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Histological determinants of the vascular surface in prostatic carcinoma

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Abstract This study was performed to analyse the correlation between vascular surface (VS), tumour grade and stage and relative proportion of tumour cells within the tumour stroma. Specimens of 41 prostatic carcinoma were immunostained using Factor VIII-related antigen. The VS was assessed by means of stereology. In tumour-free prostatic tissue the VS was 6.7 ± 0.4 mm^{-1}. In pT2 tumours this value was significantly increased to about 12 mm^{-1}. With rising pT stage the VS significantly decreased to values of 4 in pT4 tumours. In G1 tumours the VS was 14.6 mm^{-1} and significantly decreased with decreasing grade of differentiation. No significant difference was obtained between pN0 and pN+ cases. A close positive correlation ($r = 0.59$, $P < 0.001$) existed between the VS and the relative proportion of tumour cells within the tumour, whereas a strong negative correlation was found between the VS and the relative amount of tumour stroma ($r = 0.81$, $P < 0.001$). The VS mainly depends on tumour differentiation and pT stage, i.e. the tumour size and the relative proportion of stroma and tumour cells within the tumour. These results are consistent with those obtained in experimental tumours. Assessment of the VS is therefore of interest in studies of tumour biology; it is of no use in predicting lymph node metastasis.

Key words Prostatic cancer · Vascularization · Morphometry · Vascular surface

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Introduction

Based on the concept that tumour growth is angiogenesis-dependent a large number of studies concerning this aspect of neoplasia have been performed [6]. Investigations aiming at the identification of the determinants of tumour angiogenesis have predominantly been focused on biochemical and molecular aspects such as the synthesis and secretion of certain cytokines, which have been attributed a significant role in the regulation of angioproliferation [4, 5, 8, 17]. The hypothesis that quantitative parameters of the tumoural vascular bed might serve as a prognostic tool has generated several studies. Although different methodological approaches have been taken the published data suggest that tumour vascularization is of a putative prognostic significance [2, 3, 18]. The aforementioned studies were based on either random [3] or "hot spot" [18] counts of vessel profiles per unit area of tissue. However, this methodological approach has some serious disadvantages [1]. First, the number of vessel profiles per unit area of a histological section depends on the tortuosity of the vessels; in consequence what the observer estimates is not directly related to the number of vessels or the vessels' surface. In the worst case what is measured is the degree of tortuosity of the vessels. Given the case of a tortuous vessel, the number of vessel profiles per unit area additionally depends on the section angle at which the vessel is cut. Second, the dimension of the parameter "number of vessel profiles (n) per unit area (m^2)" is of no physiological significance and it is uncertain whether this parameter is significantly related to the vascular surface which serves as a target of tumour cell adhesion and penetration [1].

A second source of systematic errors is the amount of stroma within the tumours. This parameter differs significantly between and within individual tumours [1]. In recent publications we therefore proposed the stereological assessment of the vascular surface (VS) [9, 16] and correction of the data for differing amounts of tu-

mour stroma as a valuable tool for the quantitation of tumour vascularization [1, 9]. The present study analyses the correlation between VS in prostate cancer and tumour differentiation, tumour stage and nodal status. Furthermore we explicitly analyse the correlation between the amount of tumour stroma and the VS.

Materials and methods

A total of 41 male patients were studied. They were aged between 45 and 79 years and had undergone radical prostatectomy for prostatic cancer. The patients' characteristics are summarized in Table 1. Immediately after resection the prostates were immersed in a 4% buffered (pH 7.4) formalin solution for fixation. Thereafter the organs were sliced in the frontal plane to sections of about 1 cm thickness, representative tissue blocks were selected for routine investigations and processed. Whole-mount sections comprising the complete prostatic tissue obtained from surgery were prepared in order to assess tumour heterogeneity.

Tumour grading

Tumour grading was based on the principles described by Mostofi and coworkers [10, 11]. Areas composed of glands of about normal size or areas of papillary differentiation were classified as low-grade carcinomas (G1) and G2 (intermediate grade) were tumour formations with fused or cribriform glands. In G3 (high-grade) tumours the tumour tissue was composed of solid cell nests or scirrhous tumour formations with little or abortive gland formation. Of the 41 tumours investigated 19 were heterogeneous, i.e. the tumour was composed of subareas disclosing more than one grade: 15 tumours showed two different grades and four tumours showed three different grades. The prostatic tissue of 7 age matched men suffering from mild prostatic hyperplasid served as control.

