on CT scans. Disease-free survival (DFS) was defined as the interval from the date of surgery to the first recurrence, either locoregional or systemic.

IV Statistical Analysis

Statistical analysis was performed with STATISTICA 12 software (StatSoft Inc. Tulsa, OK, USA). To determine mean values of variables and standard errors of means (SE) we used the descriptive statistics. One-way ANOVA test or Student's t-test were applied to test intergroup differences in the mean values. Associations between investigated categorical parameters and clinicopathological variables were evaluated by Pearson's Chi^2 test.

The correlations between proteins expression were tested with Pearson correlation and between proteins expression and other variables with Spearman rank test. For sensitivity and specificity of each marker, receiver curve analyses (ROC curves) were performed. For the analyzed end points, prognostic factors were stratified into two groups based on cut-off points for each variable, optimized by ROC curve and the areas under the curve (AUC). Univariate- and multivariate-adjusted relative risks (RRs) and 95% confidence intervals (CIs) estimated using Cox proportional hazards regression model were applied to determine prognostic factors for OS, LRFS, MFS and DFS. Survival was estimated using the Kaplan-Meier method and tested by the log-rank test. In multivariate analysis, clinical and biological variables were tested together. Statistical significance was considered at *p* value of 0.05.

Table 2: Pre-treatment PSA level and examined proteins expression for two groups of patients having negative or positive clinical outcome after radical prostatectomy.

Characteristics	Total Mean ± SE	Seminal vesicle(s) invasion Mean (%) ± SE		Radicality of surgery Mean (%) ± SE		Positive surgical margin Mean (%) ± SE		Local recurrence Mean (%) ± SE		Metastasis Mean (%) ± SE	
		Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
	N = 130	N= 107	N = 23	N= 45	N = 85	N= 71	N = 59	N= 118	N = 12	N= 177	N = 13
PSA ng/mL	9.9 ± 0.5	9.4 ± 0.5	12.2± 1.6[a]	11.7±1.1	8.9±0.5[b]	9.0±0.5	10.9±0.9[c]	9.8 ± 0.5	10.8 ± 1.0	9.7± 0.5	11.5 ± 1.2
Ki-67 LI (%)	8.1 ± 0.6	7.0 ± 0.6	12.9± 2.0[d]	9.7±1.3	7.2±0.6[e]	7.6±0.7	8.6±1.0	7.9 ± 0.6	9.6 ± 1.6	7.7 ± 0.6	11.7± 2.8[f]
GLUT-1LI (%)	30.4± 2.1	29.1± 2.2	36.0± 5.3	30.9±3.8	30.1±2.5	32.9±2.6	27.3±3.3	29.9± 2.2	35.1 ± 7.3	30.0± 2.2	33.5 ± 5.8
VEGF LI (%)	15.1± 1.4	14.9± 1.6	15.7± 2.8	13.7±1.8	15.8±1.9	15.5±2.0	14.6±2.0	15.3± 1.5	12.6 ± 3.6	15.5± 1.5	11.3 ± 2.8
MVD/mm^2	97.1± 2.5	95.3± 2.6	105.4 ±6.6	92.2±3.2	99.7±3.2	102.7±3.5	90.3±3.3[g]	97.0± 2.6	97.9 ± 9.1	97.5± 2.6	93.4 ± 6.1
PSMA LI (%)	44.6± 2.0	43.1± 2.2	51.7± 4.1	48.3±3.1	42.7±2.5	43.0±2.7	46.6±2.8	44.1± 2.0	50.3 ± 8.4	44.6± 2.0	45.2 ± 7.2
hTERT LI (%)	56.5± 1.6	57.1± 2.9	53.5±5.3	53.3±4.6	58.2±3.0	62.1±3.4	49.7±3.7[h]	56.5± 2.6	56.3 ± 9.3	58.5± 2.6	38.7 ± 7.9[i]

[a]$p = 0.025$, [b]$p = 0.006$, [c]0.048, [d]$p < 0.001$, [e]$p = 0.048$, [f]$p = 0.048$, [g]$p = 0.012$, [h]$p = 0.015$, [i]$p = 0.019$. Differences between positive and negative subgroups (*t*-test).

Results

A total of 130 consecutive patients were included in the study. Mean age in the entire group was 62.0 (range: 41-77) years. Clinical and pathomorphological characteristics of the analyzed group is presented in (Table 1). There were 82 (63.1%) cases staged pT1-2 and 48 (36.9%) staged pT3-4. The majority (53.1%) of tumours were well-differentiated (grade group G1), 49 (37.7%) were moderately differentiated (G2-3) and 12 (9.2%) poorly differentiated (G4-5) (Table 1). The median age was

62.0 years (range: 49-77). In 85 (65.4%) patients the surgery was radical, while in 59 (45.4%) the histopathologist found positive surgical margin (PSM). In 23 (17.7%) cases seminal vesicle(s) involvement was observed (Table 1). Six patients (4.6%) had second malignancy.

