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ORIGINAL ARTICLE

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Morphometric microvascular characteristics predict prognosis in superficial and invasive bladder cancer

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Abstract Recent research has shown that neovascularization, quantitated by microvessel density (MVD), constitutes a strong prognostic indicator in patients with invasive urothelial carcinomas. These studies, however, have focused only on MVD as the only factor reflecting angiogenesis in transitional-cell carcinomas (TCCs). The objective of this report was to evaluate multiple morphometric microvascular characteristics besides MVD in superficial and muscle-invasive TCCs separately, to provide a better approach to the relationship between angiogenesis, clinicopathological parameters, and prognosis. Histologic sections from 115 TCCs [35 superficial (T1) and 80 muscle-invasive] were immunostained for CD31 and evaluated using image analysis for the quantitation of MVD, area, total vascular area, major axis length, minor axis length, perimeter, compactness, shape factor, and Feret diameter. Patients were followed-up until death (n=31) or for an average of 42.2 months (median 38.5 months). MVD increased with progressing T category (P=0.049) but area (P=0.033), major axis length (P=0.022), perimeter (P=0.043), and Feret diameter (P=0.042) were highest in T2 tumors. Area was the single independent predictor of adverse significance in T1 TCCs, whereas for muscle-invasive tumors, survival was independently predicted by MVD. Regarding disease-free survival in superficial tumors, the single significant independent parameter was compactness, whereas area was an independent favorable indicator of disease-free survival for patients with invasive TCCs. It is concluded that the prognostic significance of neovascularization is better assessed by area and shape-related morphometric characteristics, whereas MVD becomes influential only with regard to overall survival of patients with invasive tumors.

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Introduction

Angiogenesis, new blood vessel formation, is fundamental to prenatal and postnatal tissue development, reproduction, and wound healing [12]. In these situations, it is limited in time and results from an equilibrium between angiogenic stimulators and angiogenic inhibitors keeping endothelium in a quiescent state [6]. When blood vessels grow unabated, angiogenesis becomes pathologic and sustains the progression of many neoplastic and non-neoplastic disorders [10, 11].

Most tumors in humans persist for a certain period of time without neovascularization until a subset of neoplastic cells acquires an angiogenic phenotype, shifting resting endothelial cells into a phase of rapid growth. The switch to the angiogenic phenotype involves overexpression of angiogenic factors by tumor cells, mobilization of angiogenic proteins from the extracellular matrix, and recruitment of host cells (such as macrophages), which produce their own angiogenic proteins [11].

The interest of oncologists in angiogenesis stems from the widely held belief that, since tumor growth and invasion depend on neovascularization, the ability to quantitate immunohistochemically the degree of angiogenic response within or around a tumor may be of prognostic relevance. A plethora of investigations pursued to this end have been met with considerable success, verifying that increased microvessel proliferation is associated with tumor progression and decreased overall survival in a variety of malignancies [3, 14, 24, 28, 29, 30], including bladder cancer [2, 5, 7, 15, 21, 22, 25]. These studies, however, have focused only on microvessel density (MVD) as the only factor reflecting angiogenesis, overlooking other parameters that might be significant as well, such as the complexity of the microvascular network, the size and shape of microvessels, and the immunostaining intensity of endothelial cells.

The objective of this study was to evaluate multiple morphometric microvascular characteristics besides MVD in superficial and in muscle-invasive transitional-cell carcinomas (TCCs) in relation to clinicopathologic data and survival. Such information might prove valuable in clarifying certain steps in the evolution of the disease and would broaden our understanding of the relationship between angiogenesis and prognosis in these tumors.

Materials and methods

Patients

This is a retrospective study of 115 patients with primary transitional-cell carcinoma (TCC), who presented at Asklepeion Voula Hospital in Athens between 1983 and 1994. The cohort was not entirely consecutive, since adequate tumor biopsy specimens for immunohistochemistry were not available in many superficial (T1) small tumors, the respective tissue blocks having been exhausted in previous studies. Clinical data were available in all cases. There were 101 men and 14 women with a mean age of 69.4 years (range 35–92 years). The follow-up period lasted 38.15 months on average (median 36 months, range 3–120 months). By the time this study was undertaken, 31 patients had died of their disease after a mean survival of 32.2 months (median 30 months; range 6–68 months). The mean follow-up for the remaining 84 patients was 42.2 months (median 38.5 months; range 3–120 months). Fifty-nine patients relapsed after a mean period of 13.2 months (median 9 months; range 3–58 months). In survival analysis, six patients who died of other causes during the follow-up period (without evidence of bladder cancer at the time of death) were treated as censored data.

