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EXPRESSION OF KI-67 AS A PROLIFERATION MARKER IN PROSTATE CANCER

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ABSTRACT

Introduction. Prostate carcinoma (PCa) is the most common male cancer found in industrialized societies and represents a serious public health problem. Delineation of gene expression patterns in early PCa that correlate with an aggressive phenotype is a priority and may allow for radical treatment to be offered on a more selective basis to those patients with a clinically localized, yet aggressive disease. Ki-67 was recognized as associated with carcinogenesis in PCa.

Aim. The aim of this study was the immunohistochemical evaluation of Ki-67 and its expression in PCa following radical prostatectomy, and analysis of its relationship to chosen clinical and morphological parameters of such tumors.

Materials and methods. A total number of 56 randomly selected patients undergoing radical prostatectomy were investigated. The tumors, after fixation with 10% neutral buffered formalin, were completely embedded in paraffin. The sections were cut into hematoxylin-eosin staining for histological examination. The sections were also immunostained, with monoclonal antibodies against Ki-67. Immunolocalization of Ki-67 was performed using the LSAB method. Serum PSA levels were obtained from clinical information. These obtained results were statistically analyzed using the Fisher's exact test and χ^2 test.

Results and Discussion. No statistically significant correlation was found between the expression of Ki-67 and the preoperative PSA level, lymph node metastases, capsular penetration, seminal vesicle invasion, and positive or negative surgical resection margins. However, a strong statistically significant correlation between Ki-67 positive and the T stage was found. We also found a relationship between the Gleason score of 7 or above and a high expression of Ki-67 in PCa ($p < 0.004$, $p < 0.02$ respectively).

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Conclusions. It is noteworthy that a significant correlation exists between the Gleason score and the expression of Ki-67 in this present study. We observed a high expression of Ki-67 for a Gleason score of 7 or above. Our results suggest that Ki-67 may be useful to serve as a tumor marker in PCa.

Key words: prostate cancer (PCa), Ki-67, immunohistochemistry

INTRODUCTION

Prostate cancer (PCa) is the most common malignant tumor found in men. Identification of patients with aggressive rather than indolent PCa is a major challenge for optimal management purposes and is only partially met by currently available prognostic parameters. Delineation of gene expression patterns in early PCa that correlate with an aggressive phenotype is a priority and may allow for radical treatment to be offered on a more selective basis to those patients with a clinically localized, yet aggressive disease. Various indices of cellular proliferative activity have been investigated. Mitotic counts only detect cells in the M phase, are dependent on the period of time between surgical removal and fixation of the specimen, and suffer from heterogeneous distribution and confusion between mitoses and nuclear pyknosis and karyorrhexis. Ki-67 recognizes a proliferation specific nuclear antigen. It is expressed by proliferating cells in late G_1, S, G_2, and M phases, but not in resting cells in G_0. Staining is commonly nucleolar or perinucleolar. Ki-67 gives a higher labeling index than other antibodies, with good inter-reading reproducibility. It is therefore important to identify the antibody used in each study [12].

Therefore Ki-67, the most frequently used cell proliferation marker, recognized nuclear antigens as associated with all phases of the cell-cycle, except G_0 [7]. Ki-67 was recognized as associated with carcinogenesis in PCa [5, 6, 10].

In this study we evaluated the immunohistochemical profile of Ki-67 and correlated the results with chosen clinicopathological parameters in resected specimens of PCa.

AIM

The aim of this study was the immunohistochemical evaluation of Ki-67 and its expression in PCa following radical prostatectomy, and the analysis of its relationship to chosen clinical and morphological parameters of the tumors.

MATERIALS AND METHODS

All patients in this study were diagnosed and treated for PCa. A total of 56 randomly selected patients undergoing radical prostatectomy were investigated in the present study. The tumors, after fixation with 10% neutral buffered formalin, were complete-

ly embedded in paraffin. Up to three blocks from each case were obtained to provide representative material from the major foci of cancer within each prostate. The sections were cut to hematoxylin-eosin (HE) staining (Fig. 1), to AMACR examination (Fig. 2, 3), to Cytokeratin 34βE12 (Fig. 4) for pathological examination according to the TNM classification and according to the Gleason grading system. Tumors were classified as high grade when the combined Gleason score was 7 or above and as low grade when the combined score was 6 or below. Serum PSA levels were obtained from clinical information.

