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Tumor Angiogenesis in Advanced Stage Ovarian Carcinoma

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Tumor angiogenesis has been found to have prognostic significance in many tumor types for predicting an increased risk of metastasis. We assessed tumor vascularity in 43 cases of advanced stage (International Federation of Gynecologists and Obstetricians stages III and IV) ovarian cancer by using the highly specific endothelial cell marker CD34. Microvessel counts and stage were associated with disease-free survival and with overall survival by Kaplan-Meier analysis. The plots show that higher stage, higher average vessel count at 200× (200× avg) and 400× (400× avg) magnification and highest vessel count at 400× (400× high) magnification confer a worse prognosis for disease-free survival. Average vessel count of less than 16 (400× avg, $P_2 = 0.01$) and less than 45 (200× avg, $P_2 = 0.026$) suggested a better survival. Similarly, a high vessel count of less than 20 (400× high, $P_2 = 0.019$) conferred a better survival as well. The plots suggest that higher stage, higher average vessel count at 200× and 400×, and highest vessel count at 200× and 400× show a trend to worse overall survival as well. With the Cox proportional hazards model, stage was the best predictor of overall survival, however, the average microvessel count at 400× was found to be the best predictor of disease-free survival. These results suggest that analysis of neovascularization in advanced stage ovarian cancer may be a useful prognostic factor. (Am J Pathol 1995, 147:33–41)

Growth and metastatic dissemination of solid tumors requires vascular support for nutritive supply and access.[1–4] For a tumor to get much larger than 1 mm^3 neovascularization must occur.[3,5] The onset of this vascular phase marks a period of more rapid growth, local invasion, and, ultimately, metastasis.[6] Growth of tumors, both primary and metastatic, is dependent on angiogenesis[1–4,7]; thus any increase in tumor mass must be accompanied by an increase in capillary formation to supply the tumor mass.[6]

Metastasis also requires angiogenesis in addition to the three steps of invasion.[4,7] Vascularization is required to supply conduits for tumor cells to be shed into the circulation.[1,6] In addition, newly formed capillaries are leaky because of fragmented basement membranes, making them more accessible to errant tumor cells.[8]

Tumor angiogenesis has been associated with patient outcome in a number of malignancies and may be an important prognostic marker. Tumor angiogenesis was first found to have prognostic significance in cutaneous malignant melanoma.[9–11] Subsequently, clinical studies in early stage invasive breast carcinoma revealed that angiogenesis was a predictor of an increased risk of metastatic disease.[8,12–16] This prognostic feature has since been described in non-small cell lung carcinoma,[17] in prostate cancer,[18] and in testicular germ cell tumors.[19]

Little is known of the significance of neovascularization in ovarian cancer. The early and extensive metastatic dissemination of ovarian cancer suggests that angiogenesis may be an early and important event. Such information could be of use in identifying those patients who are at increased risk of relapse or more distant dissemination. This retrospective study evaluated the presence of neovascularization in advanced stage epithelial ovarian cancer patients address the prognostic significance of neoangiogenesis on overall survival (OS) and disease-free survival (DFS).

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Materials and Methods

Case Selection

Patients were treated with one of two similar chemotherapy protocols for newly diagnosed advanced stage epithelial ovarian cancer at the National Cancer Institute. All patients were referred to the Medicine Branch, National Cancer Institute, with newly diagnosed disease; the majority of patients had their initial workup and staging laparotomy at an outside institution. All patients had advanced International Federation of Gynecologists and Obstetricians stage III or IV disease. A total of 80 patients from two protocols were identified. Of the original 80 patients, 37 were excluded; 7 patients were excluded because the primary tumor was unavailable for histological confirmation, 9 because of lack of follow-up information, and 21 because the paraffin block was no longer available for study. Blocks and slides were available for 43 patients. Of these, 23 patients (53%) were treated by dose-intensive induction therapy with cyclophosphamide and cisplatin; 6 of these patients also received consolidative whole abdominal radiation.[20] The remaining 20 patients (47%) were treated on a pilot protocol of a platinum-intense induction regimen consisting of cyclophosphamide, carboplatinum, and cisplatinum.