The histological samples consisted of pretreatment transurethral (TUR) material fixed immediately after removal in 10% formalin and embedded in paraffin. Cases were assigned into two grade categories, i.e., low (n=44) or high (n=71), conforming to the 1998 World Health Organization (WHO) classification [9]. Given that accurate assessment of the vascularity of papillary tumors is not possible according to some authors [7], only cases with a solid or mixed (papillary/solid) histology were included in this study. Six cases with a predominantly papillary growth were excluded.

The staging of tumors was performed according to International Union Against Cancer (UICC) standards [13] and was based on the combination of clinical and histological data, i.e., cystoscopy, intravenous urography, computed tomography, ultrasonography, and histological examination to determine the presence of invasion of the lamina propria (T1, n=35) or invasion of the detrusor muscle (T2–T4, n=80). All cases were N_0M_0 at presentation. Superficial (T1) tumors were treated with TUR, intravesical instillations of epirubicin, or bacillus Calmette–Guerin, being administered only in case of recurrence. For invasive (T2–T4) carcinomas, TUR was followed by cystectomy, intravesical chemotherapy, or radiation. None of the patients had received intravesical instillations or radiation before TUR.

Immunohistochemistry

The tumor microvessels were highlighted using a mouse monoclonal antibody to the CD31 antigen on endothelial cells (clone 1A10; Novocastra, Burlingame Calif.). The antibody was applied at a dilution of 1:50 for 1 h. Before staining, slides were incubated four times for 5 min in citrate buffer pH 6.0 at 750 W in a microwave oven [27]. Application of the primary antibody was followed by the standard three-step streptavidin peroxidase technique (Cadenza Tag kit, Shandon Lipshaw Inc, Pittsburgh Pa.). All specimens were treated using identical procedures. Negative controls (i.e., sections in which the primary antibody was substituted with non-immune mouse serum) were also stained in each run.

Image analysis method

Images were acquired using a Zeiss Axiolab microscope (Carl Zeiss Jena GmbH, Jena, Germany) with a mechanical stage, fitted with a Sony-iris CCD videocamera (Sony Corporation, Tokyo, Japan). The videocamera was connected to a Pentium II personal computer loaded with the Image Scan Software (Jandel Scientific, Erkrath, Germany). Slides were examined carefully at a low power magnification (×40) to identify the areas with the highest density of capillaries and small vessels. In each case, the most vascularized area was selected, and a ×200 field was stored as a JPEG file [(1550×1070 pixels, 16.7 million colors (24-bit)]. In cases in which the most vascularized area was not obvious, 2–4 optical fields with the highest MVD were stored. However, only the most vascularized field was finally taken into account for further evaluation. Areas with a dense leukocytic or hemorrhagic infiltration and areas associated with ulceration or the presence of granulation tissue (indicating prior transurethral biopsy site) were excluded from the storing procedure. These regions, however, served as internal controls to verify the adequacy of endothelial cell staining. Single endothelial cells or clusters of endothelial cells positive for CD31 were considered as individual vessels. In each vessel, the outline was identified and traced (Fig. 1).

The presence of blood cells or fibrin without any detectable endothelial cells was not sufficient to define a microvessel. Vessels with muscular walls were not counted; however, there was no restriction regarding the size of the countable vessels, so as not to underestimate longitudinal sections or bifurcations of microvessels. For each countable microvessel, the following morphometric parameters were estimated: major axis length, minor axis length, area, perimeter, compactness (perimeter^2/area), shape factor (4π*area/perimeter^2) and Feret diameter $\left(\sqrt{(4 * Area)/\pi}\right)$.

The variables entered into the statistical analysis were the mean values of the above seven morphologic indices, the total count of microvessels per optical field (MVD), and the total area occupied by them (total vascular area, TVA). The whole procedure took place without any knowledge of the patients' clinicopathologic data.

Statistical analysis

Intrarater and interrater reliability were assessed by Pearson's correlation coefficient in ten randomly selected cases. Reproducibility of measurements in the same optical field or of the same individual vessel was assessed using the coefficient of variation. The normality of distributions was tested with the Kolmogorov–Smirnov test. Differences in mean values between grades were examined using the Mann–Whitney U test. Associations between microvascular parameters and stage were assessed with Kruskal–Wallis analysis of variance. Spearman's rank correlation coefficient was calculated to determine associations between numerical variables.

Survival analysis was carried out separately for superficial (T1) and muscle-invasive tumors. The effect of various parameters (age, gender, grade, T category, and the microvascular parameters) on clinical outcome was assessed by plotting survival curves according to the Kaplan–Meier method, and groups were compared using the log-rank test. The continuous variables, age, and the microvascular parameters were categorized on the basis of the median value rounded at its nearer 5%. To minimize the potential confounding effects of variations in treatment, we adjusted the P values of the log-rank test accordingly. Multivariate analysis was performed using the stepwise Cox's regression model to evaluate the predictive power of each variable independently of the others. To avoid any "data-driven" categorization, the continuous variables were entered in multivariate analysis in both continuous and categorical forms. Statistical analysis was performed using the SPSS for Windows Software (SPSS, Chicago Ill.) on an IBM-compatible computer. Differences were considered statistically significant when the P value (two-sided) was <0.05.

