Angiogenesis as a Prognostic Indicator of Survival in Non-Small-Cell Lung Carcinoma: a Prospective Study

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Background: **Tumors acquire nutrients that are essential for continued growth and an avenue for dissemination to the rest of the body by inducing angiogenesis (i.e., the formation of new blood vessels). Preliminary studies involving a number of different kinds of cancer have indicated that an assessment of tumor angiogenesis may be useful in predicting disease outcome.** ***Purpose:*** **In a prospective study, we evaluated the relationship between tumor angiogenesis and survival for 407 patients with non-small-cell lung carcinoma who were treated with potentially curative surgery.** ***Methods:*** **The study population consisted of 360 male and 47 female patients who underwent surgery consecutively at the Department of Surgery, University of Pisa, Italy, from March 1991 through December 1994. Follow-up lasted through February 1996, with a median follow-up for living patients of 29 months (range, 15-60 months). An anti-CD34 monoclonal antibody, which is specific for endothelial cells, and standard immunohistochemical techniques were used to measure angiogenesis in tumor samples. Angiogenesis was quantified in terms of microvessel counts; the counts for single, high-power microscopic fields (magnification ×250) in the three most intense areas of blood vessel growth for each sample were averaged. The median microvessel count in this series was 20, and the counts were categorized as follows: 1) low versus high (≤20 versus >20 microvessels) or 2) in five categories (1-10, 11-20, 21-30, 31-40, and ≥41 microvessels). Disease-free and overall survival during follow-up were assessed. Kaplan–Meier survival curves were modeled in a univariate analysis of patient and tumor characteristics; the Cox proportional hazards model was used in multivariate analysis. Reported *P* values are two-sided.** ***Results and Conclusions:*** **In the univariate analysis, patients with larger tumors (*P* for trend <.00001), a more advanced tumor stage (*P* for trend <.00001), a greater degree of regional lymph node involvement (*P* for trend <.00001), or more vascularized tumors (high versus low microvessel count, *P*<.00001) experienced significantly reduced overall survival. When microvessel counts were analyzed in five categories, a highly significant trend (*P*<.00001) toward worse prognosis was observed with increasing tumor vascularity. In multivariate analysis, tumor microvessel count (*P*<.00001), tumor size (*P* = .0006), and regional lymph node status (*P*<.00001) retained independent prognostic value with respect to overall survival; among these variables, tumor microvessel count, considered as a continuous variable, was the most important, with a relative hazard of death of 8.38 (95% confidence interval = 4.19-16.78) associated with the highest microvessel counts.** ***Implications:*** **An evaluation of tumor angiogenesis may be useful in the postsurgical staging of patients with non-small-cell lung carcinoma and in identifying subsets of patients who may benefit from different postsurgical treatments. [J Natl Cancer Inst 1997;89:881-6]**

Despite improvements in surgical and medical management, the clinical behavior of lung cancer, unfortunately, remains bad. Currently, the best predictor of outcome in non-small-cell lung cancer (NSCLC), which accounts for approximately 75% of the cases of lung carcinoma, is the TNM (tumor, node, and metastasis) classification of the patient (*1*). The necessity for new indicators in the assessment of this type of cancer has prompted an examination of several biologic parameters, such as oncogene and/or tumor-suppressor gene expression (*2-4*), tumor cell proliferation kinetics (*5,6*), and angiogenesis (*7-11*). Tumor-induced neovascularization (i.e., angiogenesis) is important in neoplastic development and progression, since both tumor growth and the metastatic dissemination of tumor cells depend on vascular support (*12,13*). The number of microvessels in the most intense areas of tumor-associated neovascularization has been suggested as a useful prognostic indicator for patients with malignant melanoma (*14*) and carcinomas of the breast (*15-18*), head and neck (*19*), prostate (*20*), ovary (*21*), stomach (*22*), and lung (*7-11*). Various preliminary studies (*7-11,23,24*) have been performed to assess the prognostic impact of tumor angiogenesis in lung cancer. However, no confirmatory analysis has been performed in a large and prospective series of patients to justify the introduction of microvessel count (MVC) into clinical trials. In this study, we have prospectively analyzed a cohort of 407 patients with NSCLC in an attempt to incorporate tumor MVC into a new biologic–pathologic classification of NSCLC that is clinically useful in determining patient survival subsequent to radical surgery.

