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Evaluation of selected proinflammatory factors, angiogenesis and lymphangiogenesis as potential markers of early tumor recurrence in patients undergoing surgical treatment for prostate cancer

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SUMMARY

Introduction: Up to a half of patients after radical prostatectomy due to prostate cancer have biochemical recurrence, i.e., PSA concentrations >0.2 ng/ml. In most patients, biochemical recurrence does not significantly affect survival and quality of life; however, in about one third of patients, biochemical recurrence precedes a clinically relevant recurrence of prostate cancer. Usually, based on the current criteria of biochemical recurrence, prostate cancer recurrence is diagnosed several months after surgery. This delays adjuvant treatment and worsens treatment outcomes. This study aimed to check whether preoperative concentrations of markers of angiogenesis, lymphoangiogenesis, and extracellular matrix degradation could predict biochemical recurrence of prostate cancer after radical prostatectomy.

Materials and methods: The study included 82 patients who underwent radical prostatectomy for prostate cancer (cT1-T2N0M0). Before surgery and 8-10 days after surgery, serum concentrations of PSA and markers of inflammatory processes, angiogenesis, lymphangiogenesis, and extracellular matrix degradation were measured (CRP, VEGF-A, VEGF-C, VEGF-D, TIMP-1, TIMP-2). The stage of prostate cancer was determined histopathologically (TNM classification, Gleason score) and clinically (digital rectal examination, prostate volume assed with ultrasound, pelvic magnetic resonance imaging). With the Kaplan-Meier analysis and Cox proportional hazard models, we checked whether the variables studied were associated with the risk of biochemical recurrence of prostate cancer after radical prostatectomy.

Results: During a 3-year follow-up, 27 of 82 patients (32.9%) had biochemical recurrence of prostate cancer after radical prostatectomy. The risk of biochemical recurrence of prostate cancer was not significantly associated with patient age, BMI, result of preoperative prostate digital rectal examination, cancer stage assessed with pelvic magnetic resonance imaging, number of lymph nodes removed, and cancer grade on histopathology. The risk of biochemical recurrence of prostate cancer after radical prostatectomy was higher in patients with lower prostate volume on pre-operative transrectal ultrasound, higher Gleason score in preoperative and postoperative analyses, and positive surgical margins. The concentrations of the studied markers of inflammatory processes, angiogenesis, lymphangiogenesis, and remodeling of extracellular matrix were not related to the risk of biochemical recurrence of prostate cancer after radical prostatectomy.

Key words: prostate cancer, VEGF-C, VEGF-D, TIMP-1, TIMP-2

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INTRODUCTION

Prostate cancer

Prostate cancer is an important public health problem in highly developed countries. In Western and Northern Europe, prostate cancer is the most frequent solid tumor in men (approximately 214 cases per 100,000 men) [1]. In the rest of Europe, the incidence of prostate cancer is smaller, but is still increasing. In Poland, in 2011, the number of new cases of prostate cancer increased to 10318 (14.4% of all malignant tumors), and the number of deaths caused by this cancer rose to 4085 (7.92% of all deaths due to malignant tumors). Patients with cancer of the prostate gland live progressively longer: In the years 1999-2007, the overall European 5-year survival rate increased from 73.4% to 83.4% [2].

Suspected prostate cancer can be checked on the basis of rectal prostate examination, blood PSA and Transrectal Ultrasonography (TRUS). The definitive diagnosis is based on histopathological examination of prostate biopsy specimens. PSA is a serine protease from the kallikrein family. This enzyme is produced by the glandular epithelium of the prostate and secreted into the ejaculate. PSA liquefies ejaculate and mucus in the cervical canal [3]. PSA is a biological marker specific for glandular prostate tissue. The probability of diagnosing clinically significant prostate cancer increases with increasing PSA concentration; however, the probability of diagnosing cancer in latent form decreases [4,5].