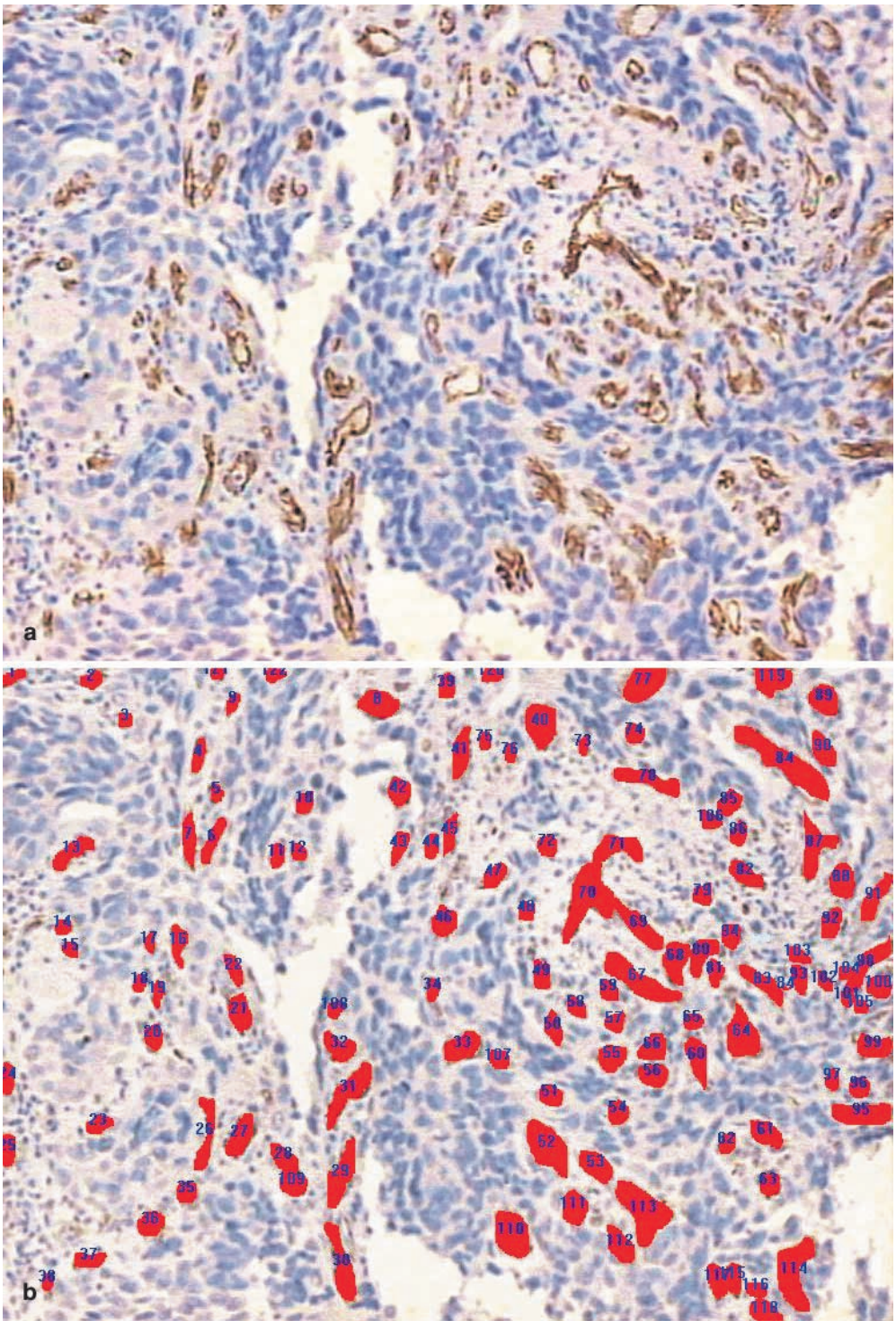


Fig. 1 **a** Immunohistochemical staining of endothelial cells with anti-CD31 in a case of high-grade transitional-cell carcinoma (TCC). **b** Same field as in (**a**). The outline of each vessel is traced, and the *red layer* represents the section area of each vessel

Results

Reliability of the methods

Intraobserver and interobserver variation for the counting method was assessed in 10 random cases. There was no significant difference between occasions (P=0.8) or between observers (P=0.9) for all the examined parameters with a reliability coefficient from 0.75 to 0.84.