Patients and Methods

Patients and Tumor Tissue

Four hundred seven patients with NSCLC who consecutively underwent curative surgical resection at the Department of Surgery of the University of Pisa from March 1991 through December 1994 were studied. Participation in the study required verbal consent. There were 360 male patients and 47 female patients, with a combined median age of 64 years (range, 40-88 years). Follow-up lasted through February 1996, with a median follow-up of 29 months for living patients (range, 15-60 months). Preoperative evaluation included the following: a detailed medical history and physical examination, a biochemical and hematologic profile, a chest x ray, computed tomography of the chest, an abdominal ultrasound scan, and common cardiopulmonary tests. The patients presented no detectable metasta-

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See ''Note'' following ''References.''

ses in distant organs at the time of surgery. They had not received either chemotherapy or radiation therapy before surgery. Tumor specimens were obtained at resection. At least four samples of neoplastic tissue were removed, depending on the tumor size and the presence of regressive alterations. Large, non-necrotic cancers allowed the collection of a greater number of samples. The samples were always removed from the periphery of the tumors, since the central region of a tumor is more often subject to regressive alterations, such as necrosis or hemorrhage. The tumor samples were fixed in formalin and embedded in paraffin for histologic and immunohistochemical processing. The pathologic features of the samples were classified according to World Health Organization histologic criteria (*25*) and to guidelines of the American Joint Committee on Cancer staging (*26*).

Immunohistochemical Methods

Immunohistochemical analysis of the formalin-fixed, paraffin-embedded tissue was performed by use of the avidin–biotin complex immunoperoxidase method (Vectastain kit; Vector Laboratories, Inc., Burlingame, CA). Five-micrometer-thick sections of the most representative paraffin blocks for each tumor were used in this analysis. The sections, mounted on glass slides, were dewaxed in xylene, dehydrated in ethanol, and then incubated in 3% hydrogen peroxide for 10 minutes at room temperature. After washing with phosphate-buffered saline (PBS) and incubation for 30 minutes in 10% normal horse serum, the sections were heated twice in citrate buffer (pH 7.6) in a microwave oven for 5 minutes at 700 W. After microwave treatment, the sections were incubated overnight at room temperature with an anti-CD34 monoclonal antibody (clone QB-END10; Novocastra, Newcastle, U.K.), which was diluted 1:100 in PBS. Next, the sections were incubated for 1 hour with a biotinylated anti-mouse immunoglobulin G (Vector Laboratories, Inc.), which was diluted 1:500 in PBS. Specific immunostaining was detected through the formation of avidin–biotin complexes and avidin-coupled peroxidase activity. Light counterstaining was performed with hematoxylin.

Microvessel Quantitation

In all cases, MVC was determined independently by two pathologists. Each pathologist evaluated the slides without any knowledge of the counts made by the other pathologist or of the clinical outcome of the patients. No significant differences in MVC were found between the two observers. In fact, the counts from the two pathologists showed a very good correlation ($r = .98$; two-sided $P<.0001$). When conflicting numbers were obtained (for 103 [25.3%] of the 407 patients), we used mean values. A single microvessel was defined as any brown-immunostained endothelial cell that was separated from adjacent microvessels, tumor cells, and connective tissue elements. Each sample was examined by each pathologist, who identified the three most intense regions of neovascularization under low microscopic power (×10 objective lens and ×10 ocular lens). A ×250 field (×25 objective lens and ×10 ocular lens; 0.74 mm^2/field) in each of the three areas was then counted, and the average counts of the three fields were recorded. Large vessels with thick, muscular walls were excluded from the counts. The presence of a lumen was not required for scoring as a microvessel. MVCs were categorized in two ways: 1) as a dichotomous variable (low versus high MVC) and 2) as a five-category variable. In the dichotomous categorization, a count of 20 microvessels (the median value obtained in this series) was used as the cutoff point to distinguish a low MVC from a high MVC. When using five categories, MVC was codified as follows: 1) one to 10 microvessels, 2) 11-20 microvessels, 3) 21-30 microvessels, 4) 31-40 microvessels, and 5) 41 or more microvessels.

Statistical Analysis

All statistical analyses were carried out by use of BMDP Statistical Software (Biomathematical Data Package Statistical Software, Inc., Los Angeles, CA). Univariate analysis was performed by modeling Kaplan–Meier survival curves (*27*). The Mantel–Cox test (*28*) was used to evaluate the statistical significance of differences in survival among prognostic groups. For multicategoric ordinal variables, a test for trend was used. Multivariate analysis was carried out by use of the Cox proportional hazards model (*29*). The Cox model was first used to select among variables that significantly affected survival in the univariate analysis and then among those variables whose prognostic role was independent. The selection of covariates was performed by use of a stepwise procedure, using a Maximum Likelihood Ratio test for backward elimination. MVC was introduced into this model as a continuous variable after log transformation, owing to its skewed distribution. For covariates retained in the final model, relative hazards with 95% confidence intervals (95% CIs) were estimated. A Cox model stratified by the five categories of MVC defined above was then constructed in the manner described by Kalbfleisch and Prentice (*30*) to plot cumulative hazard functions for each category. The same model was used to estimate the 2-year probability of death for different combinations of the major covariates. The stratified model allowed good estimates to be made, even in the presence of violations of the proportionality assumption for the variables being used to stratify.