I Biological Markers

The mean PSA level was 9.9 ng/mL. Ki-67LI, GLUT-1LI, VEGFLI, MVD, PSMALI and hTERT were 8.1%, 30.4%, 15.1%, 97.1 vessels/mm^2, 44.6%, and 56.5%, respectively (Table 2). PSA (p=0.038), Ki-67 (p=0.021), PSMA (p<0.001) showed positive, while hTERT negative (p<0.001) correlation with tumour grade. Furthermore, PSA (p=0.002), GLUT-1 (p=0.039) and PSMA (p<0.001) were positively associated with pT, whereas PSA was correlated only with Ki-67LI (p=0.014). Patients with seminal vesicle(s) involvement had significantly higher PSA level and Ki-67LI, and the same was true in patients after non-radical surgery (Table 2). Positive surgical margin was found in patients with significantly higher PSA level, lower MVD and hTERTLI (Table 2). For local recurrence each marker was predictive, however, higher Ki-67LI and lower hTERTLI were important prognosticators for metastasis (Table 2).

II Overall Survival, Local Recurrences and Metastases

Patients were followed up for an average of 79 (1-148) months, during which 55 (42.3%) died. In 12 (9.2%) patients local recurrences were diagnosed, and in 13 (10%) – distant metastases (in 6 to bones, in 5 to lymph nodes, in 2 to soft tissues, nodes and bones), which occurred on average 33.7 months and 41.8 months from surgery, respectively (Table 1). The median survival time was 102 months. Five- and 10-year OS probability were 78.8% and 43.3%, respectively. Median LRFS and MFS were not reached. Among men with local recurrence and metastasis 5- and 10-year LRFS and MFS were similar and equal to 91.0% and 88.2%, respectively.

III Adjuvant Treatment

After RP, 53 (40.8%) patients were treated with AT. Median OS in men who received AT was 108 months and in those without AT was 90 months, however, in log-rank test the difference between these two groups appeared to be statistically insignificant (p=0.072). In the group receiving AT, the biggest subgroup (20%) was treated with RT (Table 1), whereas the smallest with anti-androgen therapy, where steroidal or non-steroidal androgen receptor antagonists were given to block androgen-mediated growth and survival signaling. The highest 5-year OS (100%) was observed in men treated with adjuvant RT+HT+CHT or luteinizing hormone-releasing hormone (LH-RH) +RT, intermediate (84%) in those treated with RT, and the lowest (0%) in those receiving anti LH-RH treatment. However, there was no statistical difference in OS between the treatment subgroups (p=0.212). Late surgical treatment effect was measured by the results of the analysis of correlations between biological and clinicopathological variables and OS, LRFS, and MFS.

Table 3: Cox proportional hazards analysis of the biological and clinicopathological features of prostate cancer predicting OS in localized PCa patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	p value*	RR	95% CI	*p* value
Age (years)						
≤ 59.0	1.00	Reference				
> 59.0	1.33	0.72-2.44	0.356			
PSA						
≤ 9.2 ng/mL	1.00	Reference				
> 9.2 ng/mL	1.37	0.81-2.33	0.239			
AJCC Grade						
1-3	1.00	Reference				
4	1.55	0.66-3.61	0.313			
Clinical stage						
cT1-2	1.00	Reference	0.363			
cT3-4	1.28	0.75-2.19				
Pathological stage						
pT1-2	1.00	Reference				
pT3-4	1.28	0.75-2.19	0.357			
Radical surgery						
yes	0.70	0.41-1.19	0.186			
no	1.00	Reference				
Hist-pat surgical margins						
negative	1.00	Reference				
positive	1.16	0.68-1.97	0.590			
Seminal vesicle(s) invasion						
negative	0.92	0.45-1.89	0.833			

positive	1.00	Reference				
Adjuvant treatment						
no	1.00	Reference	0.078	1.00	Reference	
yes	0.61	0.35-1.06		0.48	0.27-0.86	0.013
Ki-67LI						
≤ 6.7 %	1.00	Reference		1.00	Reference	
> 6.7 %	1.58	0.93-2.68	0.089	2.04	1.18-3.54	0.010
GLUT-1LI						
≤ 1.4 %	1.00	Reference				
> 1.4 %	2.78	1.00-7.73	0.049			
VEGF LI						
≤ 16.3 %	1.00	Reference				
> 16.3 %	0.63	0.36-1.12	0.119			
MVD						
≤ 84.2/mm^2	1.00	Reference				
> 84.2/mm^2	1.23	0.69-2.21	0.470			
PSMA LI						
≤ 51.7 %	1.00	Reference		1.00	Reference	
> 51.7 %	0.47	0.26-0.87	0.016	0.43	0.23-0.81	0.009
hTERT LI						
≤ 20.5 %	1.00	Reference		1.00	Reference	
> 20.5%	0.49	0.27-0.91	0.023	0.39	0.21-0.75	0.004