Clinical advancement of prostate cancer is assessed according to the seventh edition of the TNM (Tumor, Nodes, Metastases) classification published in January 2010 by the American Joint Committee on Cancer. The degree of histological malignancy of prostate cancer is determined on the Gleason scale according to the guidelines published in 2005 by the International Society of Urological Pathology [6-9]. Clinical advancement on the TNM scale, Gleason score and PSA concentration in the blood allow

estimation of the risk of biochemical recurrence of prostate cancer [10].

Angiogenesis and lymphangiognesis

Angiogenesis and lymphangiogenesis, i.e. the formation of new blood and lymph vessels, are important mechanisms for the development of cancer, including prostate cancer. In the microenvironment of the tumor, these processes occur continuously in an uncontrolled manner through the host organism. Angiogenesis and lymphangiogenesis in the parenchyma and tumor environment are induced by the increased need of cancer cells for oxygen and nutrients, and by lymphatic fluid stasis caused by increased tissue pressure (tumor pressure) [11].

The formation of new blood vessels can be stimulated directly by activating proangiogenic oncogenes or indirectly *via* cytokines secreted by tumor-infiltrating immune cells. Neoangiogenesis and lympho-angiogenesis are necessary for the development of cancer, because the diffusion of oxygen and nutrients from the capillaries to the surrounding tissues occurs only within a radius of 0.1 mm-1 mm from the capillary vessel. At this stage, proliferation and death of tumor cells, as well as the activity of proangiogenic and anti-angiogenic factors are balanced. However, if a group of tumor cells secreting proangiogenic factors arise as a result of another mutation, tumor progression occurs, including formation of distant metastases [12]. In addition, angiogenic factors can be released not only by tumor cells, but also by endothelial cells, blood, stromal cells and extracellular matrix [13]. Some proangiogenic factors can directly stimulate the proliferation of cancer cells [14]. There are four groups of factors that favor the phenomenon of "angiogenic switching" [12]: factors of metabolic stress; mechanical stress factors; immunological and inflammatory factors, as well as genetic factors.

Vascular Endothelial Growth Factor (VEGF)

Vascular Endothelial Growth Factor (VEGF) is the most specific and the most important growth factor for endothelial cells. VEGF stimulates the migration and proliferation of endothelial cells. In addition, through the bcl-2 gene, VEFG inhibits the apoptosis of these cells. VEGF binds to specific endothelial membrane receptors VEGFR-1, 2 and 3 receptors of tyrosine kinases.

VEGF-C and VEGF-D are the main factors that promote lymphangiogenesis and tumor spread by lymphatic vessels. By combining with VEGFR-3, VEGF-C and VEGF-D, they increase the diameter of the lymphatic vessels in the vicinity of the tumor; induce the formation of new lymphatic vessels and abnormal connections between the lymphatic system and the circulatory system in the lymph nodes. The evoked changes in the lymphatic system not only facilitate passive tumor spread, but also actively promote dissemination through chemotactic effects on cancer cells [15].

Metalloproteinases and Tissue Inhibitors of Metalloproteinases (TIMP)

Matrix Metalloproteinases (MMPs) are proteins constituting the basic building block of the intercellular matrix, which are involved in cell migration and angiogenesis [16]. MMPs are produced by tumor cells and adjacent cells. MMPs, by proteolysis of the basement membrane (collagen IV), enable tumor spread. MMP activity is regulated at both transcription and translation levels. In addition, some proteins, e.g. Tissue Inhibitors of Metalloproteinases (TIMP) and alpha-macroglobulin, inhibit MMP activity. The activity of enzymes from the metalloproteinases family suppresses tissue inhibitors of metalloproteinases (TIMP) [17]. Currently, four TIMP proteins are known: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. TIMPs have anti-angiogenic activity because they inhibit the activity of metalloproteinases and the migration and proliferation of vascular endothelial cells. TIMP-2 also has a cytostatic effect on tumor cells, enclosing them in interstitial collagen network [17]. MMPs and TIMPs exist as enzymatically inactive complexes that must be cleaved to release active MMPs. The proteolytic activity of MMPs is not determined by the intensity of their expression, but by the relationship between MMPs and their tissue inhibitors. Disruption of this mutual relationship in cancer tissue leads to tumor progression.