Results

Patient and Tumor Characteristics

The mean age of the 360 male and 47 female patients was 63.5 years ± 7.3 years (mean ± standard deviation; range, 40-88 years; median age, 64 years). The most common histologic type of tumor was squamous cell carcinoma (55.8%) followed by adenocarcinoma (34.5%), large-cell anaplastic carcinoma (7.6%), and bronchoalveolar carcinoma (1.9%). With respect to the degree of tumor differentiation, we observed 98 (24.1%) well-differentiated (grade 1) carcinomas, 170 (41.8%) moderately differentiated (grade 2) carcinomas, and 139 (34.2%) poorly differentiated (grade 3) carcinomas. With respect to tumor size, 103 (25.3%) cancers were classified as T1, 266 (65.4%) were classified as T2, and 38 (9.3%) were classified as T3. Sixty-four (15.7%) patients showed metastatic involvement of the hilar lymph nodes (N1), while mediastinal lymph nodes (N2) were involved in 96 (23.6%) patients. No metastasis to regional lymph nodes (N0) was present in 247 (60.7%) patients. Most cases were classified as stage I (227; 55.8%), while 57 (14.0%) cases were classified as stage II and 123 (30.2%) were classified as stage III. One hundred eighty-six (45.7%) patients relapsed during follow-up; 40 (21.5%) of these individuals developed local metastases, and 146 (78.5%) developed distant metastases. At the time of analysis, 273 (67.1%) patients were alive, whereas 134 (32.9%) had died.

Association of Clinicopathologic Characteristics and MVC With Survival

Among the clinicopathologic parameters, nonsquamous histology (two-sided $P = .024$), greater tumor size (test for trend, two-sided $P<.00001$), metastatic nodal involvement (test for trend, two-sided $P<.00001$), and advanced stage (test for trend, two-sided $P<.00001$) were significantly associated with worse overall survival (Table 1). With the exception of histology, a similar statistically significant association was observed between these characteristics and disease-free survival (data not shown). MVC, analyzed both as a dichotomous variable (using the median value of 20 microvessels as the cutoff point) and as a five-category variable (according to microvessel numbers as follows: 1-10, 11-20, 21-30, 31-40, and 41 or more), was a highly significant predictor of both overall survival (Table 1; two-sided $P<.00001$) and of disease-free survival (data not shown). Fig. 1 shows Kaplan–Meier survival plots generated on the basis of tumor size (A), nodal status (B), or MVC (C and D). MVC was examined either as a dichotomous variable (C) or as a five-category variable (D). A clear trend toward worse clinical outcome is observed progressing from a lower to a higher count of microvessels (test for trend, two-sided $P<.00001$) (Fig. 1, D).

Association of Clinicopathologic Characteristics and MVC With Overall Survival in Multivariate Analysis

A multivariate analysis was performed to assess the independent prognostic role of MVC after adjusting for other significant covariates. All variables that significantly affected survival in the univariate analysis were introduced into a Cox proportional hazards model (Table 2, starting model). In this analysis, MVC was used as a continuous variable. With the use of a backward-stepwise procedure, we eliminated covariates without independent prognostic significance from the model. At the end of the stepwise process, only tumor size, nodal status, and MVC maintained their independent prognostic influence on overall survival. The relative hazard of death associated with a high MVC was 8.38 (95% CI = 4.19-16.78), and the hazards associated with larger tumors and regional lymph node involvement were 1.73 (95% CI = 1.26-2.38) and 1.60 (95% CI = 1.31-1.95), respectively. To obtain a more clinical understanding of the prognostic effect of MVC, we performed a Cox analysis using the five-category MVC as a stratification factor. This approach allowed us to plot a cumulative hazard of death function for each MVC category, adjusting for all of the other covariates in the final model (Fig. 2, A). A highly positive association was observed between MVC category and the multivariate cumulative hazard. On the basis of this model, we also estimated the probability of survival at 2 years after surgery for different combinations of the two covariates. As shown in Fig. 2, B, the determination of MVC added strong, independent prognostic information to TNM staging. In fact, in patients with early stage disease (T1, T2, or N0), the 2-year survival probability declined progressively from 95% to 50% in relation to increasing MVC. Moreover, the 2-year survival probabilities for patients with T3, N2 disease were 67% and 53% in the subgroups with the two lowest categories of tumor vascularization, falling to only 27%, 14%, and 6%, respectively, in the three subgroups with progressively more vascularized tumors.

