

VASCULARIZATION IN PROSTATIC CARCINOMA

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Abstract

Background: Angiogenesis means the creation of new blood vessels, a critical natural process that occurs in the body both in health and in disease.

Objective: Quantitative assessment of tumor vascularization in different grades of prostatic tumors.

Methods: 23 paraffin blocks of prostatic biopsies (8 cases of benign prostatic hyperplasia and 15 cases of prostatic carcinoma equally distributed in well, moderately and poorly differentiated (Gleason's grade 2, 3 and 4&5 respectively). Processed routinely and stained with hematoxylin and eosin, elastica Van Gieson, and Masson Trichrome stain. The vascular surface density (VSD), the microvessels number (NVES), and the maximum microvessels number (NVES-MAX) was assessed by means of stereology, and the

results were related to grade of tumor differentiation.

Results: NVES and NVES-MAX showed a significant increase with rising tumor grade ranging from 16.1 in BPH to 109.0 microvessels/mm² in poorly differentiated (grade 4&5) tumors. Discrimination of different tumor grades was more accurate with NVES-MAX. The VSD was significantly higher in low-grade tumors compared with BPH, whereas there was continuous decrease from low grade (11.6mm⁻¹) to high-grade tumor areas (5.1 mm⁻¹).

Conclusions: The present study shows a correlation between tumor grade and vascularization.

Key words: Prostate cancer, angiogenesis, vascularization.

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Introduction

Rapid cellular proliferation in malignant tumors requires nutritional support guaranteed by a sufficient vascular bed and unrestricted growth of tumors is dependent upon angiogenesis^[1,2]. Recent studies have clarified the molecular basis of tumor neovascularization, a process mediated by several growth factors^[3]. Angiogenic activity first appears in a subset of hyperplastic tissue before the onset of tumor formation^[4,5]. Hyperplasia *per se* does not obligate angiogenesis. Rather, a few hyperplastic tissues become angiogenic correlates closely with subsequent tumor incidence^[4,6]. The vessels surface in a tumor is a putative target of tumor cell adhesion and invasion, and consequence local and

systemic spread^[5-8]. On light microscopic level, the process of tumor angiogenesis results in an increase number of vessels within the tumor tissue, and areas of maximum vascularization, called hot spots^[9,10].

Materials & Methods

This study comprises of 23 paraffin blocks of prostatic biopsies (8 cases of benign prostatic hyperplasia and 15 cases of prostatic carcinoma equally distributed in well, moderately and poorly differentiated (Gleason's grade 2, 3 and 4 or 5 respectively). Fixed in 10% buffered formalin processed routinely and stained with hematoxylin and eosin, elastica Van Gieson, and Masson Trichrome stain.

Stereological measurements: Areas of unequivocal tumor tissue were marked on the cover slide. Within the tumor 10 areas were randomly chosen for

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assessment of the vascular surface density (VSD), microvessel number (NVES), and Maximum Microvessel number (NVES-MAX)^[5].

VSD: The vascular surface density and the stromal volume portion were assessed by means of stereology at a x160 microscopic magnification^[5].

An ocular square lattice (periplan x10, leitz Wetzlar, Germany) with 121 points composed of 11 horizontal and 11 vertical test lines with line length ($L_g=2.6875\text{Mm}$) was superimposed on the test fields to be measured, and the number of intersections (I_a) between the test lines and labeled vessels walls was counted (Figure 1). VSD was assessed according to (Barth et al 1996)^[5].

$$\text{VSD} = E I_a \cdot 2, \text{LR.Vv (STR)}$$

Microvessel number (NVES); in the same procedure step, the number of vessel (N) within the measuring field was counted and the number of vessels per mm² stroma (NVES) was computed according to

$$\text{NVES} = N/V_v \text{ (STR)}$$

Maximum Microvessel number (NVES-MAX)^[5]:

To simulate hot-spot measurements, maximum microvessels counts were computed from the arithmetic mean of microvessel counts in the tumor areas that yielded the 30% highest values.

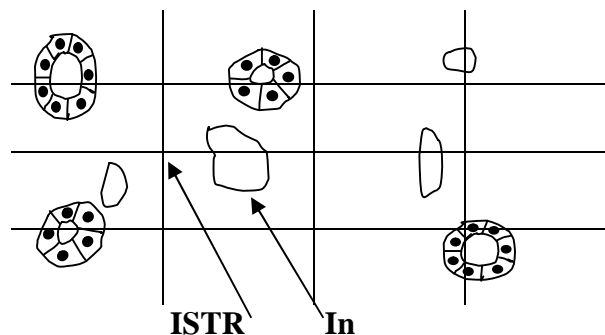


Figure1: Schematic drawing indicating the assessment of the stereological parameters VSD derived from the counts of intersections between test-lines and vessel walls (I_n) and points superimposed on the stromal compartment (ISTR).