The classic clinical and histopathological criteria used in everyday practice do not allow for the decision to initiate adjuvant treatment immediately after radical treatment in patients with prostate cancer limited to this organ. Such a decision is usually made after several months of observation of postoperative changes in PSA levels in the blood and confirmation of the biochemical recurrence criterion. Therefore, it is necessary to look for prognostic factors that would predict biochemical and/or clinical recurrence earlier than those currently used.

Since the development of cancer is closely related to inflammatory processes, neoangiogenesis, lymphangiogenesis and remodeling of the extracellular matrix, it is reasonable to check whether these factors can predict recurrence of prostate cancer after radical treatment. Previous studies evaluating markers of inflammatory processes, angiogenesis and lymphangiogenesis in patients with prostate cancer were mainly retrospective, of small size and with a short period of observation. In addition, usually these studies only assessed single markers, and their results were often contradictory. Therefore, the analysis of the "angiogenic profile" including a number of proangiogenic and anti-angiogenic factors could be more valuable [18].

Aim of the study

The main objective of the study was to investigate whether markers of inflammatory process, angiogenesis and lymphangiogenesis (CRP, VEGF-A, VEGF-C, VEGF-D, TIMP-1, TIMP-2) are predictors of early recurrence of prostate cancer in patients undergoing radical surgical treatment.

The study also looked at whether recurrence of prostate cancer after radical surgery was associated with anthropometric and clinical variables. It was also examined whether the concentrations of factors related to angiogenesis and lymphangiogenesis differ depending on clinical and histopathological variables in patients after radical prostate surgery.

MATERIAL AND METHODS

General information

The study included 114 patients aged from 44 to 78 years

(mean age 61 years) after radical prostatectomy for prostate cancer in the clinical stage of cT1-T2N0M0. Patients were treated in 2010-2011 at the Clinical Department of Oncological Urology of the Oncology Center in Bydgoszcz. The study excluded patients with diseases affecting angiogenesis (diabetes, active coronary disease, organ failure, taking anticoagulants other than acetylsalicylic acid). After exclusion of the above-mentioned patients, there remained 82 patients aged 51-78 years (mean age 62), who were observed for 36-54 months (mean follow-up time 45 months). The biochemical recurrence was defined as an increase in PSA concentration >0.2 mg/ml with two or more subsequent indicators in blood serum. The date of the recurrence diagnosis referred to the date of the first examination in which the PSA was >0.2 mg / ml. The study was approved by the Bioethics Commission at the Collegium Medicum in Bydgoszcz of the Nicolaus Copernicus University in Bydgoszcz. The condition for entering the study was the patient's giving informed consent to participate in it.

Treatment details

Concentrations of serum markers were determined using commercially available immunoenzymatic tests. The CRP, VEGF-A, VEGF-C, VEGF-D, TIMP-1, and TIMP-2 values was determined for each patient 1 day before surgery. On the 8th-10th day after radical prostatectomy, blood was collected again to evaluate the concentrations of VEGF-A, VEGF-C, VEGF-D, TIMP-1 and TIMP-2. The obtained blood serum from the first and second collection was stored at -700°C until material was collected from all patients. The determinations were made at the Department of Laboratory Diagnostics of the Oncology Center in Bydgoszcz using the immunoenzymatic ELISA method using mouse polyclonal antibodies with a spectrophotometer at wavelength λ=450 nm. Patients were monitored according to general recommendations every 3 months in the first and second year after surgery. Then, follow-up visits were held at intervals of 3 or 6 months, depending on the patient's health situation, for at least 3 years after radical treatment.

The severity of prostate cancer was assessed in the postoperative material on the basis of microscopic examination. Patients for whom post-operative histopathological evaluation showed neoplastic disease beyond grade pT2 N0 were not eligible for further studies. Patients were divided into two groups: patients with pT2a tumors (8 patients) and pT2b tumors (6 patients), and patients with pT2c tumors (68 patients). If microscopic prostate cancer cells were seen in the surgical margins outside the prostate, the condition was defined as Positive Surgical Margins (PSM). The presence of PSM did not change the degree of advancement of pT2 to higher.

