

The Prognostic Significance of Angiogenesis in Epithelial Ovarian Carcinoma¹

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ABSTRACT

The molecular biology underlying the metastatic process in ovarian carcinoma remains poorly understood. For other neoplasms, the induction of angiogenesis by malignant cells has been shown to play a pivotal role in the process of tumor proliferation and metastasis. The purpose of this study was to characterize the degree of angiogenesis in epithelial ovarian malignancies and to determine whether the degree of neovascularization has prognostic significance for survival.

Tissue sections obtained from 88 ovarian cancer patients were examined immunohistochemically for angiogenesis after staining with anti-human endothelial cell antibodies to von Willebrand factor and CD31. Light microscopy was performed, and individual microvessel counts were quantified at high power ($\times 400$). A chart review was completed, collating data regarding age, stage, grade, status of disease, and survival. Statistical exploratory methods were used to find potentially useful prognostic cutpoints for marker values of angiogenesis.

Of the total 88 patients, tissue microvessel counts from 85 were evaluated via antibodies to von Willebrand factor and 87 for CD31. Overall, median survival was 2.7 years in women with cancers containing high microvessel counts versus 7.9 years in those with low microvessel counts ($P = 0.03$). A low microvessel count was associated with better 5-year survival in both early stage (I and II) and advanced stage (III and IV) disease.

Our data suggest that the degree of neovascularization may have prognostic significance in epithelial ovarian carcinoma, especially for women with early-stage disease. In this group of women, the degree of angiogenesis may allow

the selection of women at high risk for recurrence who may benefit from aggressive adjuvant therapy.

INTRODUCTION

Ovarian carcinoma is the fourth most frequent cause of cancer death in women in the United States and causes more deaths than all other gynecological malignancies combined. Approximately 1 woman in 70 will develop ovarian cancer in her lifetime, and 1 woman in 100 will die of this disease (1). The reason for the high mortality rate is well known: the majority of ovarian cancer patients present with metastatic disease.

The growth of malignant tumors and metastatic capacity have both been shown to be highly dependent upon angiogenesis, or new blood vessel formation. In 1971, Folkman proposed that neovascularization of a tumor was required to provide essential nutrients beyond the limit of simple diffusion and to allow for growth >2 mm (2). Both tumors and host tissues produce a variety of angiogenic factors that promote the migration of endothelial cells, usually from a post capillary venule, toward the tumor, leading to the development of a capillary bed. These new vessels often have defective basement membranes and are thin walled and leaky, providing a venue through which a large number of cancer cells can enter the circulatory system and metastasize. The angiogenic process therefore not only enhances tumor growth locally but facilitates the dissemination of tumor cells to other sites (2, 3).

The induction of neovascularization by malignant cells has been shown to play a pivotal role in the process of tumor proliferation and metastasis for other types of neoplasms (3). Prior studies have demonstrated a direct correlation between the degree of angiogenesis in tumors and a more aggressive tumor biology, as manifested by adverse clinical outcomes (4). Weidner *et al.* investigated patients with primary breast cancer and found a correlation between higher microvessel counts and both an increased risk of metastasis and higher tumor grade. A greater degree of angiogenesis was predictive for death and relapse of disease, regardless of nodal status. In a multivariate analysis, including lymph node status, tumor size, and grade, the microvessel count remained an independent prognostic factor for metastasis and survival (5-7). Weidner postulated that the degree of neovascularization may identify high-risk, node-negative breast cancer patients who may benefit from adjuvant therapy (6, 7). Further investigations have demonstrated that tumor angiogenesis may have an important prognostic role in malignant melanoma, prostate cancer, bladder carcinomas, and lung cancers (8).

The role of angiogenesis in ovarian carcinoma was uncertain until Hollingsworth *et al.* (9) conducted a retrospective study on 43 advanced-stage epithelial ovarian cancer patients. Their results suggested that the degree of neovascularization was associated with overall and disease-free survival and may be a useful prognostic factor. Using a Cox proportional hazards

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model, Hollingsworth concluded that stage was the best predictor of overall survival. Tumor angiogenesis, however, was found to be the best predictor of disease-free survival. This study did not include women with early-stage disease and contained a small number of study subjects.