Table 1. Univariate analysis of the association between prognostic variables and overall survival: results expressed as observed/expected numbers of deaths

Patient and tumor characteristics	No. of cases	No. of deaths (observed/expected)	Two-sided *P*
Sex			
Male	360	115/112.0	.032
Female	47	19/12.0	
Age, y			
≤64	210	71/71.3	.959
>64	197	63/62.7	
Tumor grade*			
G1	98	30/30.4	.572†
G2	170	52/55.9	
G3	139	46/41.7	
Histology			
Squamous	228	64/76.8	.024
Nonsquamous	179	70/57.2	
Tumor size*			
T1	103	18/37.4	
T2	266	99/86.2	<.00001†
T3	38	17/10.4	
Node status*			
N0	247	58/91.6	
N1	64	24/19.1	<.00001†
N2	96	52/23.3	
Stage*			
S1	227	51/84.5	
S2	57	18/18.2	<.00001†
S3	123	65/31.3	
Dichotomous microvessel count, No.			
≤20	205	34/75.2	<.00001
>20	202	100/58.8	
Five-category microvessel count, No.			
1-10	68	11/26.7	
11-20	137	23/48.5	
21-30	65	25/21.3	<.00001†
31-40	86	46/24.4	
≥41	51	29/13.1	

*See (*25*,*26*) for information on tumor grading and staging.
†Test for trend.

Discussion

The only potentially curative treatment for NSCLC is complete surgical resection of the primary tumor and dissection of the hilar and mediastinal lymph nodes. Unfortunately, the 5-year prognosis for patients with NSCLC after surgical resection has not improved significantly over the last 10 years.

The most relevant prognostic information about NSCLC has been obtained from the TNM classification of the patient (*1*), reflecting the size of the primary tumor, the involvement of regional lymph nodes, and the presence of distant metastasis. However, the different and unpredictable outcomes for individual patients diagnosed at the same stage of disease and doubts regarding the selection of patients for aggressive adjuvant therapies have prompted the search for novel biologic and/or pathologic parameters that may help to identify patients with NSCLC who need postsurgical therapy.

The importance of tumor angiogenesis in tumor development and progression has been revealed in recent years. Several studies [reviewed in (*31*)] have suggested that MVC (as a measure of tumor angiogenesis) is a significant predictor of increased risk of metastatic disease and worse overall survival for several types of human cancer, including lung cancer. However, relatively few reports (*7-11*,*23*,*24*) have been published on the prognostic significance of MVC in lung cancer. Furthermore, some of these studies [e.g., (*8*)] have analyzed only particular subgroups of NSCLC, and, in some cases [e.g., (*9*)], a multivariate analysis to assess the independent prognostic role of MVC on relapse and overall survival has not been carried out. Finally, several antibodies against different endothelial cell markers, such as factor VIII-related antigen, CD31, or CD34, have been used with sometimes conflicting results (*16*,*32*,*33*).

In this study, we used an anti-CD34 monoclonal antibody in the immunohistochemical analysis. In previous studies (*7*,*9*), we used an anti-factor VIII monoclonal antibody on methacarn-fixed, paraffin-embedded tumor samples. In agreement with other investigators (*22*), we have found that the anti-CD34 antibody is more reliable in terms of specificity and reproducibility than monoclonal antibodies generated against other endothelial cell antigens.

To our knowledge, this study is the largest prospective analysis of the prognostic role of tumor angiogenesis in patients with NSCLC treated with curative surgery. The results clearly demonstrate that, in stage I-III operable NSCLC, MVC determined by means of specific anti-CD34 immunostaining is a statistically significant independent predictor of poor outcome and shorter survival. T and N stages were the only other two parameters that independently affected prognosis in this series of patients. The risk of death appeared to be strongly associated with the degree of neovascularization. In fact, the multivariate cumulative hazard of death showed a striking increasing trend from the least neovascularized to the most neovascularized class of tumors. Furthermore, multivariate estimates of the 2-year probability of death following radical surgery increased markedly as MVC increased in all of the T and N subgroups of patients. In this respect, a simple prognostic model that includes MVC, T size, and N status can be proposed to identify subsets of patients with a very wide range of prognostic estimates, i.e., with a 2-year probability of death spanning from 5% with the best combination of the three