STATISTICAL ANALYSIS

The values of the qualitative variables in several groups were compared using the chi-square test with Yates correction or Fisher's exact test, if small expected values appeared in the tables.

The consistency of the distribution of variables with the normal distribution was checked by the Shapiro-Wilk test. Variables deviating significantly from the normal distribution were analyzed with nonparametric tests: Mann-Whitney test (comparison of 2 groups) and Kruskal-Wallis test (comparison >2 groups).

Survival analyzes were performed using Kaplan-Meier curves (log-rank test) or the Cox proportional hazard model. p values<0.05 were considered statistically significant. Statistical calculations were made using the statistical package "R" (version 3.1.2).

RESULTS

In the examined group of patients after radical surgical treatment of prostate cancer, there was no significant relationship between anthropometric variables (age, BMI) and the risk of tumor biochemical recurrence (Cox proportional hazard model).

In the Cox proportional hazard model, the prostate volume measured by transrectal ultrasound was significantly associated with the risk of prostate cancer recurrence. An increase in prostate volume by 1 ml was associated with a reduced risk of recurrence after radical prostatectomy of 3.3% (Table 1).

Because as many as 58 patients had a Gleason score of 2-6 points, these patients were assigned to two subgroups: patients with a score of 6 points and patients with a score of 2-5 points. Because there were few patients with a Gleason score of 8, one group was formed for patients with a score of 7 or 8 points. Patients with Gleason scores of 2-5 points stayed longer without tumor recurrence than other patients, but this difference was not statistically significant (Table 2).

There was no significant correlation between tumor stage in pre-operative pelvic magnetic resonance imaging and risk of prostate cancer recurrence. Moreover, there was no significant correlation between tumor stage (T feature) in histopathological examination and risk of prostate cancer recurrence.

Tab. 1. Prostate volume in transrectal ultrasound performed before surgery and risk of prostate cancer recurrence (Cox proportional hazard model).

Variable	HR	95% confidence interval for HR		p
Volume of prostate in TRUS [ml]	0.967	0.939	0.996	**0.027**

Tab. 2. Gleason score in pre-operative biopsy samples and risk of prostate cancer recurrence

Gleason Scale (points)	n	Survival free from recurrence					p
		6 mths	12 mths	24 mths	36 mths	48 mths	
02-May	20	95.00%	95.00%	90.00%	90.00%	85.00%	
6	38	75.68%	75.68%	72.97%	62.16%	62.16%	0.132
07-Aug	24	91.67%	91.67%	70.37%	61.57%	57.18%	

Tab. 3. Presence of positive surgical margins after prostatectomy and the risk of prostate cancer recurrence

PSM	n	Survival free from recurrence					p
		6 mths	12 mths	24 mths	36 mths	48 mths	
PSM	21	75.00%	75.00%	60.00%	55.00%	49.50%	0.052
No PSM	61	88.52%	88.52%	81.87%	73.52%	71.85%	

Tab. 4. PSA concentrations and markers of inflammation and angiogenesis and the risk of prostate cancer recurrence after radical surgical treatment

Variable	HR	95 % confidence interval for HR		p
PSA before biopsy [ng/ml]	1.004	0.969	1.041	0.813
CRP 1 day before operation [mg/l]	0.956	0.866	1.056	0.376
VEGF-A 1 day before operation [pg/ml]	0.9999	0.999	1.001	0.937
VEGF-C 1 day before operation [pg/ml]	1.078	0.914	1.271	0.373
VEGF-D 1 day before operation [pg/ml]	1.001	0.999	1.003	0.427
TIMP-1 1 day before operation [ng/ml]	0.9999	0.999	1.001	0.784
TIMP-2 1 day before operation [ng/ml]	1.0003	0.999	1.001	0.607
PLT 1 day before operation [10^9/l]	0.996	0.989	1.003	0.271
VEGF-A 8-10 days after operation [pg/ml]	1.0003	0.999	1.001	0.484
VEGF-C 8-10 days after operation [pg/ml]	1.123	0.962	1.311	0.141
VEGF-D 8-10 days after operation [pg/ml]	1.001	0.999	1.003	0.293
TIMP-1 8-10 days after operation [ng/ml]	0.9999	0.999	1.001	0.553
TIMP-2 8-10 days after operation [ng/ml]	0.9997	0.999	1.006	0.552
Decrease in concentration after operation: VEGF-A [pg/ml]	0.999	0.998	1.001	0.34
Decrease in concentration after operation: VEGF-C [pg/ml]	0.928	0.733	1.176	0.539
Decrease in concentration after operation: VEGF-D [pg/ml]	0.9998	0.996	1.003	0.91
Decrease in concentration after operation: TIMP-1 [ng/ml]	1.0001	0.999	1.001	0.708
Decrease in concentration after operation: TIMP-2 [ng/ml]	1.0001	0.999	1.001	0.611