Pathological Review

All pathology was reviewed by two separate observers for histological subtype and given both an architectural and a cytological grade.[21,22] Tumors were classified according to their predominant histology, defined as a single pattern comprising over 50% of the tumor, as serous, endometrioid, transitional cell, clear cell, mucinous, or undifferentiated. Those that did not have predominant epithelial pattern were classified as mixed carcinomas. The presence or absence of vascular invasion was noted. A mean of 5.3 sections were reviewed per case (range 1 to 16). The best fixed, most representative sections were then chosen for analysis of neovascularization. When available (n = 23), a representative block of omental implant was also studied. All angiogenesis parameters were determined without prior knowledge of patient outcome.

Immunohistochemistry

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue the avidin-biotin immunoperoxidase complex technique (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA). The following antisera were used: anti-*Ulex europaeus* agglutinin (Dako, Carpinteria, CA), anti-human von Willebrand factor (vWF), previously designated as factor VIII-related antigen (Dako), and 110-kd CD34 (AMAC, Westbrook, ME). Anti-*Ulex*, the least specific to endothelial cells, reacts with native lectins. Anti-vWF, previously studied by Weidner et al in breast carcinoma,[12] reacts with vWF in endothelial cells. CD34 recognizes a cell surface antigen selectively expressed on hematopoietic progenitor cells and on some vascular endothelial cells.[23,24]

Microvessel Quantitation

Two methods were used to determine the density of the tumor-associated microvasculature. Both methods were performed on adjacent sections with each antibody, CD34, *Ulex*, and vWF. The first, a modification of the method of Weidner et al,[12] quantifies the vessels in the most vascular portion of the tumor. With a variation of their method proposed by Bosari et al,[13] sections were examined under low power (40× to 100×) to identify the region of highest vessel density (so-called hot spots). In each section, the three most vascular areas were chosen. A 200× field in each of these three regions was counted, and the highest and average counts of the three fields were recorded. This analysis was also performed in a 400× field in each of these three regions. As discussed by Bosari et al, large vessels with thick muscular walls and large vessels with lumina greater than approximately eight red blood cells were excluded from the count. A vessel lumen was not required for identification of a microvessel; single cells or cell clusters were counted. Counts are expressed as total number of microvessels per 200× or 400× field. These data will be referred to as density counts.

The second method of microvessel quantitation utilized will be referred to as volume counts, referring to the quantitation of vascular volume (VV). With a variation of the method of Vogel,[25] a modification of the method of Chalkley,[26] the VV of the tumor tissue was calculated. At a magnification of 200×, four points in the focal plane of the tissue section were randomly shifted with a mechanical stage. Coincidence of a stained endothelial cell with one of these points constituted a hit, and the procedure was continued randomly across the tissue section until 45 such hits were accumulated. The number of fields examined per section was a function of the actual VV present, ie, the smaller the VV, the greater the number of fields that had to be examined to accumulate 45 hits.[25] The accuracy of this method for determining VV is between

6 and 10% of the true value.[27] Areas of necrosis were avoided and, as above, large vessels were not included as a positive hit. Values of VV are expressed as percent of total tissue volume.[25]

Statistical Analysis

Data on all 43 patients were used in the analyses. In cases for which more than one section of tumor was examined, the average of the values obtained was used for that patient.

The Kruskal-Wallis test was used to compare values of the angiogenesis parameters between grade level and histological subtypes. The Wilcoxon rank sum test was used to compare angiogenesis parameters between stage III and stage IV patients. Spearman rank correlation coefficients were used to express the associations among the individual angiogenesis parameters.

The probability of OS or DFS was calculated by the Kaplan-Meier method,[28] and the significance of the difference between pairs of Kaplan-Meier curves was calculated by the Mantel-Haenszel procedure.[29] The median value of each angiogenesis variable was used as the cutoff category in the analysis. The Cox proportional hazards model was used to identify which factors are jointly significant in their association with OS or DFS.[30] The resulting model parameters (b_i) were converted to relative risks by computing exp(b_i) where exp(a) = 2.1783[a]. [31] The 95% confidence interval for the relative risk was computed as (exp(b_{iL}), exp(b_{iH})) where $b_{iL} = b_i - 1.96$ (estimated SE (b_i)) and $b_{iH} = b_i + 1.96$ (estimated SE (b_i)). The relative risk indicates the risk associated with dying, or relapsing while being in a greater risk category compared with that of being in a lower risk category. All P values are two-sided and denoted by P_2.