Assessment of VS: labelling of tumour vessels

Vessel walls were labelled immunohistochemically using Factor VIII-associated antigen (DAKO, Hamburg, Germany). Although *Ulex europaeus* agglutinin I (UEA) has been reported to be the superior method [12] we chose Factor VIII labelling because UEA also reacts with a varying amount of tumour cells in prostatic carcinoma.

Stereological measurement of VS

The VS and the stromal volume proportion were assessed by means of stereology at 160 × microscopic magnification using an ocular square lattice (Periplan 10 ×, Leitz, Wetzlar, Germany) composed of 11 horizontal and 11 vertical test lines with known total test line length (13750 μm = L_R, area = 0.36 mm^2, 121 grid points). The number of intersections (I_n) between the test lines and labelled vessel walls was counted and the volume proportion of stroma [$V_{(STR)}$], epithelium [$V_{(EPI)}$] and glandular lumen [$V_{(LUM)}$] was assessed by point counting (Fig. 1). The relative proportion of the stromal compartment [$V_{(STR)}$] within the microscopic measuring field was then computed according to:

$$V_{(STR)} = I_{STR}/1.21$$

(I_{STR} = the number of lattice points superimposed on prostatic stroma).
The relative volume proportion of the epithelial [$V_{(EPI)}$] and luminal compartment [$V_{(LUM)}$] were analogously computed:

$$V_{(EPI)} = I_{EPI}/1.21$$

(I_{EPI} = the number of lattice points superimposed on epithelial cells).

Table 1 Synopsis of patients data (pT, pN). pN+ comprises pN1 and pN2 cases

pN/pT	pT2	pT3	pT4
pN0	5	16	1

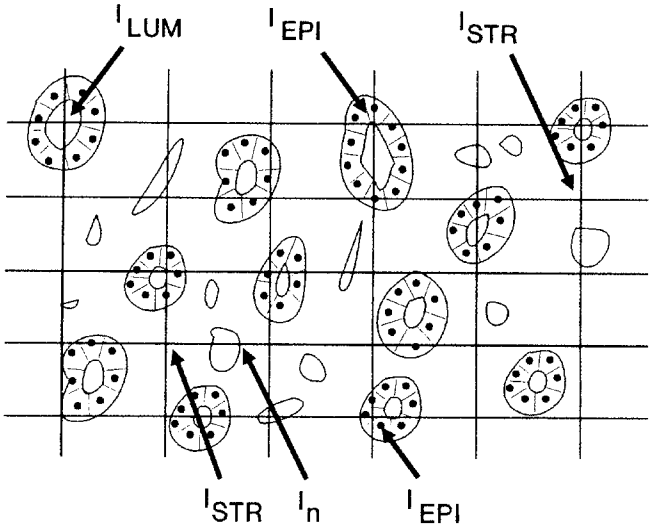


Fig. 1 The method for the stereological assessment of vascular surface and tissue composition. The test lattice is composed of horizontal and vertical lines of defined length and 121 grid points. For the computation of the vascular surface the number of intercepts between vessel walls and test lines was counted. The computation of tissue compartments was based on counts of grid points located on the different compartments. *LUM* lumen, *EPI* epithelium, *STR* stroma

$$V_{(LUM)} = I_{LUM}/1.21$$

(I_{LUM} = the number of lattice points superimposed on glandular lumina).
Thereafter the quotient of volume proportion of epithelium or tumour cells and the volume proportion of stroma was computed using the expression $V_{(EPI)}/V_{(STR)}$. This quotient quantifies the relative volume of epithelial or tumour cells per unit volume of stroma.