Tab. 5. Pre-operative concentrations of VEGF-A, VEGF-C, VEGF-D, TIMP-1, TIMP-2 and CRP depending on the condition of postoperative margins

Parameter	PSM	Average	SD	Median	Min	Max	Q1	Q3	p
VEGF-A [pg/ml]	Yes	264.05	170.53	235.97	43.53	607.35	114.4	413.84	0.341
	No	399.29	401.3	293.14	49.98	1804.82	155.44	489.96	
VEGF-C [pg/ml]	Yes	1.39	2.41	0.65	0	9.96	0.49	0.95	0.777
	No	1.15	1.65	0.72	0	11.62	0.37	1.56	
VEGF-D [pg/ml]	Yes	325.79	130.74	278.57	146.77	603.74	219.34	414.58	0.329
	No	315.83	190.66	275.05	78.5	994.53	199.3	358.11	
TIMP-1 [ng/ml]	Yes	1733.29	905.27	1883.34	259.99	3096.85	772.51	2488.62	0.6
	No	1629.72	841.55	1683.1	59.37	3120.52	1026.7	2304.31	
TIMP-2 [ng/ml]	Yes	1025.06	471.97	988.37	177.91	2364.44	828.15	1179.89	0.032
	No	1244.72	324.38	1192.14	763	2016.5	1026.1	1434.35	
CRP [mg/l]	Yes	2.28	3.8	1.21	0.1	17.82	1.05	1.78	0.897
	No	4.18	10.37	1.22	0.2	61.2	0.61	2.43	

The presence of positive surgical margins after prostatectomy was associated with a higher risk of tumor recurrence compared to the presence of tumor-free surgical margins (difference at the level of statistical trend, p=0.052, Table 3).

Patients with higher Gleason scores in the postoperative formulation (6-8 points) had a significantly higher recurrence risk than those with lower scores (2-5 points). The number of lymph nodes removed during radical prostatectomy was not significantly associated with the risk of tumor recurrence.

Serum concentrations of PSA, CRP, VEGF, TIMP and platelet count (PLT) were not significantly associated with the risk of biochemical recurrence of prostate cancer after radical prostatectomy. Also, post-operative reduction in VEGF and TIMP concentrations was not significantly associated with recurrence risk (Table 4).

The concentrations of VEGF-A, VEGF-C, VEGF-D, TIMP-1, TIMP-2 and CRP did not differ significantly depending on the Gleason score in the pre-operative prostate cancer biopsy, and did not significantly differ with the outcome in the Gleason scale in the postoperative material, and also did not differ significantly depending on the stage of the tumor in histopathological examination. Patients with Positive Surgical Margins (PSM) had significantly lower TIMP-2 concentrations than patients with clear operating margins. The concentrations of VEGF-A, VEGF-C, VEGF-D, TIMP-1 and CRP did not differ significantly depending on the state of postoperative margins (Table 5).

After taking into account anthropometric variables (age, weight, height, body mass index BMI) in Cox proportional hazard models, serum concentrations of PSA, CRP, VEGF and TIMP and their postoperative changes were not significantly associated with the risk of prostate cancer recurrence after radical prostatectomy.