Results

Patient Characteristics

Patient data are presented in Table 1. Patient age ranged from 18 to 71 years (median 53). There were 25 patients who were stage III (12 of these were optimally debulked) and 18 were stage IV. The patients received a median of three cycles of chemotherapy (mean 3.6, range 1 to 6). Median survival was 2.5 years. At the time of analysis, 5 patients were without evidence of disease, 11 were alive with disease, and 27 were dead of disease.

Primary tumors were a mean of 5.1 cm in greatest diameter (range 2 to 16 cm), and most (74%) were bilateral. Serous tumors made up the majority of cases (16, 37%), followed by transitional cell (8, 19%), clear cell (6, 14%), and mixed epithelial tumors (4, 9%), as shown in Table 1. Architectural grade III was seen in 42% of cases, with 25 and 33% of cases exhibiting architectural grades I and II, respectively; 63% of cases were cytological grade III with 16 and 21% grades I and II, respectively. Vascular invasion was observed in 11 (25%) cases.

Table 1. *Clinical Information (n = 43 Patients)*

Parameter	Number
Mean patient age (range)	49.6 (18–71)
Stage III	25
Optimally debulked	12
Suboptimally debulked	13
Stage IV	18
Cycles of chemotherapy (median (range))	3 (1–6)
Mean tumor diameter* (range)	5.1 (2–16) cm
Bilateral tumor	32
Unilateral tumor	4[†]
Tumor type	
Serous	16
Transitional cell	8
Clear cell	6
Other[‡]	13
Architectural grade I[§]	11
II	14
III	10
Cytologic grade I	7
II	9
III	27
Median survival	2.5 years
Survival NED[‖]	5
AWD	11
DOD	27

*Size in four cases is unknown.
†Bilaterality in seven cases is unknown.
‡Includes serous borderline (2), mixed epithelial carcinomas (4), endometrioid (2), serous surface (2), mucinous (1), small cell (1), and poorly differentiated (*1*).
§Transitional cell tumors given cytologic grade only.
‖NED, no evidence of disease; AWD, alive with disease; DOD, dead of disease.

Immunohistochemistry

Immunohistochemistry was performed with the three antibodies described above, *Ulex*, vWF, and CD34. Initially, counts were performed with each of the three stains on each case. Subsequently, however, it was noted that variability in staining was occurring with both *Ulex* and vWF, leading to misleading low vessel counts. In these cases, understaining of endothelium was noted in approximately 50% of the slides. Although attempts to optimize staining procedures with these antibodies were made, variability and lack of reproducibility remained a problem. The CD34 antibody stained consistently and reproducibly and did not exhibit such problems. For these reasons, data presented are from CD34 staining only.

Figure 1A shows a representative case of serous

carcinoma with a high vessel count, in comparison with Figure 1B, which is representative of a case with a low vessel count.

Angiogenesis Variables

Both density counts and volume counts were performed on all tissue sections. Vascular density was quite heterogeneous in any given tissue section, some areas being quite vascular other areas remained relatively avascular. Table 2 shows the mean number of microvessels seen in the density counts at two powers and presented as mean and highest levels observed. The mean percent VV calculated by the VV method is tabulated (Table 2). A high degree of variability was seen between the average and high vessel counts at each of the two magnifications. Comparison of the differences at 200× power ranged from 0 to 10% (21% of cases) to greater than 20% (28% of cases). More variability was noted at 400× power, where a greater than 20% difference was seen in 35% of cases and agreement (<10% difference) in only 26%. This calls attention to the fact that the so-called hot spots of neovascularization tended to have more neovessels than the rest of the tissue, even in regions that appeared vessel-rich to the observers.