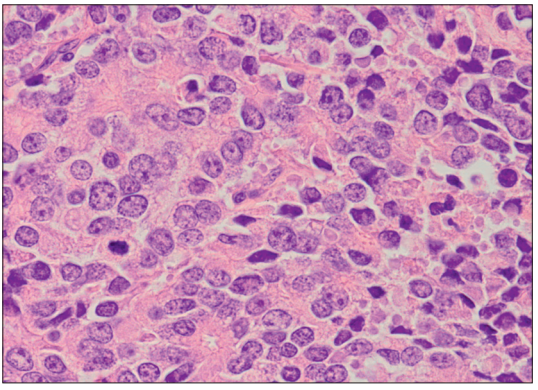

Fig. 1. Adenocarcinoma of the prostate after radical prostatectomy – Gleason score 9 [HE, magn. 400×]

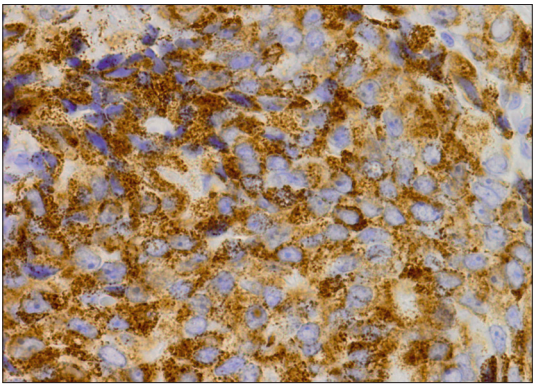

Fig. 2. Adenocarcinoma of the prostate after radical prostatectomy – Gleason score 7 [AMACR, magn. 400×]

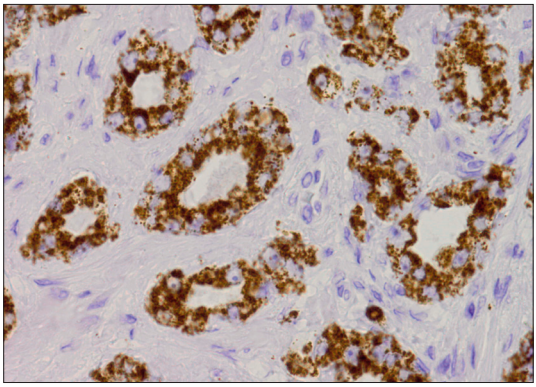

Fig. 3. Adenocarcinoma of the prostate after radical prostatectomy – Gleason score 9 [AMACR, magn. 400×]

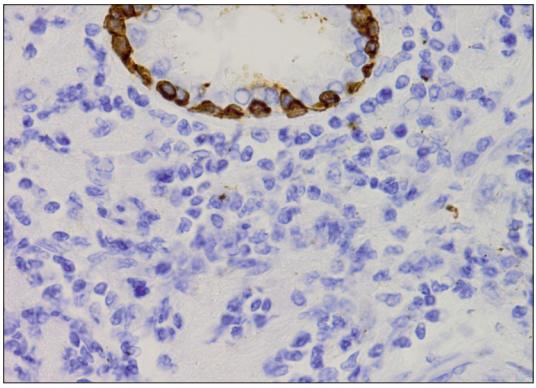

Fig. 4. Adenocarcinoma of the prostate after radical prostatectomy – Gleason score 9 [Cytokeratin 34βE12, magn. 400×]

The sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. The sections were treated with 2% H_2O_2 for 10 minutes at room temperature to inactivate endogenous peroxidase activity. The deparaffinized tissue sections were incubated in 10 mM citrate buffer, pH 6.0, and heated at 95°C for 15 minutes. The sections were stained immunohistochemically with the anti-human Ki-67, PCNA monoclonal (Dako/Ki-67, No N1574 and Dako/PCA, No M0879). The immunolocalization of Ki-67 was performed using the Labeled Streptavidin Biotin (LSAB) method. The Ki-67 expression was semiquantitatively assessed in neoplastic cells and defined as follows: Ki-67 negative (lack of reaction), Ki-67 low (reaction present in less than 10% of cells) (Fig. 5), and Ki-67 high (reaction present in more than 10% of cells) (Fig. 6).

The obtained results were statistically analyzed using the Fisher's exact test and χ^2 test.

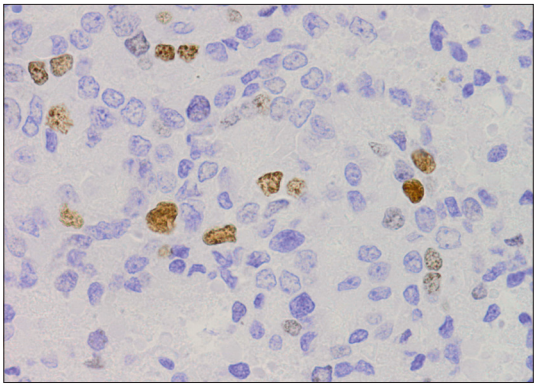

Fig. 5. Adenocarcinoma of the prostate after radical prostatectomy – Gleason score 7 [Expression of Ki-67, magn. 400×]

