

# VEGF Immunoexpression in Prostate Adenocarcinoma

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**ABSTRACT:** Angiogenesis is a basic biomolecular mechanism for tumor progression, the vascular endothelial growth factor (VEGF) being one of the main enhancers of this complex process. In this study, we analyzed VEGF-A immunoexpression in 61 prostate adenocarcinomas (PAs), related to the prognostic parameters of the lesions. VEGF scores were higher in PAs that associated serum PSA values above 20ng/ml, in tumors with pure complex or mixed growth patterns, as well as in high-grade and advanced lesions. The results obtained indicate the involvement of VEGF in prostate angiogenesis and the usefulness of the maker for the identification of aggressive lesions.

**KEYWORDS:** VEGF, Gleason score, prostate adenocarcinoma.

## Introduction

Prostate adenocarcinoma (PA) is, after lung, the second malignancy for male patients worldwide, with a maximum frequency after the age of 50 years [1,2].

However, the prognosis of PAs is relatively positive, mortality being reduced by easy access of patients to noninvasive screening programs. The introduction of serum prostate antigen (PSA) screening has led to an increase in the rate of diagnosis and thus the incidence of prostate adenocarcinoma, so that 50% of newly diagnosed cases are currently in a localized stage [2].

Most patients diagnosed with localized prostate adenocarcinoma are treated surgically and oncologically, with high cure rates, with worldwide mortality from this lesion gradually decreasing after 1990 [3].

However, there are subsets of PAs that are more aggressive, relapse, resistant to classical therapies and may metastasize. In this context, the identification of effective therapeutic targets to improve the prognosis of patients is a continuing concern. One of these mechanisms is represented by tumor angiogenesis that ensures survival and progression, including PAs, being considered a target mechanism for the treatment of lesions [4-6].

One of the most important proangiogenic factors is VEGF, respectively the VEGF-A type, with a role in increasing vascular permeability and stimulating endothelial proliferation [7].

In this study we analyzed VEGF immunoexpression in PAs in relation to the aggressiveness parameters of the lesions.

## Material and Methods

This study included 61 cases of prostate adenocarcinoma (PA) that were diagnosed in the Pathology Department of the Emergency County Hospital Craiova during a four years period (2016-2020) from operated patients in the Urology Clinic of the same hospital.

The biological material was represented by total prostatectomy specimens, which were fixed in 10% buffered neutral formalin, embedded in paraffin and stained with Hematoxylin-Eosin.

The classification and the assessment of PA was done accordingly to the criteria for tumors of the urinary system and male genital organs within the WHO (World Health Organization) issued by the specialized working group [8].

In the study were included only primitive PAs, without any history of oncological treatments or history of cancer with other locations. The analysis of VEGF immunoexpression was done in relation with the parameters of tumor aggressiveness represented by serum PSA (prostate serum antigen) values, histological type, growth pattern/classic Gleason score and tumor stage.

For the immunohistochemical detections, sections were deparaffinated in xylene, rehydrated in decreasing alcohol concentrations, blocking of endogenous enzymes and of the unspecific sites and antigen retrieval by microwaving in pH 9 Tris-EDTA buffer. The incubation with primary antibodies was done overnight at 4°C, respectively the monoclonal (clone VG1, Dako) mouse antihuman VEGF-A in dilution of 1/40. The detection system was

represented by EnVision™ FLEX System (code K8002, Dako). DAB (3,3'-diaminobenzidine tetrahydrochloride) from the same IHC detection kit was used as chromogen to visualize the reactions. To validate the IHC immunostainings, we used external positive control (kidney) and external negative control by the omission of the primary antibody.

The assessment of reactions was semiquantitative and was performed using a final staining score (FSS), which resulted in multiplying the number of labeled cells with the intensity of the reactions. According with the number of tumor labeled cells the scores were 1 (<25% positive cells), 2 (25-49% positive cells), 3 (50-74% positive cells) and 4 (≥75% positive cells), and in the case of intensity of reactions the scores were 1 (mild), 2 (moderate) or 3 (strong). For statistical analysis, VEGF immunoexpression was considered low for FSS scores with values of 1-4 and high if the scores were between 8-12.