An investigation by Abulafia *et al.* (10) evaluated angiogenesis in 42 consecutive patients with primary epithelial ovarian cancer. In 19 patients with advanced disease, the degree of neovascularization was assessed in both the primary tumor as well as metastatic omental implants. Overall, the microvessel counts of the primary tumor were not significantly related to patient age, preoperative CA 125 level, tumor stage, tumor grade, or patient survival. In contrast, in women with advanced stage disease, the microvessel counts of the omental metastases were significantly correlated with preoperative CA 125 level and were significantly predictive of survival. This study also contained a small number of subjects, and only 12 patients had early-stage disease.

The purpose of our study was to further characterize the degree of angiogenesis in epithelial ovarian malignancies in all stages of disease, to gain insight into the relevance of neovascularization in both early-stage as well as advanced stage ovarian cancer, and to determine whether the degree of angiogenesis in ovarian carcinoma has prognostic significance for survival.

MATERIALS AND METHODS

Five- μ m frozen sections obtained from tumors from 88 ovarian cancer patients chosen randomly were examined immunohistochemically for angiogenesis after staining with monoclonal mouse anti-human endothelial cell antibodies to vWF³ and CD31 (Dako Corp., Carpinteria, CA). The anti-human vWF antibody reacts with vWF, previously designated as Factor VIII-related antigen, the most specific endothelial marker. The anti-human endothelial cell antibody CD31 reacts with a M_r 100,000 endothelial cell glycoprotein. CD31 antibody is more sensitive for identification of vessels than anti-human vWF antibody, but it is not as specific and can bind to granulocytes, monocytes, megakaryocytes, platelets, and occasionally plasma cells, in addition to endothelial cells (11). Immunohistochemistry was performed using the Elite Vectastain ABC kit, as described previously (12).

Histological examination of the slides was then performed by two investigators, one of which was blinded to both the stage and grade of disease. A slide stained with nonspecific mouse IgG was used as a negative control, and slides stained with anti-cytokeratin antibody AE1, as well as H&E, were used to confirm malignant histology. Sections stained with anti-endothelial cell antibodies were then examined at low power ($\times 100$) to identify the site of maximal neovascularization. Individual microvessel counts were then quantitated at high power ($\times 400$).

A chart review was completed, collating data regarding age, stage, histological grade, status of disease, and survival. Exploratory statistical methods, based on maximizing the estimated hazard ratio for two groups, were used to find

cutpoints for the markers of angiogenesis (13). This technique necessarily gives the lowest possible “*P* value(s)” attainable; hence, statistical significance should not be interpreted in the usual way. In addition, a chosen cutpoint is generally not unique because a range of values may provide similar results. The usefulness of these markers for providing clinical prognoses depends on confirmation that the cutpoints found truly dichotomize patients into groups that differ with respect to outcome. Kaplan-Meier plots of survival were drawn, based on these cutpoints (14). The significance of the markers after adjusting for known prognostic variables was analyzed with the Cox regression model (15). The associations between angiogenesis and clinicopathological variables were analyzed using Exact tests for contingency tables.

RESULTS

Only patients with primary epithelial ovarian cancer were included in the study. Patients with borderline epithelial ovarian carcinoma and primary peritoneal carcinoma were excluded. The histological subtypes were serous, endometrioid, mucinous, clear cell, and undifferentiated carcinoma in 56, 11, 9, 2, and 1 patient, respectively. Six patients had tumors with mixed subtypes. Of the total 88 patients, 18 (20%) were stage I, 19 (22%) were stage II, 27 (31%) were stage III, and 24 (27%) were stage IV. Data on grade were available for 80 patients, with 7 (9%) grade I, 32 (40%) grade II, and 41 (51%) grade III tumors. The median age was 58 years (range, 26–91). The median survival for the overall series was 3.9 years with a median follow-up of 7.5 years. At the time of the analysis, 49 patients were dead of disease, 3 had died of other causes, and 36 were alive, 6 with known disease. In this study, patients with early-stage disease had a median survival of 7.3 years, whereas those with a stage of III or IV had a median survival of 2.2 years ($P < .001$).