Table 2. *Basic Data on Angiogenesis Variables*

	Mean	SEM	n	Range
Primary tumor				
200× average	49.4	3.3	43	16–118
200× high	61.1	4.4	43	20–174
400× average	17.5	1.3	43	6–42
400× high	21.5	1.7	43	6–53
VV (%)	7.0	0.5	43	3.1–22.5
Omental metastasis				
200× average	44.4	3.7	23	13–82
200× high	54.6	4.8	23	20–104
400× average	17.4	1.7	23	6–38
400× high	22.5	2.2	23	7–47

Although tumor growth is accelerated during the vascular phase, no significant difference in tumor size was found between the highly angiogenic tumors and those that were less angiogenic (data not shown). Analysis of the 11 cases showing vascular invasion revealed that 6 were in the high vascular density, or poor prognosis, group, and 5 were in the low vascular density, or better prognosis group, suggesting that hypervascularity and vascular invasion may be independent factors.

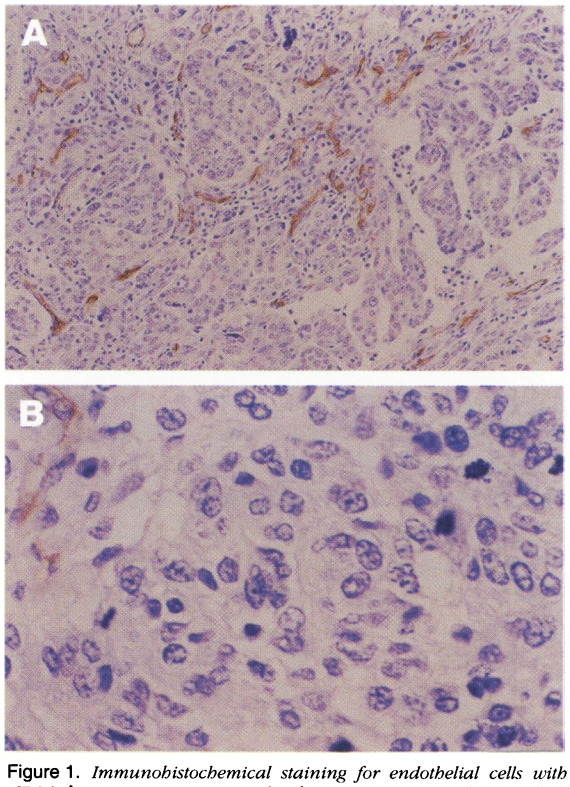

Figure 1. *Immunohistochemical staining for endothelial cells with CD34.* **A:** *Representative example of serous carcinoma showing high vascularization (400×, immunoperoxidase stain for CD34).* **B:** *Representative example of serous carcinoma showing low vascularization (400×, immunoperoxidase stain for CD34).*

Statistical Analysis

Sample statistics for each of the angiogenesis parameters are shown in Table 2. None of the angiogenesis variables were associated with either tumor type or grade. Analysis by the Kruskal-Wallis method resulted in P values between 0.05 and 0.97 for all comparisons made between angiogenesis variables and tumor type or grade. Table 3 shows that stage is statistically significantly associated with the average and high vessel counts for the primary tumor. A trend toward significance was seen for the omental vascular counts. Table 4 indicates that the four vascular density measurements are highly correlated with each other. Although still statistically significant, the correlation between vascular density and VV was less.

Kaplan-Meier plots were constructed to illustrate the effects of parameters on OS and DFS. The median value of each angiogenesis variable was used as the cutoff category in the analysis. A statistically significantly improved DFS was consistently found with all vessel count analyses (Table 5). The plots show that higher stage, higher average vessel count at 200× (200× avg) and 400× (400× avg), and highest vessel count at 400× (400× high) confer a worse prognosis for DFS (Figure 2). Average vessel count of less than 16 (400× avg, $P_2 = 0.01$) and less than 45 (200× avg,

Table 3. *Association Between Stage and Angiogenesis Variables*

Variable	Stage	$\bar{X}$	SEM	n	Wilcoxon Rank Sum P_2
Primary tumor					
400× average	III	14.7	1.4	25	
	IV	21.4	2.1	18	0.0036
400× high	III	18.2	1.9	25	
	IV	26.2	2.7	18	0.0099
200× average	III	44.0	4.3	25	
	IV	57.0	4.7	18	0.013
200× high	III	54.1	6.4	25	
	IV	70.8	5.1	18	0.0065
VV	III	6.0	0.4	25	
	IV	8.2	1.1	18	0.078
Omental metastasis					
200× average	III	36.8	5.6	8	
	IV	48.5	4.6	15	0.13
200× high	III	45.4	6.8	8	
	IV	59.5	6.2	15	0.12
400× average	III	13.6	1.6	8	
	IV	19.5	2.3	15	0.14
400× high	III	17.3	2.6	8	
	IV	25.3	2.9	15	0.075

$P_3 = 0.026$) suggested a better survival. Similarly, a high vessel count of less than 20 (400× high, $P_2 = 0.019$) conferred a better survival as well.