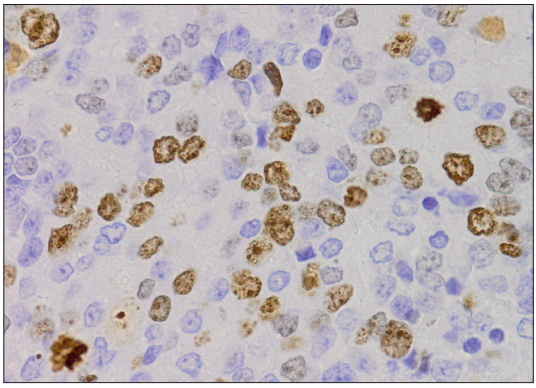

Fig. 6. Adenocarcinoma of the prostate after radical prostatectomy – Gleason score 9 [Expression of Ki-67, magn. 400×]

RESULTS

The overall results of immunohistochemical examinations have been presented in Tab. 1. The average age of the patients was 62 years. Depending on the preoperative serum PSA concentrations, we distinguished three groups: first (6 cases) when the PSA concentration was 0–4 ng/mL, second (18 cases) when the PSA concentration was 4–10 ng/mL, and third (32 cases) when the preoperative serum PSA level was 10 ng/mL or above. In the present study, following pelvic lymph node dissection and radical prostatectomy, tumor specimens were classified as pT2 (38 patients, 67.9%) and pT3 (18 patients, 32.1%). The statistically significant correlation was found between pT and Ki-67, where 16 (88.9%) out of 18 patients with a pT3 stage were Ki-67 positive and only 2 of them were Ki-67 negative. Also, no correlation was found

between the Ki-67 expression in PCa and anatomoclinical parameters such as lymph node metastases, capsular penetration, seminal vesicle invasion, surgical resection margin, and between the preoperative serum PSA levels. However, a strong association between the expression of Ki-67 in a high Gleason score is noteworthy. 24 out of 26 cases with a Gleason score of 7 or above were immunopositive for Ki-67 (Fig. 6), ($p < 0.004$, $p < 0.02$).

Tab. 1. Expression of Ki-67 and clinicopathological findings

Variable		No. of cases	Ki-67 Negative		Ki-67 High		P value
			No.	%	No.	%	
PSA	0–4	6	2	33.3	4	66.7	NS
	4–10	18	12	66.7	6	33.3	
	10	32	6	18.8	26	81.3	
pT	pT2	38	18	47.4	20	52.6	0.06
	pT3	18	2	11.1	16	88.9	
pN	Negative	48	18	37.5	30	62.5	NS
	Positive	8	2	25.0	6	75.0	
GS	<7	30	18	60.0	12	40.0	0.004
	≥7	26	2	7.7	24	92.3	
CP	Negative	24	12	50.0	12	50.0	NS
	Positive	32	8	25.0	24	75.0	
SVI	Negative	28	10	35.7	18	64.3	NS
	Positive	28	10	35.7	18	64.3	
SRM	Negative	38	16	42.1	22	57.9	NS
	Positive	18	4	22.2	14	77.8	

Comments: GS – Gleason score, CP – capsular penetration, SVI – seminal vesicles invasion, SRM – surgical resection margins, NS – not specified.

DISCUSSION

PCa is the most common solid tumor found in the Polish male population and is the second leading cause of cancer specific mortality. Neoplastic diseases, as proliferative disorders, are characterized by uncoordinated cell growth. Activation of protooncogenes and inactivation of tumor suppressor genes are the main adverse genetic and epigenetic events that are responsible for neoplastic transformation [2, 4].

Over the last few years, the proliferation rate of cancer has been assessed by means of immunohistochemical markers and exploited as a potential prognostic marker [3, 5, 6, 13]. The Ki-67 nuclear antigen, present in the G_1 through M phase of the cell cycle but not at rest, correlates well with the traditional assessment of proliferation such as bromodeoxyuridine uptake and thymidine labeling [8, 14]. In our study, Ki-67

defined as positive in more than 10% of tumor cells was found in 36 (64%) of 56 resected PCas. In this study, no statistically significant correlation was found between the expression of Ki-67 and preoperative PSA levels, lymph node metastases, capsular penetration, seminal vesicle invasion and positive or negative surgical resection margins. However, a strong statistically significant correlation between Ki-67 positive (more than 10% of neoplasmatic cells) and the T stage was found. It is similar to that shown in numerous studies, where a significant correlation between nuclear proliferation antigens, such as Ki-67, and the tumor grade in PCa was observed [9, 16]. Although Kallarury et al. [11] show a correlation between Ki-67 expression and tumor grade; that correlation did not demonstrated a significant predictive value for disease recurrence. These results are consistent with previously reported observations [1, 15], in which Ki-67 was not an independent predictor for poor survival in patients with PCa.

CONCLUSIONS

1. Significant correlation exists between the Gleason score and the expression of Ki-67.
2. A high expression of Ki-67 for a Gleason score of 7 or above was observed.
3. Ki-67 may be useful to serve as a tumor marker in PCa.

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