For the absence of reaction, the FSS score was considered negative.

For the statistical analysis we used the comparison tests represented by one-way ANOVA and chi square ( $\chi^2$ ) within SPSS 10 software and for the values of  $p < 0.05$  the results were considered significant.

A Nikon Eclipse E600 microscope equipped with Lucia 5 software was used for the images acquisition.

The study was done after receiving the written informed consent of the patients, and with the approval of the working protocol by the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

## Results

The study indicated the predominance of prostate adenocarcinomas (PA) associated with PSA values between 20-50ng/ml (59%), most of the tumors being of the conventional type (78.7%), with a mixed growth pattern (45.9%), especially in the association of patterns 4 and 5 (7 cases), while the Gleason score 8 (37.7%) and pT2/stage II (44.2%) were the most frequently observed (Table 1).

In the study, the local tumor extension (pT category) coincided with the tumor stage.

Analysis of VEGF immunoexpression indicated the presence of the staining in 55 cases, which represented 90.1% of the group, the negative cases belonging to conventional/colloid PA, with variable Gleason score between 6-8 and being mainly in pT2/stage II.

The reaction was present at the tumor level, being inconsistently identified at the level of vascular endothelium and stromal elements represented by fibroblasts, lymphocytes, plasma cells or macrophages.

Also, the VEGF reaction was present in the non-tumor glandular areas with hyperplastic appearance, the mild intensity of the reaction being focal, in less than 15% of the cells.

For the whole group of analyzed positive VEGF tumors, the number of positive cells was between 15-90%, with a mean value of  $56.7 \pm 19.4$ , the intensity of the reactions was variable and the mean value of FSS was 6.8.

The VEGF reactions showed some differences in relation to the analyzed parameters. The highest VEGF scores were identified in patients with PSA values of at least 20nm/ml.

Thus, for the group of PSA values of 20-50ng/ml, the average number of labeled cells was  $56.2 \pm 18.2$ , the intensity of the reactions was mostly moderate/strong, the average value of FSS was 5.7, while for the group with values above 50ng/ml, the number of labeled cells was  $70.8 \pm 9.5$ , the intensity of reactions mainly strong, and with an FSS value of 9.3.

For both categories, high scores predominated, respectively in 75.7% and 91.7% of cases.

By comparison, for the groups with  $PSA \leq 10$ ng/ml and 11-19ng/ml, the mean values of the number of labeled cells were  $50 \pm 21.7$  and  $28.8 \pm 14.4$ , the intensity of the reactions was variable, the mean values of FSS 5.7 and 3.2, predominating low VEGF scores, respectively in 66.7% and 75% of cases (Table 1).

**Table 1. The distribution of cases and the average values of VEGF final staining scores (FSS) depending on the analyzed parameters.**

Parameters		VEGF (FSS average value)	VEGF FSS level (No. cases)	
			Low	High
Serum PSA (ng/ml)	≤10	5.7	4	2
	11-19	3.2	3	1
	20-50	6.7	8	25
	>50	9.3	1	11
Histologic type	conventional	7.1	12	31
	ductal	9	0	4
	foamy cells	4.7	1	2
	colloid	6	0	1
	atrophic	1.5	2	0
	pseudohyperplastic	1	1	0
	sarcomatoid	9	0	1

<b>Growth pattern (pure and mixed)</b>	pattern 3	2.5	9	2
	pattern 4	7.8	3	9
	pattern 5	9.8	0	6
	mixed	7.4	4	22
<b>Gleason score</b>	score 6	2.6	9	2
	score 7	5	4	3
	score 8	8.1	3	17
	score 9	8.5	0	11
<b>Tumoral extension (pT) / Tumor stage</b>	pT1/ stage I	2.4	7	1
	pT2/stage II	6.9	6	18
	pT3/stage III	8.3	16	39

Compared to the tumor type, the highest scores of VEGF reactions were present in conventional, ductal and sarcomatoid PA, in which the mean number of labeled cells was  $59.7 \pm 19$ ,  $62.5 \pm 12.6$  and 50%, the intensity of reactions predominantly moderate/strong, with mean FSS values of 7.1, 9 and 9, in conventional PA predominating high FSS (72.1% cases), while for the other two types all positive cases

showed high FSS. For the types with foamy cells, colloid, atrophic and hyperplastic the mean values of FSS were between 1-6, with a mean number of labeled cells of  $45 \pm 5$ , 55, 22.5 and 20, with predominantly mild/ moderate reaction intensity and FSS values predominantly high for foamy cell and colloid types and low for atrophic and hyperplastic types (Table 1).