RR: risk ratio, CI: confidence interval.

Table 4: Cox univariate analysis of the biological and clinicopathological features of prostate cancer predicting Local recurrence-free survival in localized PCa patients.

Variable	Univariate analysis		
	RR	95% CI	*p* value*
Age (years)			
≤ 71.0	1.00	Reference	
> 71.0	1.85	0.40-8.48	0.426
PSA			
≤ 7.7 ng/mL	1.00	Reference	
> 7.7 ng/mL	4.32	0.95-19.75	0.06
AJCC Grade			
1-3	1.00	Reference	
4-5	1.05	0.14-8.20	0.957
Clinical stage			
cT1-2	1.00	Reference	0.715
cT3-4	1.23	0.39-3.91	
Pathological stage			
pT1-2	1.00	Reference	
pT3-4	2.55	0.56-11.6	0.228
Radical surgery			
yes	0.49	0.15-1.53	0.222
no	1.00	Reference	
Hist-pat surgical margins			
negative	0.75	0.26-2.61	0.748
positive	1.00	Reference	
Seminal vesicle(s) invasion			
negative	1.00	Reference	
positive	1.49	0.41-5.53	0.546
Adjuvant treatment			

no	1.00	Reference	
yes	4.17	1.12-15.4	0.032
Ki-67LI			
≤ 9.2 %	1.00	Reference	
> 9.2 %	2.87	0.93-8.91	0.07
GLUT-1LI			
≤ 16.0 %	1.00	Reference	
> 16.0 %	1.94	0.53-7.2	0.316
VEGF LI			
≤ 4.3 %	1.00	Reference	
> 4.3 %	0.79	0.25-2.49	0.689
MVD			
≤ 77.2/mm^2	1.00	Reference	
> 77.2/mm^2	0.38	0.12-1.20	0.100
PSMA LI			
≤ 53.3 %	1.00	Reference	
> 53.3 %	1.73	0.56-5.37	0.342
hTERT LI			
≤ 14.0 %	1.00	Reference	
> 14.0 %	0.37	0.08-1.69	0.198

RR: risk ratio, CI: confidence interval.

Univariate and Multivariate Analysis for Overall Survival, Recurrence-free, Metastasis-free and Disease-free Survival

I Overall Survival Risk Factors

In univariate Cox analysis, independent negative prognostic factors for OS were: GLUT-1LI >1.4%, PSMALI ≤51.7%, or hTERTLI ≤20.5%. However, in multivariate analysis, positive prognostic factors appeared to be: AT, lower Ki-67LI, higher PSMALI and hTERTLI (Table 3).

II Local Recurrence Risk Factors

Univariate analysis revealed that among all analyzed clinical, histological and biological parameters only AT had negative influence on LRFS (shorter time to LR, p=0.032) (Table 4). As multivariate analysis could not be performed due to low number of censored variables**,** DFS analysis was done.

III Metastasis-free Survival Risk Factors

In univariate analysis six variables: higher PSA level (p=0.020), higher grade (p = 0.041), pT (p=0.048), seminal vesicle(s) invasion (p=0.010), and higher Ki-67LI (p=0.017) or lower hTERTLI (p=0.015) were negative prognostic factors for MFS (Table 5). However, in multivariate analysis only seminal vesicle(s) involvement (p=0.007) and lower hTERTLI (p=0.015) were negative prognosticators for MFS (Table 5).

IV Disease-free Survival Risk Factors

In univariate analysis, higher PSA level (p=0.027), higher pT stage (p=0.005), seminal vesicle(s) invasion (p=0.013), AT (p=0.020), and higher Ki-67LI (p=0.027) were negative prognostic factors for DFS (Table 6). However, in multivariate analysis, only non-radical surgery (p=0.007), PSM (p=0.005), and AT (p=0.016) appeared to be independent negative prognosticators for DFS.