The Kaplan-Meier plots suggest that higher stage, higher average vessel count at 200× (200× avg) and 400× (400× avg), and highest vessel count at 200× (200× high) and (400× high) show a trend to worse OS (Figure 3). Average vessel count of less than 16 (400× avg, $P_2 = 0.064$) and less than 45 (200× avg, $P_2 = 0.059$) suggested a better survival. Similarly, a high vessel count of less than 20 (400× high, $P_2 = 0.12$) and less than 41 (200× high, $P_2 = 0.084$) portended better survival. VV counts were not found to be prognostic for either OS or DFS. No prognostic benefit was conferred by analysis of neoangiogenesis of omental implants.

The best Cox proportional hazards model for OS included FIGO stage alone. Thus, for OS, the best variable for predicting outcome from among all the angiogenesis variables, stage, and grade is International Federation of Gynecologists and Obstetricians stage.

The best Cox proportional hazards model for DFS was the average count at 400× magnification (see Table 6). Thus, for DFS, the best variable for predicting outcome from among stage, grade, tumor type, VV measurements, and the four vascular density measurements is the average count at 400× magnification (400× avg). A value of 16 or more vessels found in an average of three 400× fields yields a relative risk of disease relapse of 2.14 (95% CI = 1.08 to 4.25).

Discussion

We sought to identify the significance of neovascularization in epithelial ovarian carcinoma. The majority of women with advanced stage ovarian cancer die of progressive disease. Five year survival rates are 15 to 20% for stage III and 5% or less for stage IV.[20] Selecting out a subset of patients from this group who may have a better prognosis may be clinically useful. Most patients die of complications from local disease or peritoneal disease rather than from blood-borne metastasis, however, and it was unclear whether angiogenesis studies such as those described above in metastatic breast carcinoma would be of value.

As shown for the other malignancies, vascular density counts were predictive of both OS and DFS. VV counts, which measure the total VV of a given tissue section, were not found to have predictive value. Vascular density counts focus on the regions of highest neovascularization. As discussed by Weidner et al,[8] however, the onset of angiogenesis does not require that a tumor cells secrete angiogenic factors for neovascularization to occur. Accordingly, determination of vessel density should be in areas of most intense neovascularization, as is done with the vascular density counts, rather than in the average of an entire tissue section, as is done with the VV counts. These highly angiogenic regions are more likely the source of metastatic foci,[8] and identifying ovarian tumors with such regions may be clinically useful. CD34 was used as an endothelial cell marker, which seemed to stain more uniformly and consistently than vWF or

Table 4. *Statistically Significant Correlation for Relatedness Between Different Vessel Counts (Spearman Correlations (r) and Significance (P_2) for Association Among the Four Vascular Density Variables)*

	r; P_2			
	400× high	200× average	200× high	VV
400× average	0.98; .0001	0.90; .0001	0.87; .0001	0.47; .0014
400× high		0.87; .0001	0.84; .0001	0.46; .0019
200× average			0.96; .0001	0.44; .0031
200× high				0.40; .0076

Table 5. *Significance of Vessel Counts by Kaplan-Meier Analysis With OS and DFS (P_2 Values)*

	200× average	400× average	200× high	400× high
OS	0.059	0.064	0.084	0.12
DFS	0.026	0.01		0.019

Ulex. It should be noted that analysis of vascular density should begin with assessment of staining quality.