With the value I_n known, the VS within the measuring field (VS_{mf}) was computed according to [16]:

$$VS_{mf} = (1/L_R) \times \Sigma (I_n \times 2)$$

Finally this value was corrected for differences in the tissue composition by computing the normalized VS (i.e. the VS within the stromal compartment of the tissue) [1]:

$$VS = VS_{mf} / V_{(STR)}$$

The dimension of VS is mm^{-1} and is, therefore, equivalent to the VS per unit tissue volume ($mm^{-1} = mm^2/mm^3$).

In homogeneous tumours, i.e. tumours displaying one grade throughout the whole tumour tissue, VS, $V_{(EPI)}$, $V_{(STR)}$ and $V_{(LUM)}$ were assessed as the arithmetic mean of ten randomly chosen microscopic measuring fields. The standard error of measurements obtained by this methodology was < 10%.

Assessment of heterogeneous tumours

In heterogeneous tumours composed of tumour areas with different grades the volume proportion of different tumour grades was assessed using the whole-mount sections as follows. The tumour and grade of differentiation in different areas were marked on the

coverslip and their volume proportion in relation to the total tumour was stereologically measured by means of a rectangular grid (distance between test lines, 5 mm). The values $V_{(EPI)}$, $V_{(STR)}$, $V_{(LUM)}$ and VS were then assessed in ten microscopic fields for each grade as described above. Thereafter the parameters VS, $V_{(EPI)}$, $V_{(STR)}$ and $V_{(LUM)}$ for each patient were computed as the volume-weighted averages of the values assessed in the tumour areas of different grade.

Statistical analyses

Normal distribution of data within different groups was tested using the Kolmogoroff-Smirnov test. Differences between groups were tested by the analysis of variance procedure and consecutive Scheffé test. $P < 0.05$ was defined to be a statistically significant value. Curve fitting was performed according to the least sum of squares method.

Results

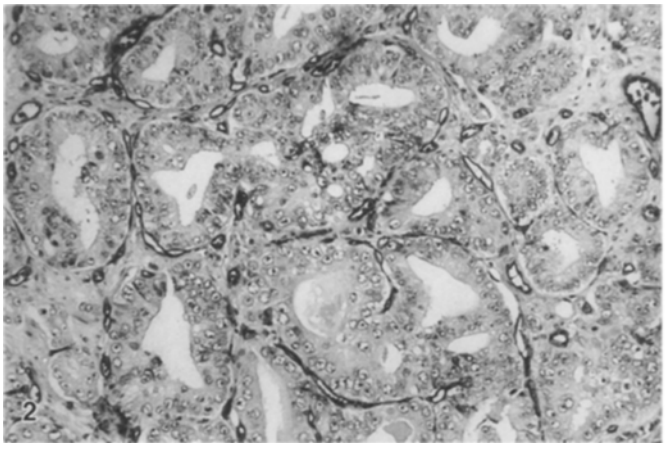
Qualitative investigations

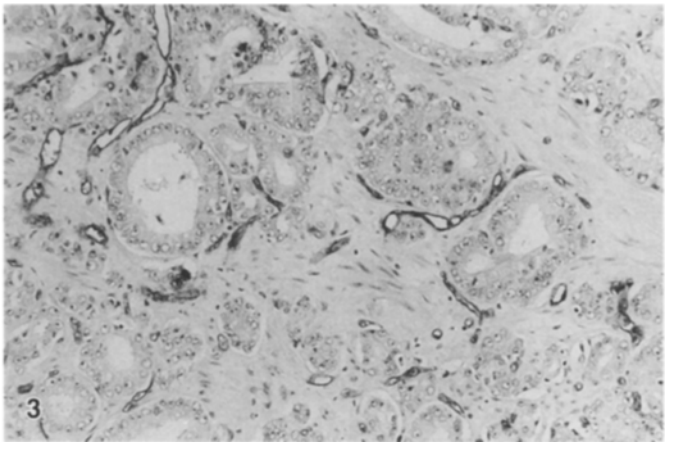
In tumour-free prostatic tissue the precapillary and capillary vessels form a well-branched network which is most dense in the subepithelial areas of the gland. In prostate cancer the vessels appear to be plumper, less branched and of a more irregular shape. In those tumours composed of closely packed glands the stroma shows a peculiar vascular network entrapping the tumour glands (Fig. 2). Tumours with an intermediate volume proportion of stroma showed a vascular bed composed of irregularly shaped, plump vessels which were predominantly localized in the vicinity of the epithelium (Fig. 3). A few tiny, randomly scattered, vessel profiles were found in those tumours with a high stromal proportion (Fig. 4).