After taking into account anthropometric variables and concentrations of all angiogenic and inflammatory factors in Cox proportional hazard models, VEGF-A concentration 1 day before

Tab. 6. Concentrations of markers of inflammation and angiogenesis and the risk of prostate cancer recurrence after radical surgical treatment after adjustment for anthropometric variables and concentrations of angiogenic and inflammatory factors

Variable	HR	95% confidence interval for HR		p
VEGF-A 1 day before operation [pg/ml]	0.994	0.99	0.999	0.021
VEGF-C 1 day before operation [pg/ml]	0.585	0.237	1.448	0.246
VEGF-D 1 day before operation [pg/ml]	1.004	0.999	1.009	0.096
TIMP-1 1 day before operation [ng/ml]	1.001	0.999	1.003	0.318
TIMP-2 1 day before operation [ng/ml]	0.998	0.995	1.001	0.256
CRP 1 day before operation [mg/l]	0.743	0.552	1	0.049
VEGF-A 8-10 days after operation [pg/ml]	1.001	0.998	1.003	0.724
VEGF-C 8-10 days after operation [pg/ml]	5.089	1.164	22.257	0.031
VEGF-D 8-10 days after operation [pg/ml]	1.002	0.998	1.007	0.275
TIMP-1 8-10 days after operation [ng/ml]	1.001	0.998	1.004	0.661
TIMP-2 8-10 days after operation [ng/ml]	1.001	0.999	1.002	0.525
Reduction in concentration after operation: VEGF-A [pg/ml]	0.999	0.998	1.002	0.716
Reduction in concentration after operation: VEGF-C [pg/ml]	1.023	0.545	1.922	0.943
Reduction in concentration after operation: VEGF-D [pg/ml]	1.001	0.997	1.006	0.602
Reduction in concentration after operation: TIMP-1 [ng/ml]	1.001	0.999	1.003	0.369
Reduction in concentration after operation: TIMP-2 [ng/ml]	0.999	0.998	1.001	0.24

Tab. 7. Concentrations of markers of inflammation and angiogenesis and the risk of recurrence of prostate cancer after radical surgical treatment after adjustment for anthropometric variables, concentrations of angiogenic and inflammatory factors and the state of operating margins

Variable	HR	95% confidence interval for HR		p
VEGF-A 1 day before operation [pg/ml]	0.993	0.982	1.004	0.2
VEGF-C 1 day before operation [pg/ml]	0.378	0.091	1.559	0.178
VEGF-D 1 day before operation [pg/ml]	1.002	0.997	1.008	0.412
TIMP-1 1 day before operation [ng/ml]	1.001	0.999	1.003	0.283
TIMP-2 1 day before operation [ng/ml]	0.998	0.995	1.001	0.175
CRP 1 day before operation [mg/l]	0.652	0.443	0.959	0.03
VEGF-A 8-10 days after operation [pg/ml]	1.001	0.998	1.004	0.659
VEGF-C 8-10 days after operation [pg/ml]	15.275	0.98	238.138	0.052
VEGF-D 8-10 days after operation [pg/ml]	1.0003	0.995	1.005	0.887
TIMP-1 8-10 days after operation [ng/ml]	1.001	0.999	1.002	0.29
TIMP-2 8-10 days after operation [ng/ml]	1.001	0.998	1.003	0.51
Reduction in concentration after operation: VEGF-A [pg/ml]	1.0002	0.998	1.002	0.825
Reduction in concentration after operation: VEGF-C [pg/ml]	0.789	0.352	1.767	0.565
Reduction in concentration after operation: VEGF-D [pg/ml]	1.001	0.997	1.005	0.621
Reduction in concentration after operation: TIMP-1 [ng/ml]	1.0003	0.998	1.003	0.841
Reduction in concentration after operation: TIMP-2 [ng/ml]	0.999	0.997	1.001	0.188

surgery, CRP concentration 1 day before surgery, and VEGF-C concentration 8-10 days after surgery were significantly associated with recurrence of prostate cancer after radical prostatectomy (Table 6).

After taking into account anthropometric variables (age, weight, height, BMI), concentration of all angiogenic and inflammatory factors and postoperative margins in Cox proportional hazard models, only CRP concentration 1 day before surgery was significantly associated with the risk of prostate cancer recurrence after radical prostatectomy (Table 7).