It has been well established that angiogenesis is an important element in solid tumor progression. Tumors initially do not require an extensive vasculature, obtaining nutrients through diffusion. This prevascular phase[3,5] can maintain the tumor only to a certain size; for the tumor to get much larger than 2 mm in diameter,[5] neovascularization must occur. The onset of the vascular phase marks a period of more rapid growth, local invasion, and, ultimately, metastasis.[6] Tumor cells secrete angiogenic factors that induce vessels to sprout into the tumor,[3,5,6] and in tumor endothelial cells elaborate growth factors that may stimulate tumor growth.[1] Growth of tumors both primary and metastatic, is dependent on angiogenesis.[3,7] Increases in tumor mass must be accompanied by an increase in its vascular supply.[6]

Induction of angiogenesis is also important in the process of carcinogenesis. Induction of angiogenesis has been shown to occur in the transition from hyperplasia to neoplasia in experimental animals,[2] and preneoplastic lesions of the breast and bladder acquire angiogenic activity before malignant transformation.[5] The acquisition of angiogenic capability can be seen in ovarian peritoneal implants, which remain avascular and small until neovascularization from adjacent peritoneal vessels occurs. Angiogenesis and neoplastic transformation are not, however, interdependent, and each can occur in the absence of the other.[1,6,7]

Metastasis is also affected by angiogenesis, although neovascularization is only one of the requirements.[7] Vascularization is usually required for tumor cells to be shed into the circulation after they acquire invasive capability.[6] In addition, newly formed tumor capillaries are leaky because of fragmented basement membranes, making them more accessible to tumor cells.[8] Angiogenesis like metastasis, requires that endothelial cells have the capability for invasive behavior, albeit in a regulated fashion. The similarity of metastasis and angiogenesis and the interdependence of the two suggested that angiogenesis would offer a good