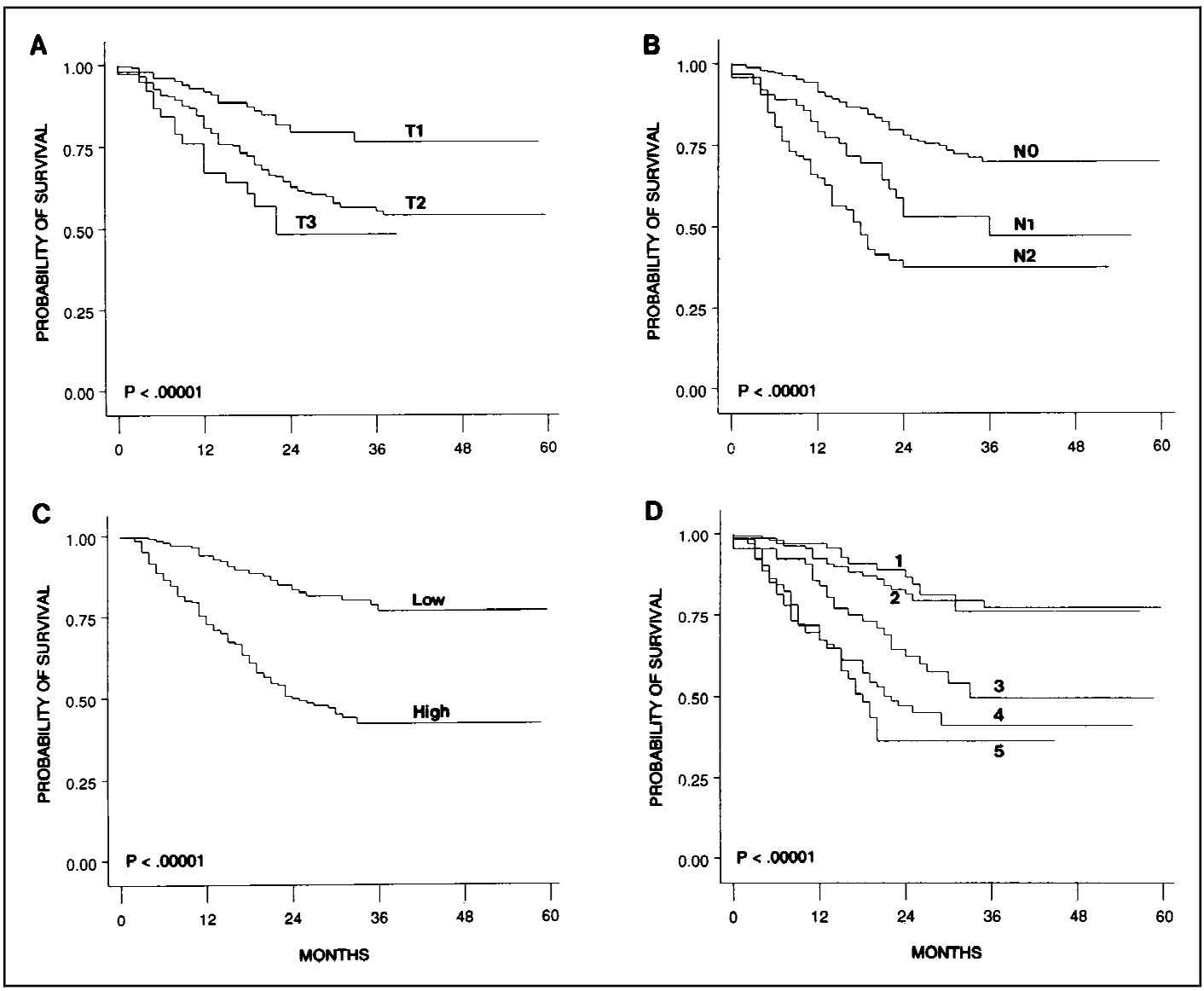


Fig. 1. Survival curves for 407 patients with non-small-cell lung cancer. The Kaplan–Meier method was used to estimate overall survival, and the logrank test was used to compare the curves. Overall survival was strongly affected by tumor size **(A),** nodal status **(B),** and microvessel count, which was stratified either into two groups (≤20 microvessels = low and >20 microvessels = high) **(C)** or five groups (according to increasing number of microvessels: 1 = 1-10, 2 = 11-20, 3 = 21-30, 4 = 31-40, and 5 = 41 or more) **(D).** The 95% confidence intervals for the survival curves at 24, 36, and 48 months, respectively, are as follows: A) T1 = 0.90-0.74, 0.87-0.67, and 0.87-0.67; T2 = 0.70-0.58, 0.63-0.51, and 0.63-0.51; and T3 = 0.66-0.30, 0.66-0.30, and 0.66-0.30; B) N0 = 0.82-0.70, 0.77-0.65, and 0.78-0.62; N1 = 0.73-0.45, 0.69-0.37, and 0.65-0.29; N2 = 0.52-0.28, 0.48-0.24, and 0.48-0.24; C) low = 0.91-0.79, 0.86-0.70, and 0.86-0.70; and high = 0.62-0.46, 0.52-0.36, and 0.52-0.36; D) 1 = 0.82-0.96, 0.70-0.92, and 0.62-0.90; 2 = 0.75-0.91, 0.68-0.86, and 0.68-0.86; 3 = 0.51-0.77, 0.39-0.69, and 0.39-0.69; 4 = 0.37-0.59, 0.29-0.53, and 0.29-0.53; 5 = 0.21-0.51, 0.21-0.51, and not available.