Association of morphometric variables with tumor grade and T category

None of the examined microvascular characteristics correlated significantly with tumor grade (Table 1). MVD gradually increased with progressing T category [Kruskal–Wallis analysis of variance (ANOVA), T1 vs T2 vs T3 and T4 P=0.049] but area, major axis, perimeter counts, and Feret diameter were higher in T2 tumors

Table 1 Vascular morphometric parameters evaluated according to grade and stage

	Histologic grade		*P*-value[a]	Stage (T category)				*P*-value[b]
	Low (*n*=44) Median (range)	High (*n*=71) median (range)		T1 (*n*=35) median (range)	T2 (*n*=63) median (range)	T3 (*n*=11) median (range)	T4 (*n*=6) median (range)	
Microvessel count	21 (5–85)	22 (4–118)	0.89	19 (5–46)	22 (4–118)	27 (6–47)	46 (15–74)	0.049
Major axis (μm)	8.68 (2.77–36.06)	7.90 (3.63–82.86)	0.92	8.57 (3.01–18.22)	8.78 (2.77–82.86)	6.61 (3.57–10.07)	6.54 (4.36–9.48)	0.022
Minor axis (μm)	4.62 (1.51–18.15)	4.12 (1.97–26.77)	0.54	4.59 (1.96–9.97)	4.66 (1.51–26.77)	3.78 (2.24–6.24)	3.60 (2.53–4.73)	0.104
Perimeter (μm)	23.61 (7.80–95.34)	21.90 (9.80–208.68)	0.94	24.02 (8.54–48.36)	24.27 (7.80–208.68)	19.08 (10.30–31.75)	17.53 (11.62–25.09)	0.043
Feret diameter (μm)	6.05 (1.99–24.22)	5.42 (2.62–44.66)	0.60	6.49 (2.36–12.15)	5.95 (1.99–44.66)	4.88 (2.80–6.73)	4.50 (3.75–6.14)	0.042
Area (μm^2)	42.43 (10.60–508.10)	41.20 (7.90–607.25)	0.42	54.95 (10.6–338.0)	42.65 (7.90–607.25)	24.99 (10.70–165.60)	28.89 (11.62–53.60)	0.033
Total area (μm^2)	862.36 (64.20–10670.16)	819.48 (63.21–7278.05)	0.52	769.1 (148.4–4526.8)	883.5 (63.2–10670.2)	557.1 (64.2–4968.0)	1158.3 (370.2–1787.4)	0.99
Shape factor	0.73 (0.49–0.86)	0.70 (0.34–0.84)	0.21	0.71 (0.50–0.84)	0.71 (0.34–0.88)	0.73 (0.39–0.80)	0.75 (0.59–0.82)	0.513
Compactness	18.18 (14.61–30.80)	19.10 (14.99–54.22)	0.11	18.17 (15.00–27.21)	18.88 (14.61–54.22)	18.35 (16.07–36.17)	17.48 (15.38–22.31)	0.537

[a] Mann–Whitney U test [b] Kruskal–Wallis analysis of variance (ANOVA)

Table 2 Univariate analysis (log-rank test) of overall survival in superficial (T1) and muscle-invasive (T2–T4) transitional-cell carcinomas (TCCs)

	T1 tumors *P*-value (adjusted to therapy)	T2–T4 tumors *P*-value (adjusted to therapy)
Age	0.0296 (0.0422)	0.2855 (0.3308)
Gender	0.2495 (0.4076)	0.6892 (0.6246)
Grade	0.2797 (0.3362)	0.0094 (0.0218)
T category	–	<0.0001 (<0.0001)
Microvessel count (<26 vs ≥26)	0.5843 (0.5602)	0.0127 (0.0147)
Major axis (μm; <11 vs ≥11)	0.0732 (0.0429)	0.1088 (0.0893)
Minor axis (μm; <4.46 vs ≥4.46)	0.2238 (0.1675)	0.4064 (0.4190)
Perimeter (μm; <22.8 vs ≥22.8)	0.1627 (0.1210)	0.5051 (0.5208)
Feret diameter (μm; <7 vs ≥7)	0.1350 (0.0795)	0.0747 (0.0969)
Area (μm^2; <41.2 vs ≥41.2)	0.0419 (0.0277)	0.0763 (0.1055)
Total area (μm^2; <830 vs ≥830)	0.4980 (0.3077)	0.1653 (0.1757)
Shape factor (<0.69 vs ≥0.69)	0.8613 (0.8758)	0.7013 (0.5795)
Compactness (<18 vs ≥18)	0.6507 (0.8436)	0.0054 (0.0085)

Table 3 Univariate analysis (log-rank test) of disease-free survi-val in superficial (T1) and muscle-invasive (T2–T4) transitional-cell carcinomas (TCCs)