Table 5: Univariate and multivariate Cox proportional hazards analysis of the biological and clinicopathological features of prostate cancer predicting MFS in localized PCa patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	p value*	RR	95% CI	p value
Age (years)						
≤ 67.0	1.00	Reference				
> 67.0	1.59	0.52-4.86	0.416			
PSA						
≤ 10.2 ng/mL	1.00	Reference				
> 10.2 ng/mL	1.32	1.22-11.47	0.020			
AJCC Grade						
1-3	1.00	Reference				
4-5	3.83	1.05-13.97	0.041			
Clinical stage						

cT1-2	1.00	Reference	0.051			
cT3-4	3.04	0.99-9.30				
Pathological stage						
pT1-2	1.00	Reference				
pT3-4	3.08	1.01-9.43	0.048			
Radical surgery						
yes	0.57	0.19-1.68	0.307			
no	1.00	Reference				
Hist-pat surgical margins						
negative	1.00	Reference				
positive	1.06	0.36-3.17	0.910			
Seminal vesicle(s) invasion						
negative	1.00	Reference	0.010	1.00	Reference	0.007
positive	4.15	1.39-12.36		4.48	1.50 – 13.34	
Adjuvant treatment						
no	1.00	Reference				
yes	2.20	0.7-6.7	0.165			
Ki-67LI						
≤ 18.0 %	1.00	Reference				
> 18.0 %	4.84	1.33-17.8	0.017			
GLUT-1LI						
≤ 19.1 %	1.00	Reference				
> 19.1 %	2.40	0.66-8.71	0.183			
VEGF LI						
≤ 11.0 %	1.00	Reference				
> 11.0 %	0.47	0.14-1.52	0.209			
MVD						
≤ 94.2/mm^2	1.00	Reference				
> 94.2/mm^2	1.10	0.34-3.57	0.876			
PSMA LI						
≤ 76.7 %	1.00	Reference				
> 76.7 %	2.72	0.60-12.30	0.193			
hTERT LI						
≤ 43.8 %	1.00	Reference		1.00	Reference	
> 43.8 %	0.23	0.07-0.75	0.015	0.22	0.07-0.71	0.011

RR: risk ratio, CI: confidence interval.

Table 6: Univariate and multivariate Cox proportional hazards analysis of the biological and clinicopathological features of prostate cancer predicting DFS in localized PCa patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	*p* value*	RR	95% CI	*p* value
Age (years)						
≤ 70.0	1.00	Reference				
> 70.0	2.25	0.83-6.06	0.109			
PSA						
≤ 8.1 ng/mL	1.00	Reference		1.00	Reference	
> 8.1 ng/mL	2.85	1.12-7.26	0.027	2.72	0.99-7.49	0.052
AJCC Grade						
1-3	1.00	Reference				
4-5	2.67	0.90-7.87	0.074			
Clinical stage						
cT1-2	1.00	Reference	0.104			
cT3-4	1.97	0.87-4.48				

Pathological stage						
pT1-2	1.00	Reference				
pT3-4	4.13	1.53-11.15	0.005			
Radical surgery						
yes	0.51	0.23-1.17	0.111	0.12	0.03 – 0.56	0.007
no	1.00	Reference		1.00	Reference	
Hist-pat surgical margines						
negative	0.90	0.39-2.07	0.817	0.09	0.02 – 0.48	0.005
positive	1.00	Reference		1.00	Reference	
Seminal vesicle(s) invasion						
negative	1.00	Reference				
positive	2.57	1.09-6.06	0.013			
Adjuvant treatment						
no	1.00	Reference		1.00	Reference	
yes	2.77	1.17-6.55	0.020	3.43	1.26-9.35	0.016
Ki-67LI						
≤ 12.8 %	1.00	Reference				
> 12.8 %	2.86	1.12-7.25	0.027			
GLUT-1LI						
≤ 16.0 %	1.00	Reference				
> 16.0 %	2.40	0.89-6.47	0.083			
VEGF LI						
≤ 16.0 %	1.00	Reference				
> 16.0 %	0.61	0.25-1.50	0.282			
MVD						
≤ 81.8/mm^2	1.00	Reference				
> 81.8/mm^2	0.60	0.26-1.38	0.228			
PSMA LI						
≤ 48.3 %	1.00	Reference				
> 49.3 %	1.84	0.81-4.20	0.147			
hTERT LI						
≤ 43.8 %	1.00	Reference				
> 43.8 %	0.48	0.21-1.10	0.083			

RR: risk ratio, CI: confidence interval.

Discussion

In comparison to similar studies, our research benefits from inclusion of tumour biological factors to the analysis of OS, LRFS, MFS, and DFS in patients after RP. Multivariate analysis revealed that tumour biological factors seemed to have better influence on survival than clinico-pathological variables, while for DFS the reverse was true.