All patients with stage II to IV disease and 16 patients with stage I disease received chemotherapy. Chemotherapy consisted of cyclophosphamide and a platinum compound in 60 (71%) patients. Additional regimens included: cisplatin, Adriamycin, and cyclophosphamide; paclitaxel and a platinum compound; i.p. cisplatin and etoposide; cisplatin; carboplatin; and melphalan and ³²P in 5, 1, 1, 5, 2, 2, and 9 patients, respectively. Forty-one patients received secondary chemotherapy, of which 24 were treated with a platinum combination.

Samples from 85 patients were evaluable for vWF, and 87 samples were evaluable for CD31. The median microvessel count for vWF was 15 microvessels/high power field (range, 3–56), and for CD31, it was 16 microvessels/high power field (2–72). To explore the relationship between tumor microvessel counts and survival, optimal cutpoints were found using the method described above to dichotomize the patients into groups that differed with respect to survival. The cutpoint most suitable for vWF was found to be 10 microvessels/high power field, whereas for CD31, it was found to be 13 microvessels/high power field. Because vessel counts measured for vWF and CD31 were highly correlated (Pearson correlation, 0.75) and comparable results for outcome were seen for the two factors, the remainder of the discussion will focus on vWF. Table 1 shows the association

³ The abbreviation used is: vWF, von Willebrand factor.

Table 1 Clinicopathological variables stratified by microvessel counts

This table demonstrates the association between the microvessel counts and the clinicopathological variables: age, stage, grade, and survival. There were no significant associations between microvessel counts and stage, age, or grade based on Fisher's Exact test. The median survival for patients with a microvessel count above 10 was 2.7 years, whereas for those with a count ≤ 10 , it was 7.9 years.

| | ≤ 10 | ≥ 10 | Total |
|----------------------|-----------|-----------|-------|
| <i>n</i> | 24 | 61 | 85 |
| Age | | | |
| <60 | 13 (54%) | 34 (56%) | 47 |
| ≥ 60 | 11 (46%) | 72 (44%) | 38 |
| Stage | | | |
| I | 8 (33%) | 10 (16%) | 18 |
| II | 4 (17%) | 15 (25%) | 19 |
| III | 9 (38%) | 15 (25%) | 24 |
| IV | 3 (13%) | 21 (34%) | 24 |
| Grade | | | |
| 1 | 4 (18%) | 3 (5%) | 7 |
| 2 | 8 (36%) | 22 (40%) | 30 |
| 3 | 10 (45%) | 30 (55%) | 40 |
| Median survival (yr) | 7.9 | 2.7 | 4.1 |

between the microvessel counts and age, stage, and grade. There were no significant associations between vWF microvessel counts and age ($P = 1.00$), stage ($P = 0.08$), and grade ($P = 0.31$) based on Fisher's Exact test.

Fig. 1 depicts the survival for patients dichotomized into prognostic groups based on the optimal cutpoints for microvessel density. For vWF, the estimated median survival for patients with a microvessel count above 10 was 2.7 years, whereas for those with a count of 10 and under, it was 7.9 years (exploratory $P = 0.03$). Table 2 shows the relationship between stage, age, grade, microvessel counts, and survival. Stage is the most significant prognostic factor in this group of patients ($P < .001$). After adjusting for stage in a Cox regression model, the microvessel counts retained marginal significance with overall survival ($P = 0.07$). Fig. 2 demonstrates the survival curves for early- and advanced-stage ovarian carcinoma stratified into low versus high microvessel count groups. For stages I and II, there is an apparent survival advantage for patients with lower microvessel counts, with an estimated 92% (95% confidence interval, 61%–99%) of the patients with counts of 10 or less alive at 5 years, compared with 61% (37%–81%) of the patients with counts > 10 ($P = 0.05$). In stages III and IV, the estimates were 42% (19%–68%) for lower counts and 28% (16%–44%) for higher counts.