	T1 tumors *P*-value (adjusted to therapy)	T2–T4 tumors *P*-value (adjusted to therapy)
Age	0.0203 (0.0259)	0.0750 (0.0389)
Gender	0.1659 (0.1461)	0.0990 (0.1227)
Grade	0.3507 (0.4438)	0.0246 (0.0093)
T category	–	<0.0001 (0.0043)
Microvessel count (<26 vs ≥26)	0.3899 (0.3268)	0.8704 (0.9145)
Major axis (μm; <11 vs ≥11)	0.2937 (0.1523)	0.0065 (0.0035)
Minor axis (μm; <4.46 vs ≥4.46)	0.3462 (0.1157)	0.1033 (0.0397)
Perimeter (μm; <22.8 vs ≥22.8)	0.8948 (0.2932)	0.0402 (0.0095)
Feret diameter (μm; <7 vs ≥7)	0.3823 (0.2688)	0.0624 (0.0332)
Area (μm^2; <4.12 vs ≥4.12)	0.7934 (0.7734)	0.0010 (0.0003)
Total area (μm^2; <841 vs ≥841)	0.8041 (0.6768)	0.6112 (0.4190)
Shape factor (<0.69 vs ≥0.69)	0.1137 (0.2728)	0.0938 (0.0589)
Compactness (<18 vs ≥18)	0.6138 (0.3428)	0.7186 (0.8064)

compared with T1 or T3 and T4 (P=0.033, P=0.022, P=0.043, and P=0.042, respectively). TVA values also tended to be higher in T2, but the difference did not reach a statistically significant level (P=0.99).

Correlations among the various morphometric variables

All morphometric parameters were significantly interrelated except for MVD and minor axis, MVD and area, minor axis and compactness, and minor axis and shape factor.

Prognostic relevance of morphometric variables in superficial tumors

After adjusting for therapy, the parameters significantly affecting overall survival of patients with T1 tumors were the age of the patient, area (P=0.0277), and major axis (P=0.0429) (Table 2, 3). Lower (i.e., <41.2 µm^2) area values and lower (i.e., <11 µm) major axis values tended to be accompanied by a better survival rate (Fig. 2). Multivariate analysis indicated that area (as a dichotomous variable) was the single independent predictor of adverse significance in patients with superficial (T1) disease. With respect to recurrence-free survival, univariate analysis showed that apart from age, no other variable was statistically significant. However, compactness (as a continuous variable) constituted a significant prognostic indicator in multivariate analysis. As evidenced from the values of the relative risk in Cox's model (Table 4), an increase in compactness implied an increased likelihood of early relapse (Fig. 3).

Prognostic relevance of morphometric variables in muscle-invasive TCCs

In univariate analysis, the parameters with a significant impact on overall survival after controlling for therapy were tumor grade, T category, MVD (P=0.0147), and compactness (P=0.0085) (Table 2, 3). An increase in MVD and compactness portended a decrease in overall survival (Fig. 4). Under the proportional hazards model, only MVD (as a continuous variable) remained statistically significant (Table 4). Accordingly, a higher likelihood of relapse was associated with advanced age of the patient, high grade, advanced T category, and lower values of major axis (P=0.0035), minor axis (P=0.0397), perimeter (P=0.0095), Feret diameter (P=0.0332), and area (P=0.0003; Fig. 5). Multivariate analysis selected only T category and area (Table 4).