prognostic factors to approximately 95% with the worst combination. This finding is very interesting from a clinical standpoint, since a similar model could be very useful in selecting among different postsurgical treatment strategies (from observation only to more aggressive experimental regimens) and in improving the counseling of patients. However, our results must be validated prospectively with other, independent series of patients before incorporating MVC determinations into current practice or using MVC as a stratification criterion for clinical trials.

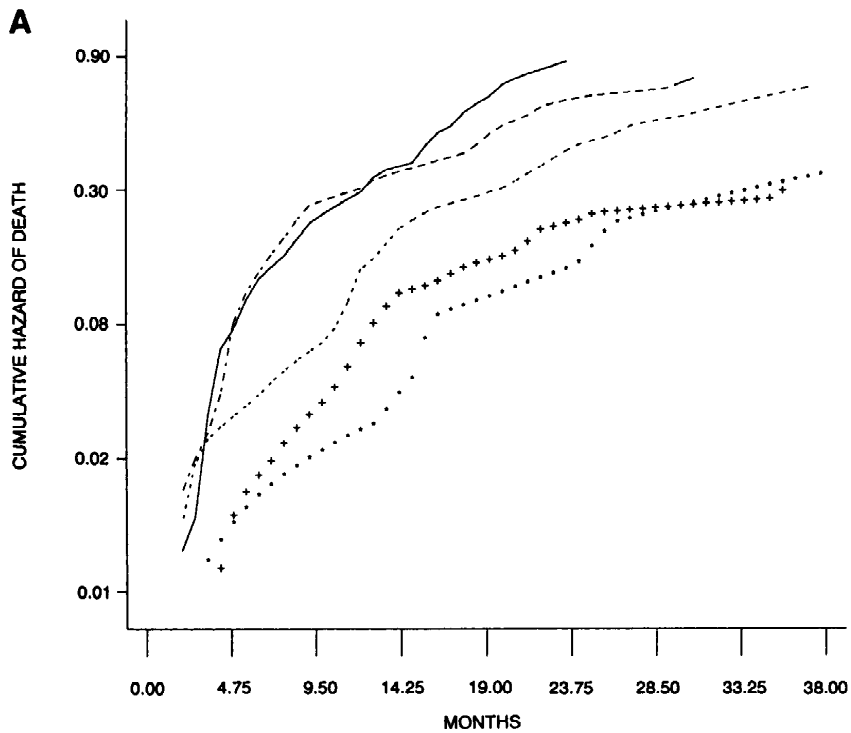
A second clinically relevant aspect of our findings deserves to be mentioned. This aspect relates to the development of new, specific antiangiogenic drugs, which

Table 2. Multivariate analysis

Covariate	χ^{2}*	*P*
	Starting model†	
Age	0.13	.7168
Sex	1.80	.1793
Tumor size	8.44	.0037
Node status	5.51	.0189
Stage	0.01	.9175
Histology	1.59	.2072
Microvessel count	4.19	<.00001

Variable	χ^{2}*	*P*	Relative hazard‡	95% CI§
		Final model†		
Tumor size	11.68	.0006	1.73	1.26-2.38
Nodal status	21.20	.00001	1.60	1.31-1.95
Microvessel count	40.29	.00001	8.38	4.19-16.78

*χ^{2} = chi-squared.
†Cox proportional hazards model (*29*).
‡Of death associated with larger tumors, regional lymph node involvement, or high microvessel counts.
§95% CI = 95% confidence interval.