DISCUSSION

Angiogenesis has been demonstrated by prior studies to have prognostic significance in a variety of solid neoplasms including breast, prostate, bladder, lung, and malignant melanoma (4–7). The investigation conducted by Hollingsworth on advanced ovarian carcinoma suggested that neovascularization may also be a useful prognostic indicator for overall survival and disease-free survival in ovarian cancer. Their data were stratified to divide the patients into two groups: those with tumors with vessel counts ≥ 16 and those with a count < 16 . An average vessel count < 16 ($\times 400$) was associated with a better

Table 2 Cox regression analysis

This table demonstrates the relationship between stage, age, grade, microvessel counts, and survival in a Cox regression model. After adjusting for stage in this model, the microvessel counts retained marginal significance with overall survival ($P = 0.07$).

| Univariate Cox regression analysis | | | |
|------------------------------------|-----------------------|----------------|-------------------|
| | Category | Score χ^2 | <i>P</i> |
| Stage | I + II, III + IV | 13.1 | < 0.001 |
| Age | < 60 , ≥ 60 | 3.6 | 0.06 |
| Grade | 1 + 2, 3 | 0.05 | 0.83 |
| vWF | ≤ 10 , ≥ 10 | 4.8 | 0.03 |
| Bivariate Cox regression analysis | | | |
| | Category | Score χ^2 | Adjusted <i>P</i> |
| Age | < 60 , ≥ 60 | 4.7 | 0.03 |
| Grade | 1 + 2, 3 | 0.4 | 0.53 |
| vWF | ≤ 10 , ≥ 10 | 3.2 | 0.07 |

* Each variable adjusted for stage.

survival and was shown to be the best predictor of disease-free survival. That study, however, included only 43 cases, and all patients had advanced disease (III and IV; Ref. 8). Our study sought to further investigate the prognostic significance of angiogenesis in epithelial ovarian carcinoma throughout all stages of disease.

Our results are in agreement with those of Dr. Hollingsworth in that we have found that the degree of neovascularization does appear to have prognostic significance in epithelial ovarian carcinoma. A higher microvessel count was associated with a poorer prognosis independent of stage of disease and grade. Our findings confirm Dr. Abulafia's results that microvessel counts were not significantly related to age, tumor stage, or tumor grade. In contrast to Abulafia, who suggests that the degree of neovascularization of primary ovarian neoplasms does not have prognostic significance, we found that the degree of angiogenesis was predictive of survival in women with early-stage disease (confined to the ovaries or pelvis). Our study included 37 patients who had stage I and II disease. Abulafia's investigation had only 12 patients with early-stage disease, which may be too small of a sample to demonstrate an association between microvessel counts and survival. Regardless, stage was the most important prognostic factor for survival in patients with advanced disease. After adjusting for stage in this group of patients, the relationship between the degree of angiogenesis and survival did not retain a significant difference but did reveal a trend toward significance. Abulafia found that the microvessel counts of omental metastases were significantly predictive of patient survival ($P = 0.013$), which may be used to identify patients with more aggressive metastatic disease (10).

In our study, which included patients with stages I through IV, a high microvessel count was associated with a poor prognosis after accounting for stage. This suggests an important role for angiogenesis in the clinical behavior of ovarian tumors. The marked difference in survival between patients with low and high tumor microvessel counts in our study suggests that the degree of angiogenesis may be a marker for the biological aggressiveness of ovarian cancers.