Pattern of failure

During the follow-up of all 82 patients (mean follow-up time 45 months), biochemical recurrences were found in 27 patients (32.9%, recurrence onset time: 6-36 months, average 16 months).

DISCUSSION

Prostate cancer is a serious public health problem in highly developed countries. In Western and Northern Europe, prostate cancer is the most frequent solid tumor in men, and its incidence is increasing. The results of treatment of patients with prostate cancer are better when the cancer is diagnosed early and the treatment started early. Similarly, a faster diagnosis of prostate cancer recurrence is associated with a better prognosis. After effective radical prostatectomy, the PSA concentration should be undetectable after 6 weeks from surgery. In clinical practice, an early symptom of recurrence is an increase in serum PSA in subsequent determinations (>0.2 mg/ml) the so called biochemical recurrence [8]. Biochemical recurrence after radical radiotherapy is diagnosed when the PSA concentration rising in successive determinations exceeds the nadir (regardless of its value) by >2 mg/ml [9]. Biochemical recurrence is not identical to clinical recurrence, i.e. the appearance of prostate cancer foci in imaging studies; these foci may or may not cause symptoms. The use of current biochemical recurrence criteria allows the diagnosis of prostate cancer recurrence most often after many months after surgery. This delays the start of adjuvant treatment

and worsens the treatment results. Currently, the D'Amico scale is used to predict biochemical recurrences of prostate cancer, which includes clinical advancement on the TNM scale, Gleason score on malignancy, and blood PSA concentration [10]. The aim of this study was to check whether preoperative concentrations of markers for angiogenesis, lymphangiogenesis and extracellular matrix degradation can predict biochemical recurrence of prostate cancer after radical prostatectomy. The validity of the study results from the fact that inflammatory processes affect the proliferation of cancer cells, angiogenesis, infiltration and formation of distant metastases [19]. Because previous studies have suggested that C-reactive protein, VEGF, metalloproteinases and tissue inhibitors of metallopreoteinases are involved in the progression of tumors, these factors were analyzed in the study.

In this study, during an average follow-up of 45 months, the biochemical recurrence criterion was met in 27 out of 82 patients, which is 32.9% and is consistent with the reports of other authors (range 27%-53%) [20].

As in previous studies, cancer stage in a histopathological examination of the tumor (pT) was not associated with a risk of biochemical recurrence of prostate cancer. For example, in a study by Freedland et al., patients with pT2aN0 and pT2bN0 prostate cancer had a similar risk of biochemical recurrence after radical tumor removal [21]. This study confirms earlier observations that a higher Gleason score in preoperative biopsy (statistically insignificant relationship) and in postoperative material (statistical trend), as well as the presence of positive operating margins, are associated with a higher risk of biochemical recurrence of prostate cancer. In the Hashimoto et al. study, five-year survival without a biochemical recurrence of prostate cancer after radical prostatectomy was about 80% in patients with negative surgical margins compared to about 60% in patients with positive surgical margins [22]. Similarly, in the study of Blute et al., conducted in over 2,500 patients with prostate cancer after radical prostatectomy, more than 80% of patients with positive surgical margins experienced a biochemical recurrence compared to less than 70% in patients with negative surgical margins [23].