I Overall Survival

Our study revealed that lower tumour cell proliferation (Ki67LI ≤6.7%), AT and high expression of PSMA (PSMALI >51.7%) or hTERT (hTERTLI >20.1%) had positive impact on OS. For MFS, only seminal vesicle(s) invasion or lower hTERTLI were negative prognostic factors. However, higher PSA level (borderline significance), non-radical surgery, AT or PSM appeared to be correlated with shorter DFS time. For DFS, both tumour grade and stage were important, but only in univariate analysis, which may indicate the specificity of qualification of early-stage tumours to RP or the importance of the analyzed tumour biological factors.

We showed that PSA level had no influence on OS and poorly (borderline significance) predicted progression of the disease (shorter DFS). We reported earlier the predictive power of PSA level >8 ng/mL for biochemical recurrence, what is in line with other studies [3, 8, 9]. Generally, PSA is considered a poor prognostic factor for OS, because several factors other than prostate malignancy can increase its level [10]. Hypoxia is a clinically important determinant of disease progression in PCa; it contributes to cancer aggressiveness, resistance to treatment, and dissemination [11, 12].

In our previous studies [6, 8] we showed that hypoxic tumour microenvironment correlates with increased tumour invasiveness and biochemical recurrence after RP, however, recently we have shown in univariate analysis that even low level of GLUT-1 (>1.4%) might have negative impact on OS. This might suggest a switch from oxidative to glycolytic metabolism in tumour cells, what may promote tumour growth [13]. However, hypoxia lost its significance in multivariate

analysis (in favour of Ki-67), therefore, we think that in PCa patients hypoxia contributes to disease progression and is not associated with treatment (RP) response. This may support experimental data which suggest that hypoxia alone is insufficient to permit metastasis [11].

Our study shows that tumour proliferation seems to have better impact on OS than hypoxia. Higher Ki-67LI (>6.7%) appeared to be an independent negative prognostic factor. Therefore, we can support the suggestion of Tollefson and colleagues that Ki-67expression should be incorporated into routine clinical management of PCa patients [14]. The authors indicated that Ki-67 expression of 6.0% appeared to be important for cancer-specific survival. There are no studies showing positive correlation between PSMA expression and OS in patients with locally advanced tumours after RP, apart from those concerning metastatic castration-resistant PCa [15].

In our study, PSMA overexpression had positive effect on OS, however, in another study the predictive power of this biomarker was not confirmed for cancer-specific mortality [16]. It is known that PSMA expression significantly increases with tumour grade and during PCa progression and is considered a marker of malignancy [7, 16]. It is utilized in PCa diagnostics and radioligand therapies for metastasized castration-resistant PCa [15, 17]. Therefore, a question arises: how to interpret our results? We have to remember that PSMA shows enzymatic activity modified by several nutritional and environmental factors contributing to the degree of aggressiveness of PCa [18].

Having that in mind, we suggest 5 possible explanations of positive correlation between PSMALI and survival: (1) Perhaps the enzymatic function of PSMA decreases the ability of PCa cells to invade the extracellular matrix, what was shown experimentally in PCa cell lines [18]. (2) Activation of tumour-directed cytotoxic T lymphocytes in PSMA-overexpressing cells is also possible (the antigen has T lymphocyte-restricted peptide epitopes) [19]. (3) PSMA can regulate IL-6 gene expression – a cytokine involved in regulation of immune reaction, cell growth, and differentiation – showing multifunctional responses ranging from inhibition of proliferation to promotion of cell survival [20]. In PCa cell line it was shown that IL-6 causes growth arrest and induces differentiation [20]. (4) PSMA expression in PCa is strongly associated with DNA repair deficiency [21]. An association exists between expression of this protein and defective DNA damage repair (DDR) [21]. If it is true, significantly higher OS in PSMA-overexpressing cases in our series may be caused by DDR deficiency, leading to cell death in unfavorable hypoxic microenvironment and, hence, better survival. (5) Finally, reported intra- and intertumour heterogeneity in PSMA expression [21]. Some high-grade cancers may show low PSMA expression, what has been recently revealed based on molecular analysis of metastatic castration-resistant PCa and in PCa diagnostics before radionuclide treatment [17, 21]. Therefore, great heterogeneity in PSMA expression within one patient and between different patients can limit the usefulness of PSMA scans and PSMA-targeted therapies [21], what we suggested earlier [7].

