# Angiogenesis is not a Prognostic Marker in Endometrial Carcinoma

***Taner Turan, *Fırat Ortaç, *Mete Güngör, **Aylin Heper, **Arzu Ensari**

Ankara University, Faculty of Medicine,
*Department of Obstetrics and Gynecology, Gynecologic Oncology Unit
**Department of Pathology, Ankara, Turkey

**Corresponding author:** Mete Güngör, Konutkent 2, A 8 Blok, No. 46, 06530, Çayyolu, Ankara, Turkey; e-mail: mtgungor@superonline.com

## Özet

**Amaç:** Bir tümör anjiogenez ölçümü olan mikrodamar dansitesinin, tümör büyüme ve metastazında prognostik bir belirteç olduğuna inanılmaktadır. Bu çalışmanın amacı, endometrial kanserlerde mikrodamar dansitesinin bir prognostik faktör olup olmadığının değerlendirilmesidir.

**Materyal ve Metod:** 1988 den 1996 yılına kadar, başlangıçta histerektomi ve pelvik/paraaortik lenfadenektomi ile tedavi edilen evre I,II ve III 57 endometrial kanser vakası histolojik olarak tekrar gözden geçirildi. Bütün histerektomi spesimenleri immunohistokimyasal olarak CD 34 antijeni için boyandı. Mikrodamar dansitesi, 200X büyütmede, en aktif neovaskülarizasyon alanlarında sayıldı. Sonuçlar, herhangi bir 200X büyütmede tanımlanan en yüksek mikrodamar sayısı olarak sunuldu. İstatistiksel analizlerde, Mann-Whitney U testi, Kruskal Wallis testi kullanıldı. Surviv Kaplan-Meier metodu kullanılarak ve surviv farkları log-rank testi kullanılarak değerlendirildi. Mikrodamar dansitesi ve diğer prognostik parametreler ile hastalıksız surviv ve ortalama surviv arasındaki korelasyon multivaryant analiz yapılarak değerlendirildi.

**Sonuç:** Mikrodamar dansitesi ile diğer prognostik parametreler (histolojik ve nükleer grade, myometrial invazyon, lenf nodu tutulumu ve lenfovaskular alan invazyonu) arasında bir korelasyon yoktu. Ek olarak, mikrodamar dansitesi ile hastalıksız surviv ve ortalama surviv arasında da bir korelasyon yoktu. Ancak, lenf nodu metastazi, myometrial invazyon ve lenfovasküler alan invazyonu ile hastalıksız surviv arasında ve lenf nodu metastazi ile ortalama surviv arasında anlamlı korelasyon vardı.

**Yorum:** Bu çalışmada, mikrodamar dansitesi, endometrial kanserli hastalarda ,hastalıksız ve ortalama surviv için prognostik bir parametre olarak bulunmamıştır.

**Anahtar Kelimeler:** anjiogenez, endometrial kanser

## Summary

**Objective:** Microvessel density ( MVD ), a measure of tumor angiogenesis, is believed to be a prognostic indicator associated with tumor growth and metastasis . The purpose of this study was to evaluate MVD as a prognostic factor in endometrial cancer.

**Material and Methods:** From 1988 through 1996, 57 cases of FIGO stage I, II, and III endometrial carcinomas treated initially by hysterectomy with pelvic and paraaortic lymphadenectomy were reviewed histologically. All hysterectomy specimens were stained immunohistologically for CD-34 antigen. MVD was counted in a X200 field in the most active area of neovascularization. Results were expressed as the highest number of microvessels identified within any single X200 field. Statistical analyses included the Mann-Whitney-U test, Kruskal Wallis test of variance. Survival was calculated using the Kaplan-Meier method and differences in survival were analyzed using log-rank test. MVD and several other prognostic parameters were examined for their correlation with progression free survival and overall survival by a multivariate analysis according to the Cox proportional hazards model.

**Results:** There was no correlation between MVD and the other prognostic parameters; histologic and nuclear grade, myometrial invasion, lymph node status and lymphovascular space invasion. In addition, there was no correlation between MVD and progression free survival (PFS) and overall survival(OS). However, there was significant correlation between lymph node metastasis, myometrial invasion and lymphovascular space invasion with PFS and lymph node metastasis with OS.

**Conclusion:** In this study, MVD was not found to be a prognostic parameter for progression free and overall survival in patients with endometrial carcinomas.