The analysis of the reactions in relation to the growth patterns indicated in the case of pure pattern 3 an average number of marked cells of  $31.4 \pm 14.5$ , intensity of reactions especially mild/ moderate, and with the average FSS value of 2.5, 81.8% of cases, presenting low VEGF scores (Figure 1A).

By comparison in the pure and mixed patterns 4 and 5 we found the predominance of high VEGF scores, respectively in 75%, 100% and 84.6% of cases. In these cases the mean number of labeled cells was  $65 \pm 15.7$ ,  $73.3 \pm 16.6$  and  $60.4 \pm 13.5$ , predominantly moderate/strong reaction intensity and mean FSS values of 7.8, 9.8 and 7.4 (Figures 1B-D).

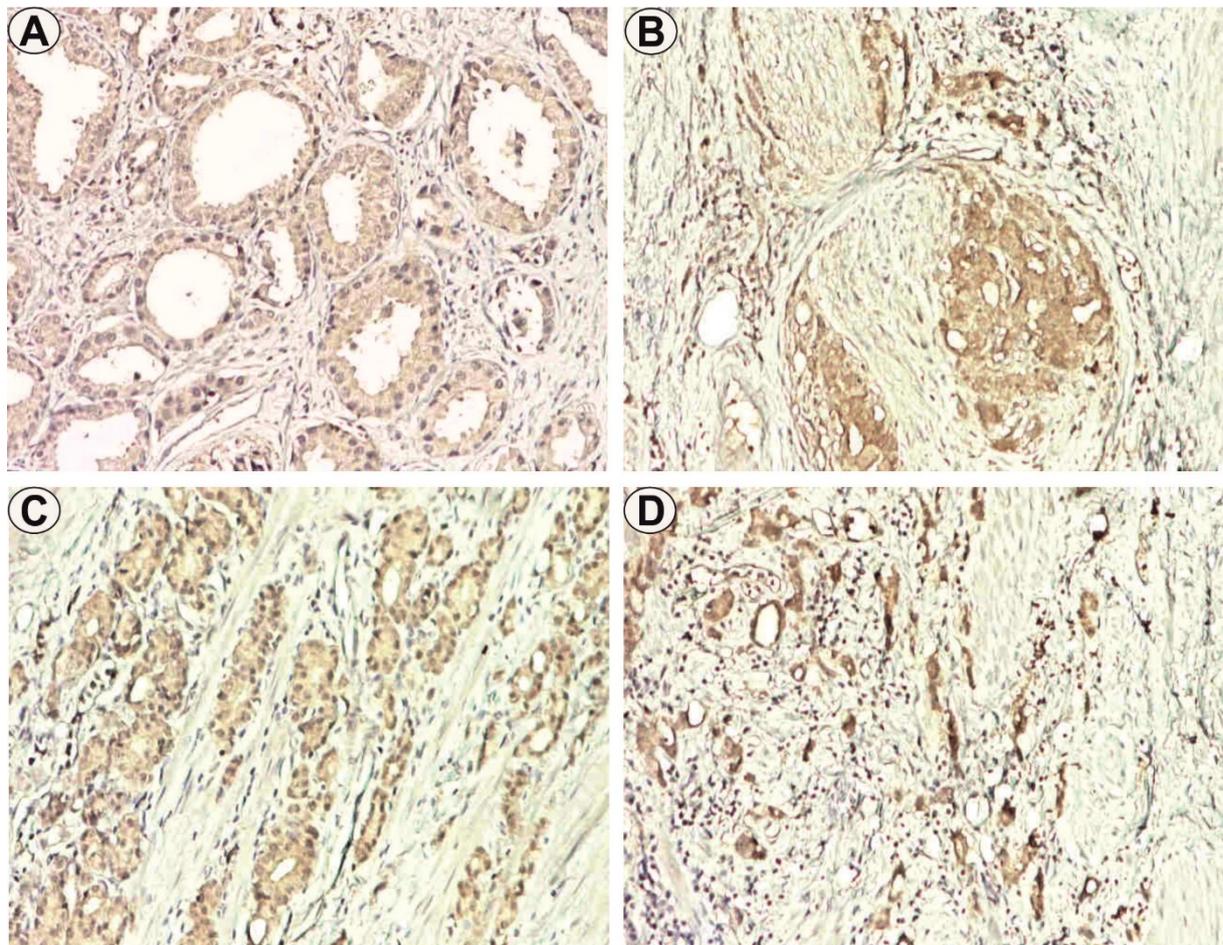


Figure 1. Prostate adenocarcinoma, VEGF immunostaining, x100. A. Growth pattern 3; B. Growth pattern 4; C. Mixed growth pattern 3 and 4; D. Mixed growth pattern 3 and 5.

Compared to the Gleason score, we found differences between the values 6-7 and 8-10.

Thus, for Gleason 6 and 7, the mean number of labeled cells was  $32 \pm 15.1$  and  $49.3 \pm 8.4$ , with predominantly mild/moderate reaction intensity and mean FSS values of 2.6 and 5, with most cases having low VEGF scores, respectively in 81.8% and 57.1% of cases.