In PCa, a large number of endogenous markers is up-regulated under hypoxic conditions, which modulates key genes posing biological effects [12]. Human telomerase reverse transcriptase seems to be one of the markers mostly effected by hypoxia. The changes in oxygen availability result in oxidative stress and damage [22]. Reactive oxygen species (ROS) are key mediators in the regulation of cell death and survival. An imbalance, due to either increased ROS production (not detoxified by cellular antioxidants) or decreased degradation, can cause ROS accumulation and cell damage [23]. Depending on the severity of damage (of cellular DNA, proteins and lipids), these phenomena result in various types of cell death: autophagy (a pro-survival mechanism enabling cells to maintain homeostatic functions in response to cellular stress, including mitochondrial dysfunction), apoptosis or necrosis [23, 24].

It was suggested that telomerase activity during tumorigenesis may serve both for stabilization of telomeres (nuclear localization) and protection against antioxidative stress, improvement of mitochondrial function and better cell survival (mitochondrial localization) [24, 25]. As indicated, hTERT overexpression not only reduces the cellular ROS levels, but also inhibits endogenous ROS production and modulates cell response to apoptotic stimuli [26]. Therefore, to explain the positive correlation between hTERT overexpression and MFS and OS in our study, we suggest several possibilities: (1) An increase in intracellular hydrogen peroxide level can lead to cytosolic acidification creating a permissive environment for the execution of the death signal [26]. (2) Telomerase has been implicated to participate in cellular response to genotoxic stress [24]. However, there are opposing findings related to the protection or promotion of DNA damage and cell death as induced by hTERT, particularly as it relates to oxidative stress [24, 27]. (3) Autophagy can lead to the degradation of catalase, a key enzymatic ROS scavenger, which disrupts the intracellular ROS balance [23]. (4) The outcomes of autophagy modulation, including those mediated by ROS, can either promote cell survival or may be associated with cell death [22, 24]. Decreased autophagy may allow for the accumulation of dysfunctional mitochondria (impaired autophagic clearance of damaged organelles, mitophagy, ROS clearance), perhaps channeling the cells to apoptosis [24]. (5) ROS from dysfunctional mitochondria may attack genomic DNA, especially telomeres, and induce DNA damage response and cell senescence [28].

Prostate cancer occurs predominantly in older people. Cells accumulate various types of damage over the years as a result of inefficient or less active self-repair mechanisms (lower levels of antioxidant enzymes, inefficient in eliminating ROS formed during the aerobic cell metabolism and environmental stress [hypoxia] that damages cell DNA). This may lead to mitochondrial dysfunction, which is a delayed result of DDR. It was shown that elevated ROS production might, in turn, contribute to DNA damage and DNA damage response, thus forming a positive feedback loop leading to tumour cell death and better survival [28].

In our study, a total of 55 of the 130 men enrolled (42.3%) died during follow-up. Mean 5-year OS of 78.8% was comparable to other authors' results [29]. However, 10-year OS was lower than cancer-specific mortality obtained by other authors [2, 29, 30]. In our series, AT had significant positive impact on OS, what supports earlier results [30]. However, we did not observe statistically significant difference in OS in patients offered different adjuvant therapeutic options. The highest probability of survival was observed in patients after RT with either LH-RH agonists or antagonists causing inhibition of testicular androgen

production. The largest hereby analyzed subgroup (20%) was adjuvantly treated with RT. As a limited number of our patients was offered AT, it was difficult to compare the results with other studies analysing larger patients' series and more advanced tumours.

II Local or Systemic Progression

The cumulative incidence of metastases (19.2%) was similar to other studies [2, 29]. Ten-year LRFS of 74.2-77.7 and 10-year MFS of 88% were comparable to other authors' findings [2, 30]. In our patient's series, 5-year DFS was 83.1% and similar to other analysis (83.6%) [30]. Ten-year DFS was 77.6% and corresponding to other studies (84.1%) [30]. We also showed that negative prognosticators for DFS were mainly clinical parameters, including higher pretreatment PSA level (borderline significance), radicality of surgery, PSM and AT. We showed that lack of radicality posed higher risk for DFS (of about 88%), what confirms earlier results of studies evaluating recurrence risk after RT [9]. Adjuvant treatment also shortened DFS (and LRFS in univariate analysis), what may suggest induction of proliferation in tumor cells remaining after RP.