**Keywords:** angiogenesis, endometrial carcinoma

## Introduction

Angiogenesis is the growth of new blood vessels toward and within a tumor, and it has been shown that tumors do not grow beyond a size of 2 to 3 mm3 unless they are able to recruit the growth of new capillaries from the existing vascular network (1). It is unclear whether angiogenesis regulates tumor behaviour or is only an indicator for the growth and metastatic potential of a tumor (2, 3). There is accumulating evidence that indicates a correlation between the degree of tumor angiogenesis and prognosis (1). Clinical studies have shown that the intensity of angiogenesis , expressed as microvessel density , predicts the probability of metastasis and survival in breast (2), prostate (4), lung (5), and recently cervical (6) and ovarian carcinomas (7).

The purpose of this study was to evaluate angiogenesis as a prognostic factor in endometrial carcinoma.

## Material and Methods

One hundred and nineteen patients with clinical stage I , II and III (according to the FIGO) endometrial carcinoma were

initially treated by surgery with/without radiotherapy at the Department of Obstetrics and Gynecology of Ankara University Hospital from 1988 through 1996. Initial surgery comprised of abdominal hysterectomy and bilateral salpingo-oophorectomy with pelvic and paraaortic lymphadenectomy. Of 119 cases, 62 were excluded for various reasons. Among the 62 excluded cases, there were 33 cases in which pathologic material was lost or the pertinent paraffin block was not available for immunohistochemical staining after histologic review and 18 cases in which follow -up was lost and 11 in which the hysterectomy specimen showed no residual tumor. Therefore, the remaining 57 cases were selected for this study. Both pelvic and paraaortic lymphadenectomy were performed in all 57 cases. Patients with myometrial invasion of > 50%, or retroperitoneal lymph node metastasis were treated with adjuvant external beam radiation therapy to the whole pelvis.

Histologically, the slides of the hysterectomy specimens were evaluated for the pathologic stage and grade of the carcinoma, the depth of myometrial invasion, and the presence or absence of pelvic and/or paraaortic lymph node metastasis, lymphovascular space invasion and histologic subtype.

Immunohistochemical staining for endothelial cells was performed on paraffin embedded tissue sections with the monoclonal anti CD-34 antibody, using the streptavidin-biotin peroxidase technique (Immunon Maxitags Kit). Microvessel density was determined by first scanning sections at low power then locating the areas of highest concentration of staining ('hot spots'). After the area of the highest neovascularization was identified, individual microvessel counts were made on a X 200 field without knowledge of the patient's outcome. Results were expressed as the highest number of microvessels identified within any single X 200 field.

Survival rates were estimated using the Kaplan-Meier method, and log rank tests were performed to test equality of the survivor functions in univariate analyses. The Cox proportional hazards model was used to evaluate the effect of angiogenesis on progression free survival (PFS) and overall survival (OS), adjusting for the effects of other variables, and to multivariately estimate survivor functions. In angiogenesis, 60, 70, 80 cells were used as a cut-off for high versus low microvessel count. The Kruskal-Wallis test of variance and Mann-Whitney-U tests were used to express the association among the intratumoral neovascularization and the other prognostic parameters. $P < 0.05$ was regarded as statistically significant.

## Results

The mean age of the 57 patients was 58.7 (range 32-76). The mean follow-up was 26.5 months. Microvessel counts ranged from 23 to 200, with a mean of 79.1.

There was no correlation between microvessel count and histologic grade, nuclear grade, myometrial invasion, lympho-vascular space invasion, lymph node status. **Table 1** summarizes the patient characteristics associated with MVD.
There was no correlation between MVD and overall survival (OS) ($p > 0.05$). The overall survival of patients with high MVD (≥ 60) and that of patients with low MVD (< 60) was 65.5 months and 70.9 months, respectively ($p > 0.05$). In addition , there was no correlation between lympho-vascular space invasion, myometrial invasion, histologic grade, nuclear grade and overall survival in univariate analysis. There was significant correlation between lymph node status and overall survival ($p < 0.05$) (*Tab. 2*).

**Table 1:** Association between Microvessel Density and Clinicopathologic Variables

| Variables | No( 57 ) | Mean MVD | p value |
|---|---|---|---|
| **Stage** | | | |
| I | 27 | 83.7 | p>0.05 |
| II | 9 | 89.9 | |
| III | 21 | 68.5 | |
| **Tumor Grade** | | | |
| 1 | 29 | 85.3 | p>0.05 |
| 2 | 14 | 76.8 | |
| 3 | 14 | 68.6 | |
| **Nuclear Grade** | | | |
| 1 | 9 | 90.9 | p>0.05 |
| 2 | 26 | 85.7 | |
| 3 | 22 | 66.5 | |
| **Myometrial Invasion** | | | |
| No | 22 | 80.2 | p>0.05 |
| <1/2 | 15 | 79.7 | |
| ≥1/2 | 20 | 77.5 | |
| **LVSI †** | | | |
| ( - ) | 29 | 78.1 | p>0.05 |
| ( + ) | 28 | 80.0 | |
| **Lymph node status** | | | |
| ( - ) | 38 | 82.9 | p>0.05 |
| ( + ) | 19 | 71.4 | |