In contrast, in the case of Gleason scores 8-10, the mean number of marked tumor cells was  $65.3 \pm 15.2$ ,  $64.5 \pm 9.1$  and  $73.3 \pm 16.3$ , the predominantly moderate/strong reaction intensity and mean FSS values of 8.1, 8.5 and 9.8, the most cases presenting high VEGF scores, respectively 85%, 75% and 100% of cases.

VEGF expression in pT1/stage I tumors showed a mean number of labeled cells of  $35 \pm 31.3$ , mild/rarely moderate intensity

reactions, with a mean FSS value of 2.4, 87.5% of cases and low VEGF scores.

By comparison, in the case of pT2/stage II and pT3/stage III tumors, the mean number of positive cells was  $56.9 \pm 18.1$  and  $65.4 \pm 14$ , with predominantly moderate/strong reaction intensity and mean FSS values of 6.9 and 8.3, the most cases presenting high VEGF scores, respectively 75% and 86.9% of cases.

The statistical analysis indicated non-significant differences of VEGF scores in relation to tumor type ( $p=0.132$ ,  $\chi^2$  test) and significant differences in relation to PSA values ( $p=0.011$ ,  $\chi^2$  test), tumor growth pattern ( $p<0.001$ ,  $\chi^2$  test), Gleason score ( $p<0.001$ ,  $\chi^2$  test) and pT/tumor stage ( $p<0.001$ ,  $\chi^2$  test), high scores being associated with PSA values above 20ng/ml, growth patterns 4, 5 and mixed, Gleason scores 8-10 and pT/tumor stage I/II (Figures 2 A-D)