The role of RP for locally advanced PCa was previously considered controversial due to high incidence of PSM [30]. Nowadays, the lack of PSM after RP represents the most important surgery-related oncological outcome of RP. In our study, PSM rate was 45.4% and within the range (10-48%) given by other authors [31, 32]. Our results demonstrate that histopathologically confirmed PSM is closely associated with poor DFS (increased risk of 90%), what was also shown in another study [32]. Prognostic value of PSM in patients after RP remains controversial, as it is associated with surgical experience [32]. Therefore, with advances in surgical techniques a significant reduction in the risk of PSM, especially in high-risk disease, might be expected after robot-assisted RP. Our study is limited by its retrospective nature and low number of high-grade tumours. Therefore, it does not necessarily reflect natural biology of PCa, but rather of the early-stage tumours qualified to RP.

Conclusion

The following independent positive prognostic factors for OS were identified: AT, lower Ki-67LI, and PSMA or hTERT overexpression. Seminal vesicle(s) involvement and lower hTERTLI were negative prognostic factors for MFS. Finally, positive prognostic factors for DFS were: radical surgery, negative surgical margins and lack of AT.

Author Contributions

Protocol/project development: J. Jaszczynski, A Gasinska. Data collection: J Jaszczynski, M. Pogodzinski, M. Palaczynski, A. Gasinska. Data analysis: J. Jaszczynski, A Gasinska, M. Palaczynski. Manuscript writing/editing: all authors. A. Gasinska is acknowledged for supervision.

Conflicts of Interest

None.

Ethical Approval

The study protocol was approved by the local Ethical Committee at the Regional Medical Chamber in Cracow. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individuals included in the study.

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REFERENCES

1. Mahal BA, Yang DD, Wang NQ, Alshalalfa M, Davicioni E et al. (2018) Clinical and genomic characterization of low-prostate-specific antigen, high-grade prostate cancer. *Eur Urol* 74: 146-154. [Crossref]
2. Bill-Axelson A, Holmberg L, Garmo H, Rider JR, Taari K et al. (2014) Radical prostatectomy or watchful waiting in early prostate cancer. *N Engl J Med* 370: 932-942. [Crossref]
3. Hupe MC, Philippi C, Roth D, Kumpers Ch, Ribbat-Idel J et al. (2018) Expression of Prostate-Specific Membrane Antigen (PSMA) on biopsies is an independent risk stratifier of prostate cancer patients at time of initial diagnosis. *Front Oncol* 8: 623. [Crossref]
4. Jia Z, Zhu J, Zhuo Y, Li R, Han Qu et al. (2019) Offsetting expression profiles of prognostic markers in prostate tumor vs its microenvironment. *Front Oncol* 9:539. [Crossref]
5. Thysell E, Vidman L, Ylitalo EB, Jernberg E, Crnalic S et al. (2019) Gene expression profiles define molecular subtypes of prostate cancer bone metastases with different outcomes and morphology traceable back to the primary tumor. *Mol Oncol* 13: 1763-1777. [Crossref]
6. Luczynska E, Gasinska A, Wilk W (2012) Expression of Ki-67 (MIB-1) and GLUT-1 Proteins in Non-advanced Prostatic Cancer. *Pol J Pathol* 63: 272-277. [Crossref]
7. Gasinska A, Luczynska E, Wilk W, Cichocka A (2013) Expression of Telomerase and Prostate-Specific Membrane Antigen in Non-advanced Prostatic Cancer. *Folia Histochem Cytobiol* 51: 66-72. [Crossref]
8. Gasinska A, Jaszczynski J, Rychlik U et al. (2019) Prognostic significance of serum PSA level and telomerase, VEGF and GLUT-1 protein expression for the biochemical recurrence in prostate cancer patients after radical prostatectomy. *Pathol & Oncol Res*.[Crossref]
9. Budaus L, Isbarn H, Eichelberg Ch, Lughezzani G, Sun M et al. (2010) Biochemical recurrence after radical prostatectomy: multiplicative interaction between surgical margin status and pathological stage. *J Urol* 184: 1341-1346. [Crossref]
10. Duffy MJ (2019) Biomarkers for prostate cancer: prostate-specific antigen and beyond. *Clin Chem Lab Med* 58:326-339. [Crossref]
11. Bharti SK, Kakkad S, Danhier P, Wildes F, PenetM-F et al. (2019) Hypoxia patterns in primary and metastatic prostate cancer environments. *Neoplasia* 21: 239-246. [Crossref]