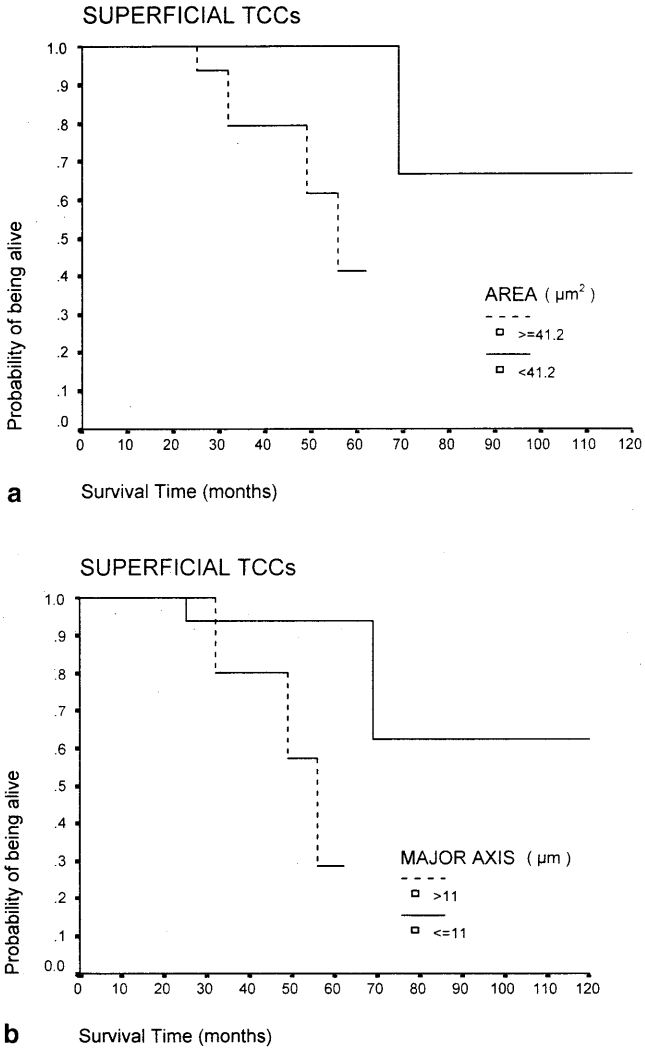


Fig. 2 Overall survival in superficial (T1) transitional-cell carcinomas (TCCs) in relation to area (**a**) and major axis length (**b**). (Kaplan–Meier curves)

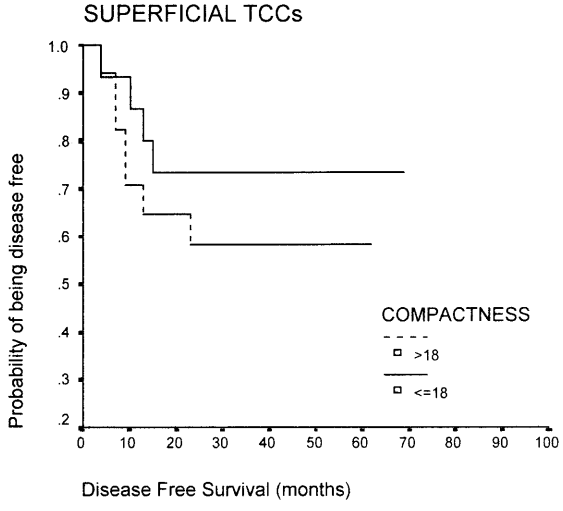
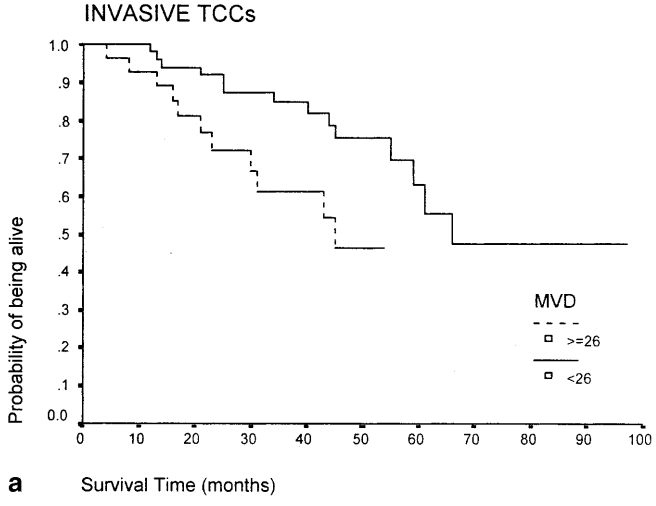


Fig. 3 Recurrence-free survival in superficial (T1) transitional-cell carcinomas (TCCs) in relation to compactness. (Cox's model)

Table 4 Cox's proportional hazard estimation of overall and disease-free survival in superficial (T1) and muscle-invasive (T2–T4) transitional-cell carcinomas (TCCs)

	Covariate	Coefficient	Standard error	*P*-value	Relative risk	95% Confidence limits for relative risk
Superficial TCCs						
Overall survival	Area (μm²; <41.2 vs ≥41.2)	0.0582	0.0303	0.0500	1.0599	1.0001–1.1247
Disease-free survival	compactness	0.2928	0.1075	0.0065	1.3402	1.0856–1.6545
Invasive TCCs						
Overall survival	Microvessel count	0.0192	0.0078	0.0139	1.0194	1.0039–1.0351
Disease-free survival	T category	1.1048	0.4580	0.0159	3.0186	1.2301–7.4073
	Area (μm²; <41.2 vs ≥41.2)	–1.0891	0.3195	0.0007	0.3365	0.1799–0.6294