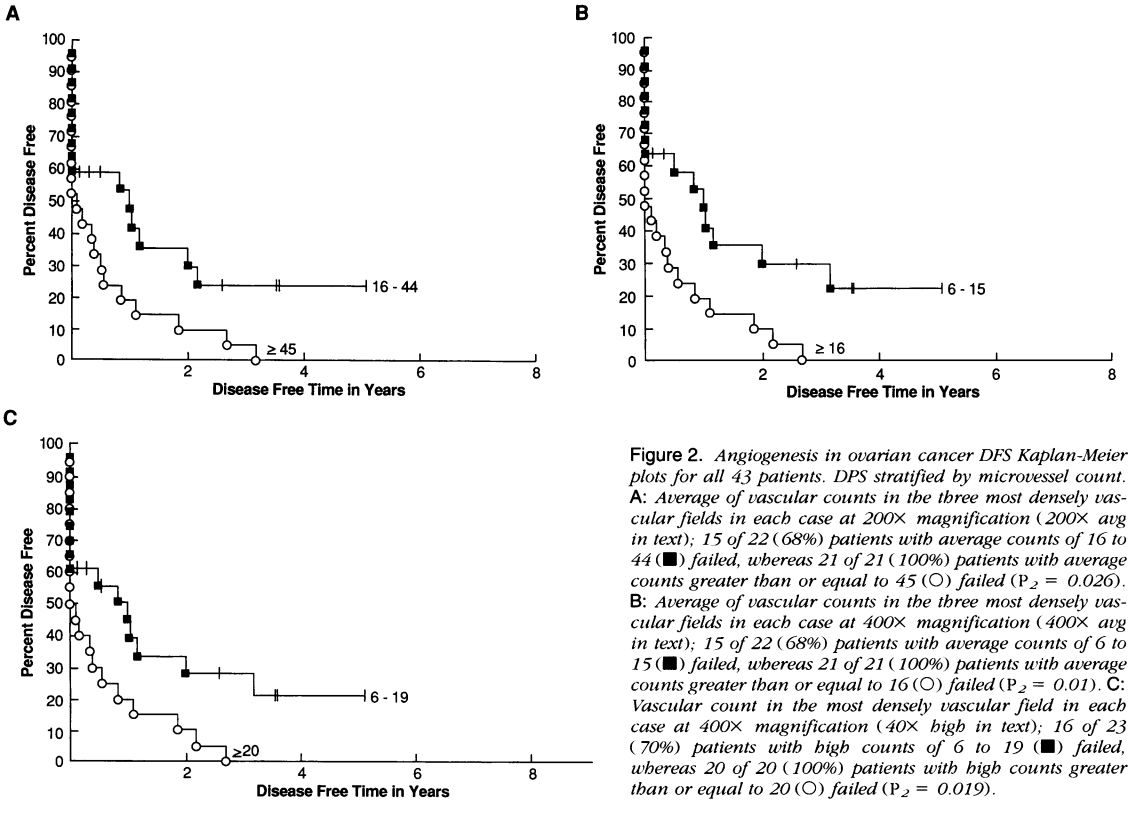

Figure 2. *Angiogenesis in ovarian cancer DFS Kaplan-Meier plots for all 43 patients. DPS stratified by microvessel count.* **A:** *Average of vascular counts in the three most densely vascular fields in each case at 200× magnification (200× avg in text); 15 of 22 (68%) patients with average counts of 16 to 44 (■) failed, whereas 21 of 21 (100%) patients with average counts greater than or equal to 45 (○) failed (P_2 = 0.026).* **B:** *Average of vascular counts in the three most densely vascular fields in each case at 400× magnification (400× avg in text); 15 of 22 (68%) patients with average counts of 6 to 15 (■) failed, whereas 21 of 21 (100%) patients with average counts greater than or equal to 16 (○) failed (P_2 = 0.01).* **C:** *Vascular count in the most densely vascular field in each case at 400× magnification (40× high in text); 16 of 23 (70%) patients with high counts of 6 to 19 (■) failed, whereas 20 of 20 (100%) patients with high counts greater than or equal to 20 (○) failed (P_2 = 0.019).*