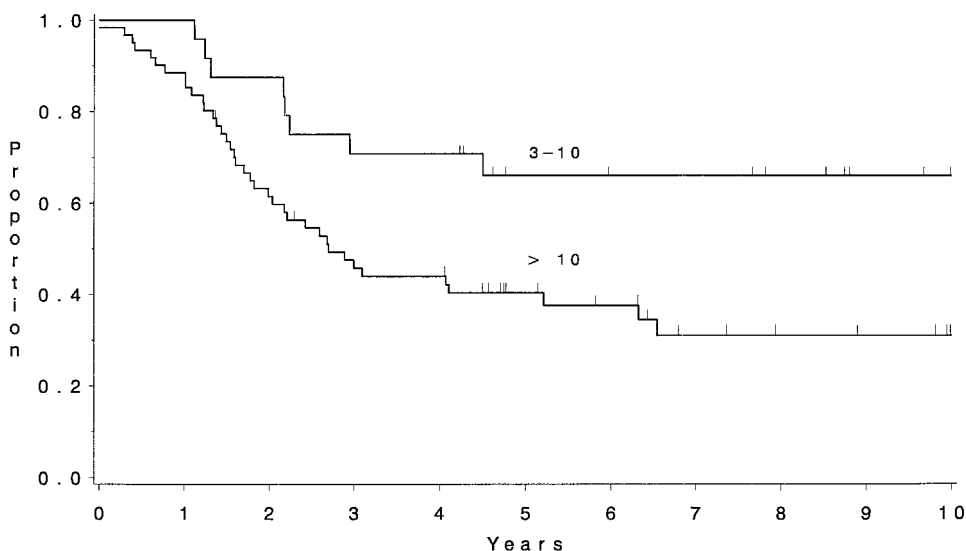
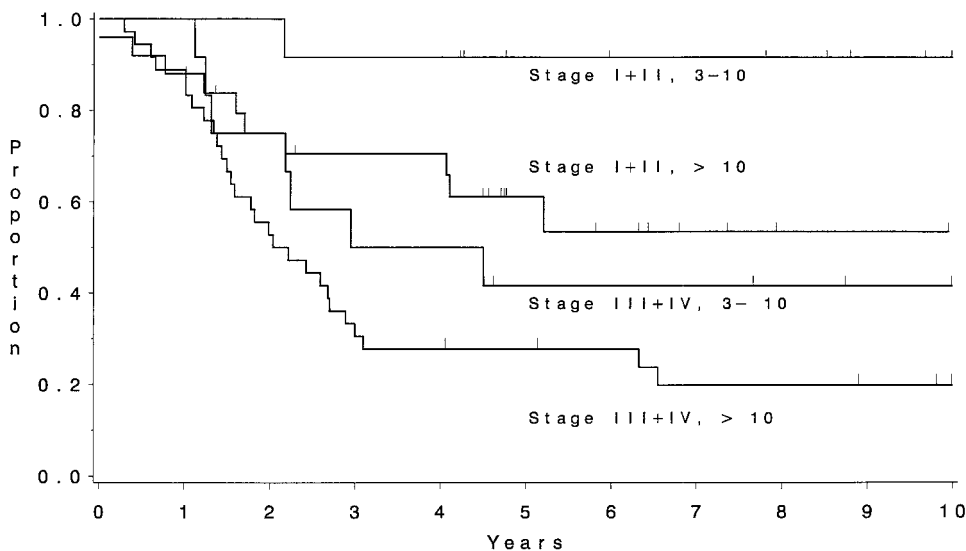


Fig. 1 Survival by microvessel count. Patients with microvessel counts >10 (n = 61) have a shorter survival than patients with lower counts (n = 24). The median survival times were 2.7 and 7.9 years, respectively (exploratory P = 0.03).

Fig. 2 Survival by stage and microvessel count. Stage I + II patients with microvessel counts >10 (n = 25) have shorter survival than stage I + II patients with lower counts (n = 11; unadjusted P = 0.05). There is no significant difference between the groups for stage III + IV patients (P = 0.19).



Ovarian malignancies are presumed to metastasize through the process of exfoliation of tumor cells from the primary tumor, followed by dissemination of cells throughout the peritoneal cavity, implantation, and subsequent growth. Most patients who succumb to this disease ultimately die from starvation and inanition due to bowel obstruction caused by i.p. metastases. In this regard, the degree of angiogenesis of ovarian cancers may directly influence the clinical course of the disease. Highly angiogenic tumors may: (a) facilitate a rapidly increasing volume of tumor cells, thereby facilitating the dissemination of the cells within the abdominal cavity and accelerating the clinical development of bowel obstruction; (b) provide access to the circulatory system; and (c) promote the development of lymphatic channels. Conversely, a lack of angiogenic capability in ovarian tumors may serve to prevent the rapid development of a large i.p. tumor burden, which is a prerequisite for bowel ob-

struction, thereby resulting in improved median survival for women with ovarian carcinoma.

In conclusion, our study suggests that the degree of neovascularization has prognostic significance for survival in epithelial ovarian carcinoma. A greater degree of neovascularization was associated with a poorer overall prognosis after adjusting for stage in a multivariate analysis. It may be possible to use the degree of angiogenesis to identify a subset of patients with early-stage disease who are at high risk for recurrence and who may benefit from aggressive adjuvant chemotherapy. The role of angiogenesis in epithelial ovarian carcinoma deserves further consideration, not only to confirm the association with overall survival suggested in this investigation but also for elucidation of the role of neovascularization in the metastatic process of this malignancy. Finally, given the striking difference in survival associated with low

versus high angiogenic counts in our study, it is interesting to speculate that antiangiogenic agents may have utility in the treatment of ovarian cancer.

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