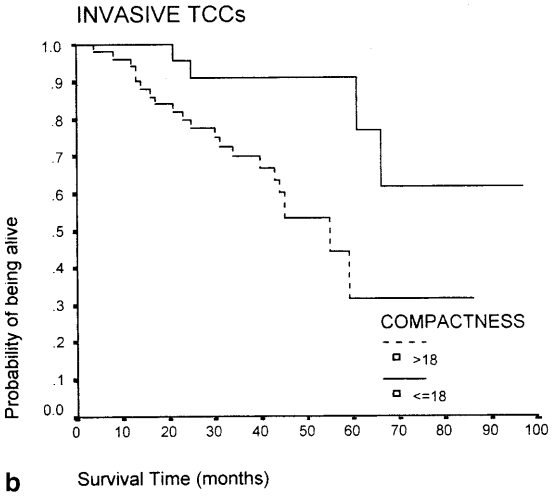
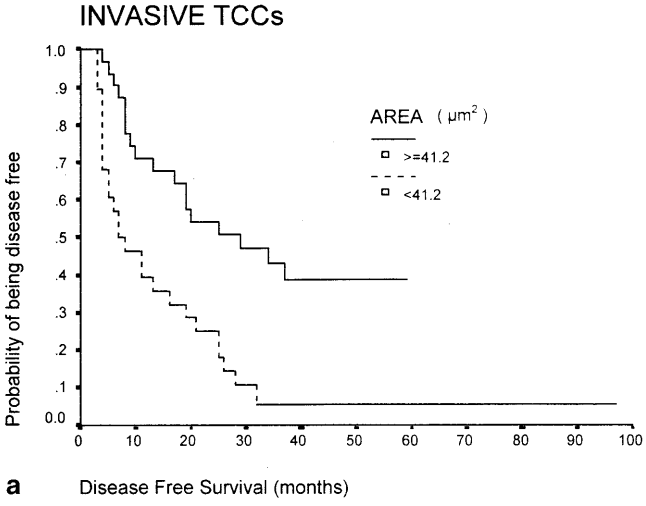


Fig. 4 Overall survival in muscle-invasive (T2-T4) transitional-cell carcinomas (TCCs) in relation to microvessel density (MVD) counts (**a**) and compactness (**b**). (Kaplan–Meier curves)



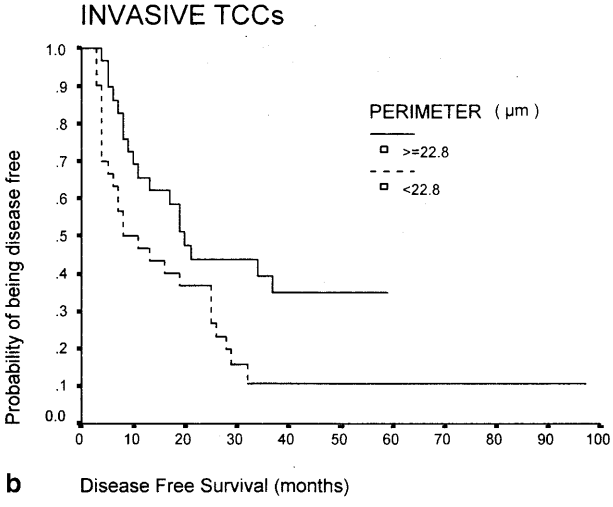


Fig. 5 Recurrence-free survival in muscle-invasive (T2-T4) transitional-cell carcinomas (TCCs) in relation to area (**a**) and perimeter (**b**). (Kaplan–Meier curves)

Discussion

Angiogenesis research in urological oncology has only recently begun to progress beyond the observational phase. Experimental data indicate that urothelial malignancies, like all malignancies, can induce angiogenesis which contributes to their malignant phenotype [4]. Fluorescein angiography reveals markedly increased uptake in papillary tumors and carcinoma in situ compared with normal urothelium, confirming what urolo-

gists have suspected, namely that these are vascular tumors [31].

Tumor growth beyond 2 mm depends on the ingrowth of new capillaries, and bladder cancer is no exception. Therefore, the gradual increase in vessel counts with progressing disease stage that we and other authors [25] have observed is not surprising, when it is considered that stage is a composite variable depending partly on tumor size [25]. Other investigators [2, 7, 15], however, have failed to establish such a relationship. These discrepancies could be attributed to differences in methodology, such as highest versus average vessel count, size of the fields (ranging from ×100 to ×400) and different antibodies (factor VIII, CD31, or CD34).

We chose to assess the highest vascularity within our cases, because it has been previously suggested that vascular "hot spots" are biologically important, providing a route through which tumor cells can metastasize [16]. In addition to the quantitative variation of neovascularization observed in our study, the analysis of morphometric parameters revealed a morphological variability of the vascular pattern among different stages. Thus, large caliber microvessels presented a peak in T2 stage compared with T1 and T3 and T4. This finding is in agreement with the concept that angiogenesis is primarily initiated by angiogenic factors, such as vascular endothelial growth factor, as an outburst in the formation of wide sinusoidal spaces, a phenomenon called hyperfusion [8]. According to our findings, this step coincides with the transition from superficial to muscle-invasive urothelial disease, although it is not yet clear whether hyperfusion is a prerequisite for, or simply accompanies, the evolution towards a more aggressive tumor. Then, the vessel caliber appears to be gradually restricted in relation to the exponential increase of tumoral parenchyma, while the rate of new vessel formation is maintained. The combination of increased MVD and decreased area might be indicative of the participation of an intussusceptive angiogenic mechanism at this stage of the disease [23]. By contrast, all morphometric parameters remained unaffected through increasing grade.