Quantitative analyses

Gland tissue composition

The volume proportion of glandular lumen, stroma and epithelial or tumour cells respectively showed a wide scatter in tumour-free prostatic tissue and prostatic cancer. The stroma made up between 38.7% and 64.9% (median 53.6%) of the tissue volume, whereas the glandular lumen ranged from 1.1% to 19.4% (median 6.6%). The volume proportion of epithelial and tumour cells lay between 29.4% and 49.0% (median 40.1%) of the glands. No statistically significant correlation was found between the amount of stroma and epithelial cells and whether the lesion was malignan or the degree of tumour differentiation, tumour stage and nodal status. The volume proportion of the glandular lumen was significantly higher in tumour-free prostatic tissue.





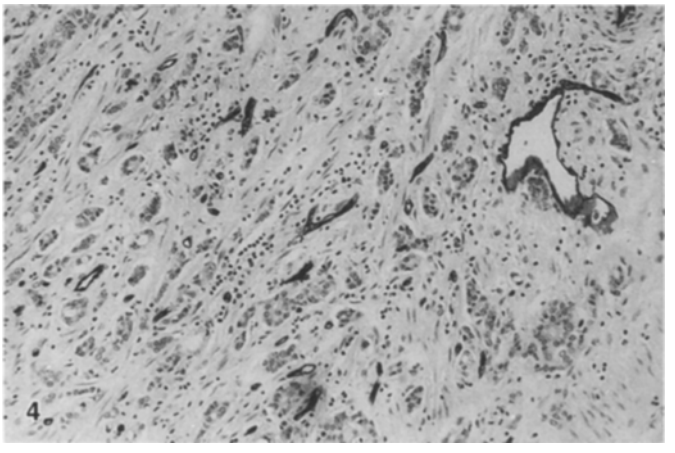


Fig. 2 Photomicrograph of a well-differentiated tumour (G1) with high volume proportion of tumour cells ($V_{(EPI)}$ = 48%). The stroma is densely vascularized [vascular surface (VS) = 22 mm^{-1}], the vessels are slender and form a peculiar network. ×160

Fig. 3 Photomicrograph of a tumour with an intermediate volume proportion of tumour cells ($V_{(EPI)}$ = 38%, VS = 10.2 mm^{-1}). ×160

Fig. 4 Photomicrograph of a scirrhous tumour with a high volume proportion of stroma ($V_{(STR)}$ = 43%) and low VS of 4.1 mm^{-1}. ×160

Within the prostatic cancer group this value was independent of the grade of differentiation, tumour stage and nodal status.

Correlation between staging, nodal status, tumour differentiation and VS

Assessment of the VS yielded the following results. In pT2 tumours the VS reached values of about 12 mm^{-1}, values significantly higher compared with tumour-free prostatic tissue (6.7 ± 0.4 mm^{-1}). In pT3 (8.2 ± 3.1 mm^{-1}) and pT4 tumours (3.9 ± 0.6 mm^{-1}) significantly lower values were obtained, with the pT3 and pT4 tumours showing no significant difference compared with controls (Table 2).

The assessment of the VS did not result in a statistically significant difference between the pN0 and pN+ group ($P = 0.59$), although a slight increase in the VS was found (Table 3).

Compared with tumour-free prostatic tissue, in G1 tumour areas the VS was significantly higher (14.6 ± 2.2 mm^{-1}). Significantly lower values were obtained for G2 (8.5 ± 1.4 mm^{-1}) and G3 tumour areas (5.4 ± 1.5 mm^{-1}). VS was independent of the heterogenity of the tumour (Table 4).