Statistical analysis: Differences between study groups were performed by the analysis of variance procedure; $P < .05$ was defined to be a statistically significant value.

Results

In low-grade tumor, the vessels formed well-branched network of small capillary vessels, whereas in the intermediate and high-grade tumor the vascular bed was mainly composed of plump less branched vessels (Figures 2 and 3). In BPH, the average number of the vessels (NVES) and (NVES-MAX) was found to be $15.4 \pm 1.12 \text{ mm}^{-2}$ and 16.1 mm^{-2} respectively.

In prostatic carcinoma, NVES and NVES-MAX increased significantly with rising tumor grade. However, no statistically significant difference could be confirmed between moderately (G3) and poorly differentiated (G4&5) carcinoma (Table 1).

Assessment of the VSD yielded completely different results. In well-differentiated tumors, the VSD reached value significantly higher compared with BPH (11.6 Vs 5.2). While in moderately and poorly differentiated carcinoma, significantly lower values compared with well-differentiated tumors were obtained, and not significantly different from that of BPH (Table 1).

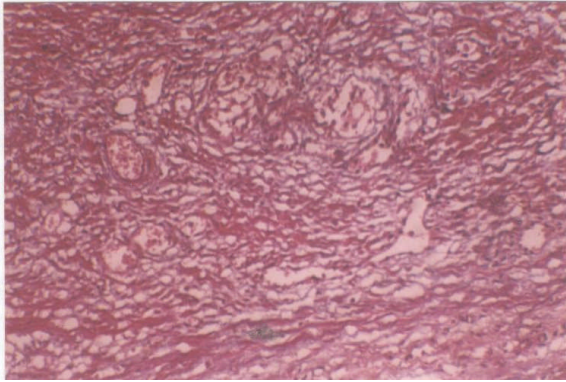


Figure 2: Morphology of low-grade prostatic carcinoma (G2). In this area the NVES is 58, the VSD is 16.3mm^{-1} (Trichrome stain)

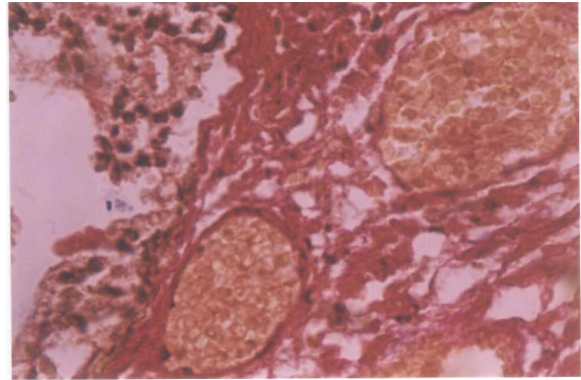


Figure 3: Morphology of high-grade prostatic carcinoma (G4). In this area the NVES is 110, the VSD is 7.3mm^{-1} (Trichrome stain)

Table 1: Vessel Parameters in Different Prostatic carcinoma grades

Method	BPH	Well differentiated carcinoma (G2)	Moderately differentiated carcinoma (G3)	Poorly differentiated carcinoma (G4&5)
VSD(mm^{-1})	5.2+0.2	11.6+1.3	9.2+3.1	5.1+0.6
NVES(mm^{-2})	15.4+1.1	52.7+23.6	75.7+22.6	105.0+0.4
NVES-MAX(mm^{-2})	16.1+2	69.8+21.1	97.5+19.6	109.0+0.4

P values < 0.5.

Discussion

The present study shows a correlation between tumor grade and vascularization, In low grade lesions (G1) the microvessel number increased with increasing tumor grade and data concerning the maximum microvessel number correspond well to those already published^[5,7-11]. In contrast to counts of vessel profiles per area, the assessment of VSD showed no significant correlation to tumor grade. This behavior of the vascular surface density is not specific for prostate cancer and also has been reported in renal cell carcinoma^[5,9]. In contrast to the microvessel number, the VSD concerns the physiological status of the vasculature as it quantifies the vascular surface available for substrate diffusion^[4,5].

In normal tissue and low-grade tumors, the microvessel are well branched and show a high surface/vessel ratio,

whereas the microvessel in high grade lesions are plump and non-branched, disclosing a low surface/vessel ratio^[5,11]. Although in high-grade lesions the vascular surface is decreased, the vascular bed serves its function to supply the tumor tissue^[5,8]. Possible explanations are an increased permeability of the vessel wall and changes of the tumoral vascular bed, which compensates the reduced vascular surface^[5].

Counts of microvessel profiles per area unit tissue section provide a significant parameter for the prediction of tumor grade, whereas the VSD primarily reflects the vascular geometry and is only weakly related to the tumor grade. Nevertheless, both methods provide valuable information in studies concerning tumor angiogenesis.

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