12. Fraga A, Ribeiro R, Pau Príncipe P, Lopes C, Medeiros R (2015) Hypoxia and prostate cancer aggressiveness: A tale with many endings. *Clin Genitourinary Cancer* 13: 295-301. [Crossref]
13. Shiraishi T, Verdone JE, Huang J, Kahlert UD, Hernandez JR et al. (2015) Glycolysis is the primary bioenergetic pathway for cell motility and cytoskeletal remodeling in human prostate and breast cancer cells. *Oncotarget* 6: 130-143. [Crossref]
14. Tollefson MK, Karnes RJ, Kwon ED, Lohse CM, Rangel LJ et al. (2014) Prostate cancer Ki-67(MIB-1) expression, perineural invasion, and gleason score as biopsy-based predictors of prostate cancer mortality: the Mayo model. *Mayo Clin Proc* 89: 308-318. [Crossref]
15. Thang SP, Violet J, Sandhu S, Iravani A, Akhurst T et al. (2019) Poor outcomes for patients with metastatic castration-resistant prostate cancer with low Prostate-specific Membrane Antigen (PSMA) expression deemed ineligible for ^{177}Lu-labelled PSMA radioligand therapy. *Eur Urol Oncol* 2: 670-676. [Crossref]
16. Kasperzyk JL, Finn SP, Flavin R, Fiorentino M, Lis R et al. (2013) Prostate-Specific Membrane Antigen protein expression in tumor tissue and risk of lethal prostate cancer. *Cancer Epidemiol Biomarkers Prev* 22: 2354-2363. [Crossref]
17. Yakar D, Noordzij W, Kwee TC (2020) Potential causes of false-negative interpretations in 68Ga-PSMA PET/CT for the detection of local andrecurrent prostate cancer: An Underexposed Issue. *Clin Nucl Med* 45: e32-e35. [Crossref]
18. Ghosh A, Wang X, Klein E, Heston WD (2005) Novel role of prostate-specific membrane antigen in suppressing prostate cancer invasiveness. *Cancer Res* 65: 727-731. [Crossref]
19. Totterman TH, Loskog A, Essand M (2005) The immunotherapy of prostate and bladder cancer. *BJU Int* 96: 728-735. [Crossref]
20. Culig Z, Steiner H, Bartsch G, Hobisch A (2005) Interleukin-6 regulation of prostate cancer cell growth. *J Cell Biochem* 95: 497-505. [Crossref]
21. Paschalis A, Sheehan B, Riisnaes R, Rodrigues DN, Gurel B et al. (2019) Prostate-specific Membrane Antigen Heterogeneity and DNA Repair Defects in Prostate Cancer. *Eur Urol* 76: 469-478. [Crossref]
22. Scherz-Shouval R, Elazar Z (2011) Regulation of autophagy by ROS: physiology and pathology. *Trends Biochem Sci* 36: 30-38. [Crossref]
23. Yu L, Wan F, Dutta S, Welsh S, Liu Z et al. (2006) Autophagic programmed cell death by selective catalase degradation. *Proc Natl Acad Sci USA* 103: 4952-4957. [Crossref]
24. Green PD, Sharma NK, Santos JH (2019) Telomerase impinges on the cellular response to oxidative stress through mitochondrial ROS-mediated regulation of autophagy. *Int J Mol Sci* 20: E1509. [Crossref]
25. Ahmed S, Passos JF, Birket MJ, Beckmann T, Brings S et al. (2008) Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. *J Cell Sci* 121: 1046-1053. [Crossref]
26. Indran IR, Hande MP, and Pervaiz S (2011) hTERT overexpression alleviates intracellular ROS production, improves mitochondrial function, and inhibits ROS-Mediated apoptosis in cancer cells. *Cancer Res* 71: 266-276. [Crossref]
27. Santos JH, Meyer JN, Skorvaga M, Annab LA, Van Houten B (2004) Mitochondrial hTERT exacerbates free-radical-mediated mtDNA damage. *Aging Cell* 3: 399-411. [Crossref]
28. Passos JF, Nelson G, Wang Ch, Richter T, Simillion C et al. (2010) Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol Syst Biol* 6: 347. [Crossref]
29. Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C et al. (2016) 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. *N Engl J Med* 375: 1415-1424. [Crossref]
30. Fahmy O, Khairul-Asri MG, Hadi SHSM, Gakis G, Stenzl A (2017) The role of radical prostatectomy and radiotherapy in treatment of locally advanced prostate cancer: A systematic review and meta-analysis. *Urol Int* 99: 249-256. [Crossref]
31. Eastham JA, Kattan MW, Riedel E, Begg CB, Wheeler TM et al. (2003) Variations among individual surgeons in the rate of positive surgical margins in radical prostatectomy specimens. *J Urol* 170: 2292-2295. [Crossref]
32. Zhang L, Wu B, Zha Z, Zhao H, Yuan J et al. (2018) Surgical margin status and its impact on prostate cancer prognosis after radical prostatectomy: a meta-analysis. *World J Urol* 36: 1803-1815. [Crossref]