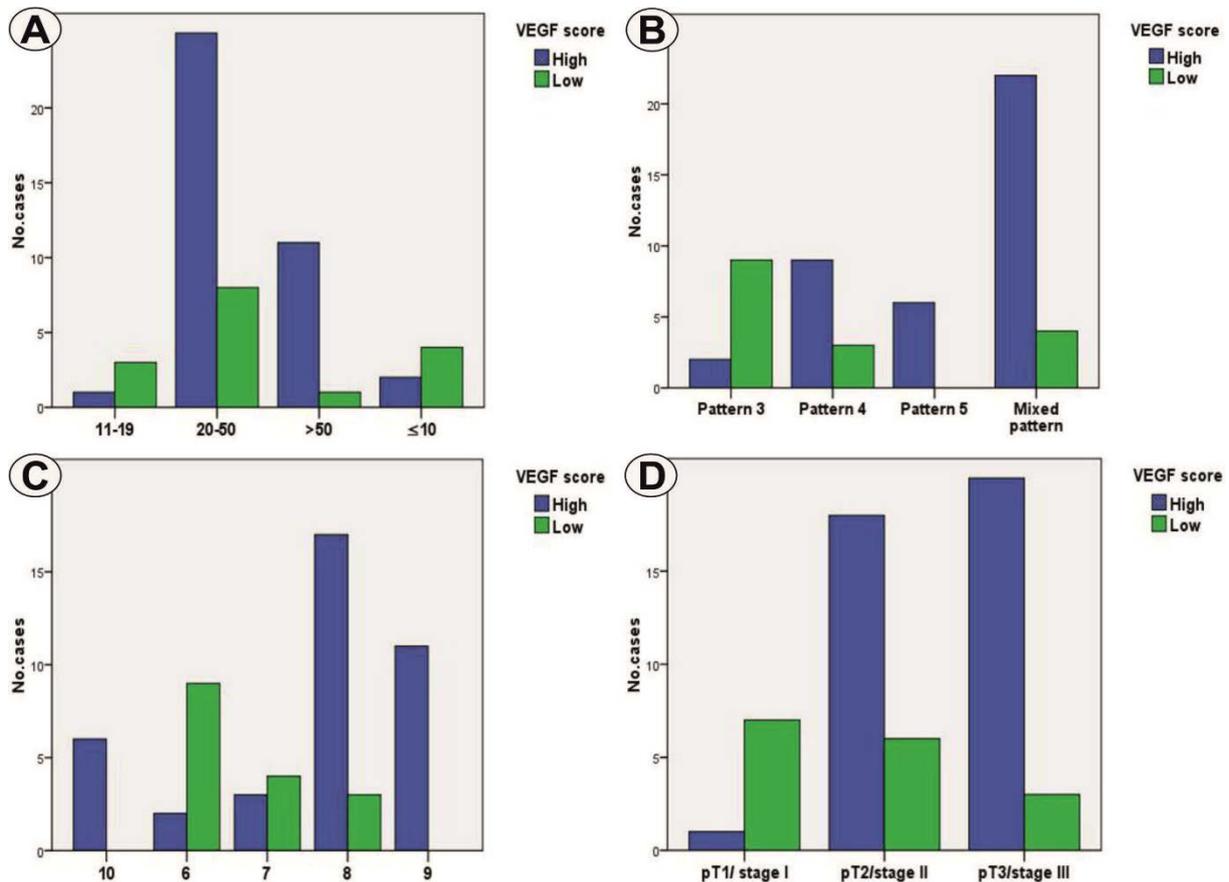


Figure 2. Distribution of cases depending on VEGF scores and PSA levels (A), growth pattern (B), Gleason score (C) and pT/tumor stage (D).

## Discussions

Tumor growth is angiodependent, after the appearance of a tumor, any subsequent growth of it, requiring the appearance of new blood vessels [9].

Experimental studies have shown that primitive non-angiogenic tumors remain clinically undetectable in size, and metastases are restricted in size and functionally dormant [9].

Tumor angiogenic switch occurs when proangiogenic factors are no longer counteracted by angiogenesis inhibitory factors, respectively when there is an imbalance between pro-angiogenic and antiangiogenic factors released by tumor cells and stromal cells from the tumor microenvironment [9].

In the animal model, the angiogenic switch proved to be a slow process, which develops during carcinogenesis and tumor progression and which is triggered from the stage of precancerous lesions [10].

In the vast majority of human tumors it has been found that the angiogenic phenotype is expressed after the tumor acquires all the characteristics of the malignant phenotype [11].

There are numerous proangiogenic factors that potentiate the formation of new vessels at the tissue level, including VEGF (vascular endothelial growth factor) and analogous receptors (VEGFR1, VEGFR2), FGF (fibroblastic growth factors  $\alpha$  and  $\beta$ ), PDGF (platelet-derived growth factor), ANG (angiopoietins 1 and 2), tumor necrosis factor (TNF $\alpha$ ), interleukin 8 (IL-8), granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), lectin, proliferin [7].