* Kruskal-Wallis test of variance
** Mann-Whitney U test of variance
† Lympho-vascular space invasion

There was no correlation between MVD and progression free survival (PFS). The progression free survival of patients with high MVD (≥ 60) and that of patients with low MVD (< 60) was 64.2 months and 62.2 months, respectively ($p > 0.05$). In addition, there was no correlation between histologic grade, nuclear grade and PFS in univariate analysis. However, myometrial invasion ($p < 0.05$), lympho-vascular space invasion ($p < 0.05$) and lymph-node metastasis ($p < 0.01$) were significantly related to PFS.

Multivariate analysis revealed a prognostic significance in lymph-node metastasis. In the current study, in endometrial carcinomas, microvessel density (MVD) is not an independent prognostic factor for progression free survival (PFS) and overall survival (OS).

## Discussion

New capillary blood vessels are needed if a tumor is to expand in three dimensions beyond 2 mm or so (8). Recent evidence suggests that the induction of angiogenesis by preneoplastic tissues is an important step in the conversion of the tissue to a cancer (8). Recent evidence also supports the

Table 2: Overall Survival( OS ) and Progression Free Survival( PFS ) Analyses for Prognostic Factors using Log Rank Test

| Variables | No | OS ( months) | p value | PFS ( months ) | p value |
|---|---|---|---|---|---|
| **MVD** | | | | | |
| < 60 | 18 | 70.9 | p>0.05 | 62.2 | p>0.05 |
| ≥ 60 | 39 | 65.5 | | 64.2 | |
| **Myometrial invasion** | | | | | |
| No | 22 | 71.7 | p>0.05 | 58.2 | p<0.05* |
| <1/2 | 15 | 59.4 | | 56.5 | |
| ≥1/2 | 20 | 65.4 | | 53.2 | |
| **LVSI †** | | | | | |
| ( - ) | 29 | 70.5 | p>0.05 | 70.0 | p<0.05* |
| ( + ) | 28 | 63.5 | | 53.9 | |
| **Lymph node status** | | | | | |
| ( - ) | 38 | 73.0 | p<0.05 * | 70.7 | p<0.01* |
| ( + ) | 19 | 46.9 | | 32.4 | |
| **Histologic grade** | | | | | |
| 1 | 29 | 66.7 | p>0.05 | 61.3 | p>0.05 |
| 2 | 14 | 69.4 | | 69.2 | |
| 3 | 14 | 56.7 | | 52.5 | |
| **Nuclear Grade** | | | | | |
| 1 | 9 | 64.3 | p>0.05 | 47.9 | p>0.05 |
| 2 | 26 | 63.6 | | 63.4 | |
| 3 | 22 | 64.4 | | 61.6 | |

* statistically significant, † Lympho-vascular space invasion

notion that metastasis of some tumor cells is correlated with the extent of the vascularity feeding the primary tumor (2). Given the obvious clinical implications, there has been intense effort into finding therapeutic agents capable of inhibiting tumor angiogenesis with the hope of limiting tumor growth and metastasis (9). A variety of animal studies have demonstrated that angiogenesis inhibitors such as TNP-470 and angiostatin mediate the inhibitory effect on tumor growth and metastasis (10,11). The inhibitory effect of Medroxyprogesterone acetate on angiogenesis induced by human endometrial carcinoma was also reported (12). Such agents may prove to be valuable antitumor chemotherapeutic drugs. Studies of many tumors (eg. carcinomas of the breast (2), prostate (4), lung (5), cervix (6) and ovary (7)) have demonstrated that increasing intratumoral microvessel density is associated with the development of metastases and other features of tumor aggresiveness. Kaku et al evaluated angiogenesis in endometrial carcinoma and reported that microvessel counts of endometrial carcinoma specimens were higher than those of controls, and in addition they suggested that MVD is an independent prognostic factor for PFS and OS in endometrial carcinoma (13). Abulafia et al demonstrated that angiogenesis in endometrial carcinoma correlates with tumor grade and depth of invasion (14).

In this retrospective study, tumor angiogenesis, as assessed with quantitative pathology using microvessel counting was found not to be an independent and important prognostic indicator of endometrial carcinoma.

Although angiogenesis may play a role in the initial growth and invasion of endometrial cancer, it is not yet predictive of metastatic spread of endometrial cancer. We believe that evaluation of the significance of angiogenesis in endometrial cancer needs further prospective investigations.

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