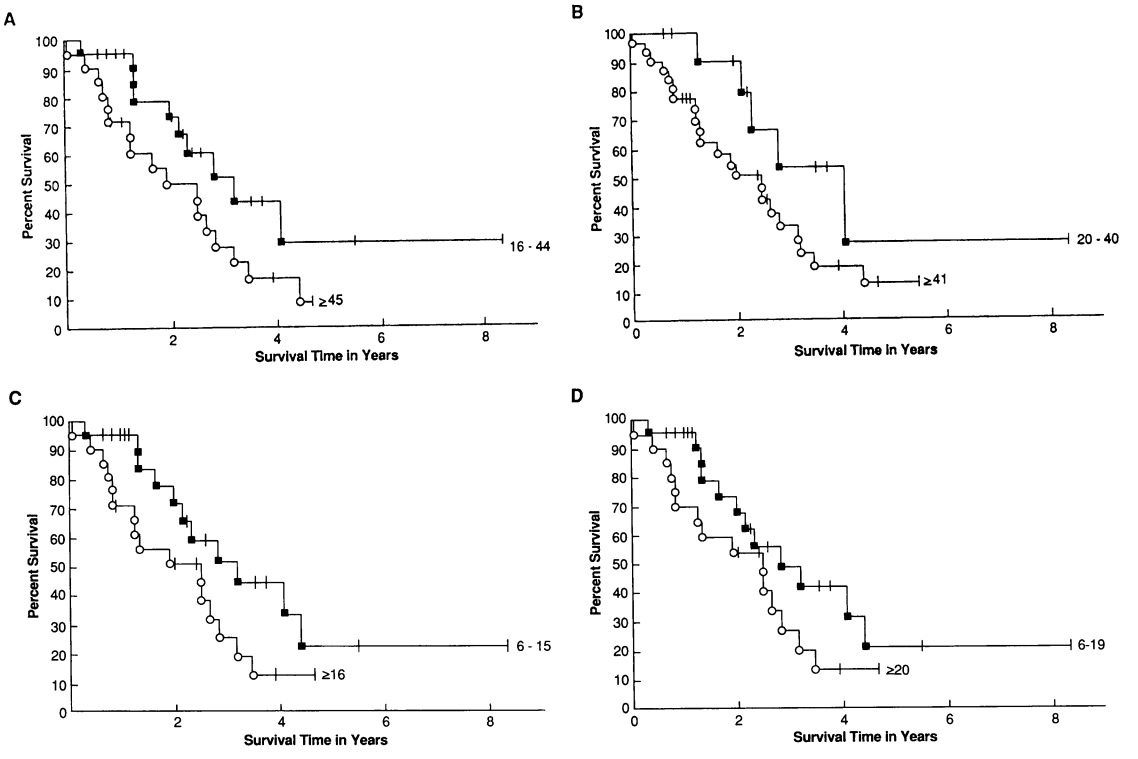

Figure 3. *Angiogenesis in ovarian cancer OS Kaplan-Meier plots for all 43 patients os stratified by microvessel count.* **A:** *Average of vascular counts in the three most densely vascular fields in each case at 200× magnification (200× avg in text); 10 of 22 (45%) patients with average counts of 16 to 44 (■) failed, whereas 17 of 21 (81%) patients with average counts greater than or equal to 45 (○) failed* ($P_2 = 0.059$). **B:** *Vascular count in the most densely vascular field in each case at 200× magnification (200× high in text); 5 of 12 (42%) patients with high counts of 20 to 40 (■) failed, whereas 22 of 31 (71%) patients with high counts greater than or equal to 41 (○) failed* ($P_2 = 0.084$). **C:** *Average of vascular counts in the three most densely vascular fields in each case at 400× magnification (400× avg in text); 11 of 22 (50%) patients with average counts of 6 to 15 (■) failed, whereas 16 of 21 (76%) patients with average counts greater than or equal to 16 (○) failed* ($P_2 = 0.064$). **D:** *Vascular count in the most densely vascular field in each case at 400× magnification (400× high in text); 12 of 23 (52%) patients with high counts of 6 to 19 (■) failed, whereas 15 of 20 (75%) patients with high counts greater than or equal to 20 (○) failed* ($P_2 = 0.12$).

Table 6. *Cox Proportional Hazards Model for DFS*

Variable	Parameter estimate	*P* value	Relative risk	95% CI for relative risk
400× average 16^+ (versus 6 to 15)	0.76	0.030	2.14	(1.08, 4.25)

marker for invasive cancers like ovarian cancer. Endothelial cells, like tumor cells, secrete collagenases and other degradative enzymes that also facilitate vascular sprout formation and tumor cell escape into the circulation.[4,8] In the process of metastasis, the number of tumor cells released is related to the density of blood vessels in the tumor.[6] Angiogenic activity may also be necessary for metastatic implants to grow.[1,7] A primary tumor with a high proportion of angiogenic cells is likely to give rise to metastatic implants that are already angiogenic, enabling them to grow in the target organ.[8]

This experimental evidence as to the importance of angiogenesis in tumor development has been applied to human tumors in recent years. The first quantitative studies showed that angiogenesis could predict metastasis in cutaneous malignant melanoma.[9–11] Subsequently, Weidner et al[12] showed that assessment of angiogenesis was an independent predictor of metastatic disease in patients with breast carcinoma. An expansion of that study with a different group of patients showed that microvessel density was the only statistically significant predictor of OS among node-negative women with breast cancer.[8] They proposed that microvessel density could be useful in selecting those node-negative patients who were at risk for metastatic disease, giving them the opportunity to receive adjuvant chemotherapy. Bosari et al[13] confirmed the prognostic independence of microvessel density and vascular invasion in patients with node-negative invasive

breast cancer. Several subsequent studies have also confirmed these findings,[14–16,32] although one study found no prognostic significance of angiogenesis in breast carcinoma.[33] Gasparini et al[34] investigated the prognostic value of several different markers in early stage breast carcinoma, including p53 expression, c-*erb*B-2, cathepsin D, DNA ploidy, epidermal growth factor receptor, and tumor angiogenesis. They concluded that tumor angiogenesis was the most important factor, in both univariate and multivariate analysis, for relapse-free survival and for overall survival.[34] Microvascular density in prostate cancer has been shown to be an independent predictor of pathological stage.[35] Angiogenesis has recently been shown to have predictive value for metastasis in prostate cancer of both low and high Gleason's score,[18,36] in non-small cell lung cancer,[17,37] and in testicular germ cell tumors.[19]

Tumor angiogenesis is of prognostic significance in several human cancers including breast, prostate, and non-small cell lung carcinomas. In this study of 43 ovarian cancer patients with advanced stage disease, a low average microvessel count at 400× (<16) with CD34 as a marker was found to be a statistically significant predictor of improved DFS, suggesting that the analysis of neovascularization may be a useful prognostic factor. Our results suggest that additional study of angiogenesis in ovarian cancer is warranted.

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