Although a small number of recent reports [2, 5, 7, 25] have looked at angiogenesis as it relates to survival of patients with invasive TCCs, the significance of this process in superficial TCCs appears less well defined [1, 21, 22]. Nevertheless, it seems that different growth factors mediate angiogenesis in superficial and invasive bladder tumors [20]. The current study is the first to assess the prognostic significance of various morphologic parameters related to the size and shape of microvessels, besides MVD, in muscle-invasive and in superficial TCCs.

Our data, indicating that increased MVD is associated with poorer survival in patients with invasive TCCs, are consistent with the findings of other investigators [2, 5, 7, 25]. There are several lines of evidence supporting a link between angiogenesis and tumor aggressiveness. The induction of new microvessels appears to be necessary to alleviate growth restriction placed on a tumor by its dependence on diffusion for nutrient delivery and waste removal and might eliminate normal barriers preventing tumor invasion and the establishment of micrometastases [9, 18, 19]. Proliferating blood vessels may also serve to allow increased access to the systemic circulation [18]. This likelihood further increases, because newly formed vessels have leaky and weak basement membranes that tumor cells can penetrate more easily than those of mature vessels [19]. Given these data, it follows that the larger the microvessel size, the higher the chance for tumor cells to enter the circulation. Alternatively, the association between tumor aggressiveness and large microvessels (represented by high values of area) may express a normal sequence of events, as aggressive, rapidly growing neoplasms soon lead their environment to hypoxia, which, being the main stimulus for the production of angiogenic factors, will result in excessive angiogenesis [11]. These hypotheses are confirmed by our results of multivariate analysis with regard to overall survival in superficial tumors, revealing that area was the single parameter of prognostic value, supporting similar findings recently reported in colorectal cancer [24]. The insufficiency of MVD to provide significant prognostic information in superficial tumors was also noted by Babkowski et al. [1].

A rather unexpected finding was the favorable effect of area on disease-free survival for patients with invasive tumors. Although this is seemingly at discrepancy with the aforementioned data, it could be argued that it reflects the association of area with early (i.e., T2) muscle-invasive disease, also evidenced by the fact that T category is included in Cox's model. Regarding reccurence-free survival in superficial tumors, it appears that the presence of flattened vessel sections (higher values of compactness) increases the likelihood of early relapse. This finding obviously relates to the distinct microvascular patterns that characterize superficial (as opposed to muscle-invasive) tumors [17] and to the consequent hemodynamic interactions. Other investigators [21, 22] also pointed out the predictive role of angiogenesis in the recurrence of superficial tumors, lending support to our findings.

Most studies evaluating the association between tumor progression and angiogenesis have reported results after dividing patient populations into two groups, on the basis of an optimal microvessel count cut-off. However, angiogenesis might not be an all-or-nothing phenomenon with respect to tumor growth. Progressive increases in aggressive behavior exhibited by tumors may be associated with increasing degrees of angiogenesis. Our data are compatible with this latter view, because decreasing overall survival in muscle-invasive TCC patients was noted with increasing values of microvascular parameters in Cox's model. This eliminates from a clinical perspective the need for cut-off points.

It should be stressed that the evaluation of bladder cancers for MVD presents certain complications not en-

countered with other types of tumors. The pathologic diagnosis is usually made from tissue removed by means of TUR, which causes tissue injury characterized by substantial neovascular response. Failure to identify areas of prior TUR may result in erroneously high estimates of angiogenesis parameters, since microvessels in these regions are not proliferating in response to signals associated with the tumor and will not accurately reflect the tumor's angiogenic potential [2].

An issue that remains to be addressed is the fact that the average observation period in our study did not exceed 3 years. It should be acknowledged, however, that the vast majority of TCCs progress clinically during the first 3 years after presentation [26], which allows meaningful conclusions to be drawn at the end of this period. The confirmation of the adverse prognostic effect of a notorious group of universally established prognostic factors (grade, T-category) proves that our cohort was quite representative and that survival analysis was valid. A drawback, nevertheless, remains insofar as the patients in our study were not uniformly treated. However, treatment was neither a significant factor in multivariate analysis nor did it have a sizeable influence on the results of univariate analysis when used as a stratum, which argues against a significant bias due to differences in treatment.

Although pathologic grade and stage are currently the primary variables that dictate treatment strategies in bladder cancer, patient selection for more aggressive therapy remains controversial, especially for T1 tumors. Our data indicate a potential role for angiogenesis in the evaluation of superficial (T1) and muscle-invasive bladder cancer. More importantly, this study demonstrates that the prognostic significance of neovascularization is better assessed by vascular area and shape-related morphometric characteristics, whereas MVD becomes influential only with respect to overall survival of patients with muscle-invasive tumors.

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