Table 2 Vascular surface (mm^{-1}) in different pT stages. Data are represented by arithmetic mean ± 1 standard deviation. The right column indicates *F*- and *P*-values obtained from analysis of variance and statistically significant differences computed by consecutive Scheffé test

Control	pT2	pT3	pT4	*F*(*P*)
6.7 ± 0.4	11.6 ± 3.5	8.2 ± 3.1	3.9 ± 0.6	5.20 ($P < 0.05$); pT2 > pT3, pT4, Control

Table 3 Vascular surface (mm^{-1}) and nodal status, pN1 and pN2 cases are combined in the pN+-group. The right column indicates *F*- and *P*-values obtained from analysis of variance and statistically significant differences computed by consecutive Scheffé test

pN0	pN+	*F* (*P*)
7.9 ± 2.9	8.9 ± 3.8	0.39 ($P = 0.59$)

Table 4 Vascular surface (mm^{-1}) in tumour areas of different grade. The right column indicates *F*- and *P*-values obtained from analysis of variance and statistically significant differences computed by consecutive Scheffé test

Control	G1	G2	G3	*F* (*P*)
6.7 ± 0.4	14.6 ± 2.2	8.5 ± 1.4	5.4 ± 1.5	127.47 ($P < 0.05$), G1 > G2 > G3, Control

Correlation between tissue composition and VS

The plot of VS versus the volume proportion of epithelial cells or tumour cells, respectively, is depicted in Fig. 5. The lowest VS values of about 5 mm^{-1} were found in tumours comprising less than 35% of epithelial cells. With rising epithelial proportion the VS rose to values of about 15 mm^{-1}. Correlation analysis revealed a high positive correlation between the amount of the epithelial compartment ($r = 0.59$, $P < 0.001$) (Fig. 5). Using the stromal portion as the independent variable an inverse behaviour was found. In this case the highest VS values ranging between 15 mm^{-1} and 20 mm^{-1} were found in tumours with a relatively low stromal proportion of about 40%. With rising stromal proportion the VS values decreased to about 5 mm^{-1}. Correlation analysis based on an exponential model revealed a negative correlation ($r = -0.81$, $P < 0.001$) (Fig. 6).

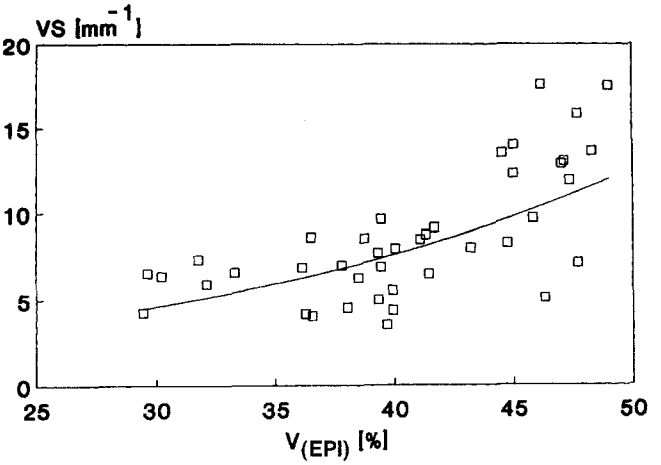


Fig. 5 Scatter plot of VS versus the volume proportion of epithelial or tumour cells together with the exponential regression curve ($y = a \times x^b$, $a = 5.34 \times 10^{-3}$, $b = 1.97$, $r = 0.59$, $P < 0.001$)

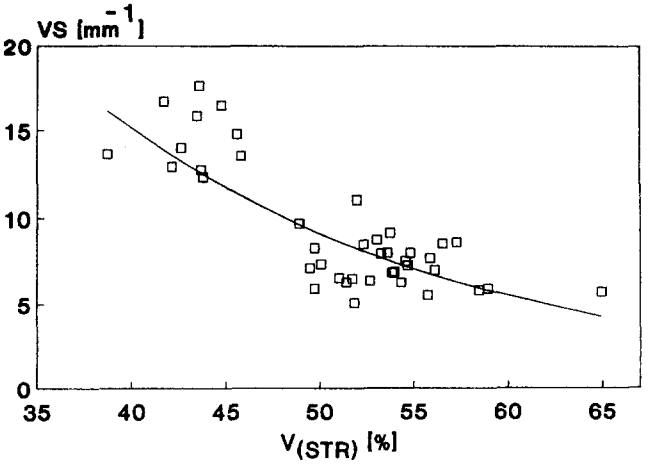


Fig. 6 Scatter plot of VS versus the volume proportion of stroma together with the exponential regression curve ($y = a \times x^b$, $a = 1674.04 \times 10^3$, $b = -3.12$, $r = -0.81$, $P < 0.001$)