In our study, a smaller volume of prostate gland was associated with a higher risk of biochemical recurrence. Previous research suggests that there is a correlation between a smaller prostate volume and more aggressive tumor, the occurrence of histological stage, and the presence of positive surgical margins after prostatectomy. In a study by Newton et al., conducted in nearly three thousand patients with prostate cancer, the size of the prostate gland measured during prostatectomy was negatively related to the histopathological stage of the cancer-the smaller the volume of the gland, the more malignant the tumor [24]. In a subsequent study, similar observations were made-the larger the prostate gland in the TRUS study, the less likely it was to diagnose prostate cancer in a biopsy, and the lower the Gleason score [25]. Perhaps the smaller prostate is the result of a low androgenic microenvironment, which favors less androgen-dependent prostate carcinomas with higher malignancy. In a study by D'Amico et al., conducted in more than 800 patients after radical prostatectomy, prostate volume over 75 ml was a beneficial prognostic factor and was associated with a lower risk of biochemical recurrence-none of the patients with a prostate volume above 75 ml had a biochemical recurrence over four years years after surgery [26]. On the other hand, a larger tumor volume is associated with a higher risk of biochemical recurrence of prostate cancer. In Ates et al., patients with recurrent biochemical prostate cancer had significantly higher tumor volume compared to patients without biochemical recurrence [27]. In univariate analyzes corrected for age and BMI, preoperative and postoperative concentrations of the investigated markers of inflammatory processes (CRP), angiogenesis and lymphangiogenesis (VEGF-A, VEGF-C, VEGF-D) and remodeling of extracellular matrix (TIMP-1, TIMP-2), were not associated with a risk of biochemical recurrence of prostate cancer after radical surgical treatment. The concentrations of these markers were not related to the Gleason score in the pre-operative biopsy and postoperative material, or the stage of cancer in histopathological examination.

The results of this study do not support the observation that the concentration of markers of inflammatory processes, angiogenesis and lymphangiogenesis, such as CRP and VEGF, is associated with an earlier biochemical recurrence of prostate cancer. For example, in a study by Nordby et al., conducted in over half a thousand patients after radical prostatectomy for prostate cancer, high expression of VEGF-A and VEGFR-2 in the tumor parenchyma was associated with almost twice the risk of biochemical recurrences [28]. Similarly, in a study by Hall et al., conducted among radiotherapy patients for prostate cancer, increased CRP concentration was associated with a shorter survival period free from biochemical recurrence. Similar conclusions can be drawn from a meta-analysis involving nine studies among almost 1,500 patients with prostate cancer-this meta-analysis showed that increased CRP concentration was associated with a worse prognosis after surgical removal of prostate cancer. Moreover, in the present study, the levels of these markers of inflammation and angiogenesis were not related to the histological parameters of prostate cancer (pT, Gleason score). Only TIMP-2 concentration was higher in patients with negative surgical margins than in patients with positive surgical margins. Perhaps a higher concentration of TIMP-2 lowering the activity of metalloproteinases inhibits the migration of tumor cells beyond the prostate gland. In analyzes corrected for anthropometric variables and concentrations of other inflammatory and angiogenic markers, preoperative VEGF-A and CRP levels, and VEGF-C concentration after surgery, were associated with the risk of biochemical recurrence of prostate cancer; however, these relationships were negligible after correcting the analysis for the presence of positive surgical margins and Gleason score. Therefore, the results of this study indicate that the concentrations of the tested markers (CRP, VEGF-A, VEGF-C, VEGF-D, TIMP-1, TIMP-2) are not independent predictors of biochemical recurrence of prostate cancer after radical surgical treatment. This study had limitations. A relatively small group of patients with prostate cancer was included in the study, and perhaps that is why there were no statistically significant relationships of the studied variables with the risk of biochemical recurrence of prostate cancer after radical prostatectomy. However, the study did not even notice any statistical trends that could suggest that in a larger test group, the markers of angiogenesis, lymphangiogenesis and

extracellular matrix degradation would prove to be predictors of biochemical recurrence of prostate cancer after surgery. The study did not include a control group of healthy volunteers, because the aim of the study was to determine whether the tested markers could predict the recurrence of prostate cancer, not to check whether patients with prostate cancer and healthy people have different concentrations of the tested markers. The advantage of this study is its prospective character, determination of concentrations of many markers and performing a multivariate analysis. In conclusion, the results of this study suggest that preoperative and postoperative concentrations of the investigated markers of inflammatory processes (CRP), angiogenesis, lymphangiogenesis (VEGF-A, VEGF-C, VEGF-D) and remodeling of extracellular matrix (TIMP-1, TIMP-2) are not significant predictors of biochemical recurrence of prostate cancer after radical prostatectomy. Further research is needed to identify patients with an increased risk of prostate cancer recurrence after radical prostatectomy that might benefit from earlier treatment.

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