The autocrine and paracrine mechanisms involved in tumor angiogenesis, including in the prostate, allow the release of proangiogenic factors by both tumor cells and stromal elements of the tumor microenvironment, represented by lymphocytes, plasma cells, mast cells, macrophages, fibroblasts and even endothelial cells [11,12].

The family of proangiogenic dimeric polypeptides VEGF includes the seven described members, of which VEGF-A is the most important promoter of prostate tumor angiogenesis, with a role in stimulating vascular permeability and endothelial cell proliferation [7,13].

In the form of various isomorphs, VEGF-A, also called vascular permeability factor (VPF),

performs its functions by binding to receptors 1 and 2 (VEGFR1/Flt1 and VEGFR2/Flk1) [11].

Angiogenesis is an important process for the growth, progression, and metastasis of prostate adenocarcinomas.

The quantification of this process is achieved by assessing the tumor microvascular density, which seems to be stimulated by numerous proangiogenic factors including VEGF [14].

Some studies performed on prostate cancer cell lines have indicated that VEGF is involved in stimulating cell proliferation and angiogenesis, one of the mechanisms being the stimulating effect of tumor necrosis factor found in the stroma of PA [15].

At the prostate level, VEGF expression is also mediated by the androgen receptor and may involve activation of oncogenes [4].

Data from the literature on the significance of VEGF immunorexpression in prostate adenocarcinomas are controversial.

Thus, although VEGF values are higher in cases of metastasis compared to localized disease, the appearance does not seem to have a prognostic potential [16].

Other studies did not identify relationships of VEGF expression with age, serum PSA level and Gleason score [17].

Also, Pan L et al. did not identify any association of intensity of VEGF immunoreactions with prognosis, metastatic disease or biochemical failure in PAs [18].

In our study, PAs that were VEGF positive accounted for 90.1% of the analyzed group, the reactions being higher in number of labeled cells compared to the hyperplastic areas associated with tumors.

The percentage is similar to other studies that indicated a positivity rate more frequently between 80-100% [15,19].

At the same time, there is a certain consensus of the results in the literature in the sense of superior positive VEGF reactions in the case of cancers compared to normal areas, benign nodular hyperplasia or precancerous lesions [15,19].

In our study, higher VEGF scores were associated with high serum PSA values, high or mixed growth patterns, high Gleason scores, and advanced stages.

There are numerous studies that have indicated the association of VEGF expression with lesion aggression parameters.

Thus Rivera-Pérez J et al. indicates intense and diffuse reactions of VEGF in the case of high-grade tumors, respectively in Gleason 8-10,

without being able to differentiate between degrees 6-7 and benign nodular hyperplasia [5].

Other studies have also identified the association of VEGF overexpression with high Gleason score, vascular invasion and metastatic PA compared to those localized, after radical prostatectomy [19-21].

In an extensive study in which markers of prostatic hypoxia and tumor angiogenesis were analyzed, Vergis R et al. indicated that increased VEGF expression is associated with biochemical failure, independent of tumor stage, Gleason score and status of resection margins after prostatectomy [22].

These contradictory results may be due to the small and heterogeneous groups investigated, VEGF clones or different methods of semi-quantitative quantification [18].

Although there is controversial data on the prognostic significance of tumor microvascularization and VEGF expression in prostate adenocarcinomas related to aggression and metastasis, there is clinical evidence that antiangiogenic therapy can be used in the treatment of these lesions [14,23].

This therapy, which can inhibit proangiogenic growth factors and their receptors, seems to genomically normalize endothelial cells, thus cytotoxic therapy being one with superior efficacy, although there are some data related to resistance to antiangiogenic agents [24].

## Conclusions

In this study, VEGF immunoexpression was present in most cases, the high scores reaction being associated with high PSA values, pure complex or mixed tumor growth patterns, and high-grade, advanced-stage tumors.

The results obtained indicate the involvement of VEGF in prostate angiogenesis and suggest the usefulness of the marker in assessing the angiogenic potential and aggressiveness of lesions.

## Conflict of interests

None to declare.

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