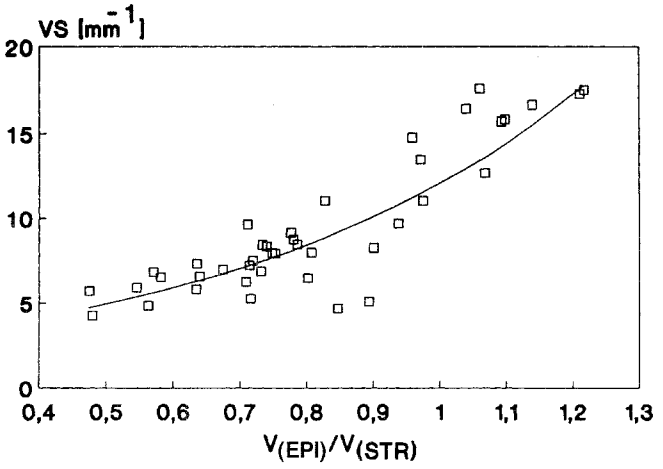


Fig. 7 Scatter plot of VS versus the quotient $V_{(EPI)}/V_{(STR)}$ together with the exponential regression curve ($y = a \times x^b$, $a = 10.95$, $b = 1.45$, $r = 0.74$, $P < 0.001$)

Statistical analyses were completed using the quotient of $V_{(EPI)}/V_{(STR)}$ as an independent variable. In this model the lowest VS values of about 5 mm^{-1} were found for $V_{(EPI)}/V_{(STR)} = 0.5$, i.e. the stromal compartment constituted about double the epithelial compartment. With an increasing $V_{(EPI)}/V_{(STR)}$ quotient, i.e. an increasing relative amount of epithelial compartment, the VS rose to values of about 20 mm^{-1}. For these assumptions the correlation analysis detected the highest positive correlation, with $r = 0.74$ ($P < 0.001$) (Fig. 7).

Discussion

In the present study we stereologically assessed the VS in order to avoid systematic errors that occur when counts of vessel profiles per area section are performed. In highly vascularized tumours showing a vascular network as depicted in Figs. 2 and 3 counts of vessel profiles are practically impossible. In those cases the stereological assessment of VS is the only worthwhile approach for the quantification of tumour vascularization. Furthermore, the amount of stroma differs between individual tumours and within one tumour. Therefore it is necessary to normalize the VS for this parameter. The methodology described here follows these recommendations and has proved a valuable tool for the assessment of vascularization [1, 9].

In the present study VS decreases with rising pT, i.e. tumour size, whereas no significant correlation was found between the nodal status and VS. These findings agree with data obtained in experimental tumours. Vogel [14, 15] quantified the vascularization in 72j mammary carcinoma in mice and reported a widening of diameter with growth, a decrease in vessel length and surface density from 46 mm^{-1} to 14 mm^{-1}. In the cited study the vascular volume did not change, a finding which is consistent with a significant change of the vessel geometry in tumour vessels. Similar results were obtained by Hilmas and Gilette [7] who investigated mammary carcinoma grown in the gastrocnemius muscle of C3H/BL mice. In this model the vascular volume also remained constant during growth, whereas VS decreased with tumour growth. Interestingly the absolute data of VS reported by Vogel and by Hilmas and Gilette are similar to those found in the present study, a further indication that data obtained in experimental models may be applied to solid tumours such as prostate cancer in humans.

The present study also demonstrates that VS is positively related to the relative amount of tumour cells within the tumour tissue. This suggests that tumour vascularization is predominantly determined by the need for nutritional support of the tumour cells. This assumption correlates with the finding that nutritional deprivation of normal or neoplastic tissues induces vasoproliferation mediated by vascular endothelial growth factor (VEGF) [13].

We conclude that VS is mainly determined by local parameters such as tumour growth and tissue composition; the precise stereological assessement of VS is obviously of no use in the prediction of lymph node metastasis.

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