B

Two-year Survival Probability

	MVC Category:	1	2	3	4	5
N0	T1	0.95	0.92	0.83	0.76	0.67
N0	T2	0.91	0.86	0.73	0.62	0.50
N0	T3	0.84	0.77	0.58	0.43	0.30
N1	T1	0.92	0.88	0.76	0.65	0.54
N1	T2	0.86	0.79	0.62	0.47	0.34
N1	T3	0.77	0.66	0.43	0.27	0.16
N2	T1	0.88	0.81	0.65	0.52	0.39
N2	T2	0.79	0.70	0.47	0.32	0.19
N2	T3	0.67	0.53	0.27	0.14	0.06

Fig. 2. Multivariate Cox analysis stratified by five microvessel count (MVC) categories. **A)** Multivariate cumulative hazard of death function according to MVC (• = MVC 1-10 microvessels, + = MVC 11-20 microvessels, −− = MVC 21-30 microvessels, –•–• = MVC 31-40 microvessels, and —— = MVC 41 or more microvessels. **B)** Estimates of the 2-year probability of survival were calculated by the following mathematic formula: $S(2\text{ year}, z) = [S_0(2\text{ year})]^{\exp(\beta 1 z1 + \beta 2 z2)}$, where S(2 year, *z*) is the 2-year (60 months) survival probability for covariate vector *z; z* is a given covariate combination; *z*1 is a value of tumor size codified as 1, 2, or 3 (T1, T2, or T3); β1 is the regression coefficient for tumor size and equals 0.5452; *z*2 is the value of nodal status codified as 0, 1, or 2 (N0, N1, or N2); S0 is the estimated baseline survival at 2 years for each stratum of MVC. *See* (*25*,*26*) for information on T and N classification.

are currently being introduced into clinical research [e.g., (*34*)]. Since angiogenesis seems to play such an important role in the biologic aggressiveness of NSCLC, this disease appears to be a perfect candidate to test the efficacy of these drugs in the clinical setting.

In conclusion, in our series, MVC is the strongest independent prognostic indicator for patients with NSCLC who have been treated with radical surgery. The future role of MVC in selecting subsets of patients for inclusion in clinical trials of adjuvant chemotherapy and/or radiation therapy must be investigated thoroughly. Finally, MVC may help to identify subsets of patients in whom the activity of specific antiangiogenic drugs can be tested.

References

(*1*) Mountain CF. A new international staging system for lung cancer. Chest 1986;89(4 Suppl): 225s-233s.

(*2*) Slebos RJ, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, Meijer CJ, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 1990;323: 561-5.

(*3*) Quinlan DC, Davidson AG, Summers CL, Warden HE, Doshi HM. Accumulation of p53 correlates with a poor prognosis in human lung cancer. Cancer Res 1992;52:4828-31.

(*4*) Harpole DH Jr, Herndon JE 2nd, Wolfe WG, Iglehart JD, Marks JR. A prognostic model of recurrence and death in stage I non-small cell lung cancer utilizing presentation, histopathology, and oncoprotein expression. Cancer Res 1995;55:51-6.

(*5*) Fontanini G, Macchiarini P, Pepe S, Ruggiero A, Hardin M, Bigini D, et al. The expression of proliferating cell nuclear antigen in paraffin sections of peripheral node-negative non-small cell lung cancer. Cancer 1992;70: 1520-7.

(*6*) Scagliotti GV, Micela M, Gubetta L, Leonardo E, Cappia S, Borasio P, et al. Prognostic significance of Ki67 labelling in resected non small cell lung cancer. Eur J Cancer 1993;29A: 363-5.

(*7*) Macchiarini P, Fontanini G, Hardin JM, Squartini F, Angeletti CA. Relation of neovascularisation to metastasis of non-small-cell lung cancer. Lancet 1992;340:145-6.

(*8*) Yamazaki K, Abe S, Takakawa H, Sukoh N, Watanabe N, Ogura S, et al. Tumor angiogenesis in human lung adenocarcinoma. Cancer 1994;74:2245-50.

(*9*) Fontanini G, Bigini D, Vignati S, Basolo F, Mussi A, Lucchi M, et al. Microvessel count predicts metastatic disease and survival in non-small cell lung cancer. J Pathol 1995;177:57-63.

(*10*) Angeletti CA, Lucchi M, Fontanini G, Mussi A, Chella A, Ribechini A, et al. Prognostic significance of tumoral angiogenesis in completely resected late stage lung carcinoma (stage IIIA-N2). Impact of adjuvant therapies in a subset of patients at high risk of recurrence. Cancer 1996;78:409-15.

(*11*) Harpole DH Jr, Richards WG, Herndon JE 2nd, Sugarbaker DJ. Angiogenesis and molecular biologic substaging in patients with stage I non-small cell lung cancer. Ann Thorac Surg 1996;61:1470-6.

(*12*) Folkman J. What is the evidence that tumors are angiogenesis dependent? [editorial] J Natl Cancer Inst 1990;82:4-6.

(*13*) Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 1989; 339:58-61.

(*14*) Srivastava A, Laidler P, Davies RP, Horgan K, Hughes LE. The prognostic significance of tumor vascularity in intermediate-thickness (0.76-4.0 mm thick) skin melanoma. A quantitative histologic study. Am J Pathol 1988; 133:419-23.

(*15*) Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. N Engl J Med 1991;324:1-8.

(*16*) Horak ER, Leek R, Klenk N, LeJeune S, Smith K, Stuart N, et al. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet 1992;340: 1120-4.

(*17*) Gasparini G, Harris AL. Clinical importance of the determination of tumor angiogenesis in breast carcinoma: much more than a new prognostic tool. J Clin Oncol 1995;13:765-82.

(*18*) Toi M, Inada K, Suzuki H, Tominaga T. Tumor angiogenesis in breast cancer: its importance as a prognostic indicator and the association with vascular endothelial growth factor expression. Breast Cancer Res Treat 1995;36: 193-204.

(*19*) Gasparini G, Weidner N, Maluta S, Pozza F, Boracchi P, Mezzetti M, et al. Intratumoral microvessel density and p53 protein: correlation with metastasis in head-and-neck squamous-cell carcinoma. Int J Cancer 1993;55:739-44.

(*20*) Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 1993;143:401-9.

(*21*) Hollingsworth HC, Kohn EC, Steinberg SM, Rothenberg ML, Merino MJ. Tumor angiogenesis in advanced stage ovarian carcinoma. Am J Pathol 1995;147:33-41.

(*22*) Tanigawa N, Amaya H, Matsumura M, Shimomatsuya T, Horiuchi T, Muraoka R, et al. Extent of tumor vascularization correlates with prognosis and hematogenous metastasis in gastric carcinomas. Cancer Res 1996;56: 2671-6.

(*23*) Giatromanolaki A, Koukourakis M, O'Byrne K, Fox S, Whitehouse R, Talbot DC, et al. Prognostic value of angiogenesis in operable non-small cell lung cancer. J Pathol 1996;179: 80-8.

(*24*) Macchiarini P, Fontanini G, Dulmet E, de Montpreville V, Chapelier AR, Cerrina J, et al. Angiogenesis: an indicator of metastasis in non-small cell lung cancer invading thoracic inlet. Ann Thorac Surg 1994;57: 1534-9.

(*25*) World Health Organization. The World Health Organization. Histological typing of lung tumours. Am J Clin Pathol 1982;77:123-36.

(*26*) American Joint Committee on Cancer. Manual for a staging of cancer. 4th ed. 1992:115-22.

(*27*) Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.

(*28*) Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163-70.

(*29*) Cox DR. Regression model and life tables. J R Stat Soc 34 [series B]; 1972:187-220.

(*30*) Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: Wiley, 1980.

(*31*) Weidner N, Folkman J. Tumoral vascularity as a prognostic factor in cancer. In: De Vita VT Jr, Hellman S, Rosenberg SA, editors. Important advances in oncology 1996. Philadelphia: Lippincott-Raven, 1996:167-90.

(*32*) Van Hoef ME, Knox WF, Dhesi SS, Howell A, Schor AM. Assessment of tumour vascularity as a prognostic factor in lymph node negative invasive breast cancer. Eur J Cancer 1993;29A: 1141-5.

(*33*) Anthony PP, Ramani P. Endothelial markers in malignant vascular tumours of the liver: superiority of QB-END/10 over von Willebrand factor and Ulex europaeus agglutinin 1. J Clin Pathol 1991;44:29-32.

(*34*) Ingber D, Fujita M, Kishimoto S, Sudo K, Kanamaru T, Brem H, et al. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth. Nature 1990;348: 555-7.

Note

Manuscript received November 7, 1996; revised March 3, 1997; accepted April 14, 1997.