Microvessel Density as a Marker of Tumor Angiogenesis in Nonsmall Cell Lung Cancer

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Abstract

Objective: To investigate the relation between microvessel density in surgical specimen and, histopathology and differentiation of tumor, nodal metastasis and stage of disease in patients with non-small cell lung carcinoma.

Design: Retrospective analysis of clinical and pathologic data abstracted from hospital charts.

Setting: Government referral-based research hospital.
Patients: Twenty two epidermoid and 16 adenocarcinoma cases who underwent lung surgery.

Measurements: The microvessels in tumor specimens were demonstrated by staining endothelial cells immunohistochemically for factor VIII using streptoavidin-biotin, alkaline phosphatase complex technique.

Results: Microvessel counts were significantly higher in adenocarcinoma than in epidermoid carcinoma (p=0.0006). In poor differentiated epidermoid carcinoma specimens, angiogenesis was more intense than well and intermediate differentiated specimens (p=0.0064). Although the intensity of angiogenesis was high in patients with mediastinal lymph node metastasis when compared with non-metastasizing cases, the difference was not significant.

Conclusion: The intensity of angiogenesis is correlated with the grade of differentiation, the lower the differentiation the higher the angiogenesis, and is high in adeno-type of tumor.

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Key words: *Microvessel density, Angiogenesis, Lung cancer, Differentiation*

Introduction

Survival in patients with non-small cell lung carcinoma (NSCLC) depends on the stage of disease; and operation remains the mainstay of treatment for resectable or marginally resectable NSCLC. Unfortunately less than 40% of patients fit operation at time of initial diagnosis, and their outcome is largely dependent on the primary tumor and pathologic lymph node status(1). In approximately half of the operated patients, relapses and metastases usually occur within 2 years after operation(2).

The intensity of angiogenesis has been reported to correlate with the probability of metastases and relapses in NSCLC as well in melanoma and breast carcinoma(3,4,5). High vascularity increases the vascular area, facilitating the escape of tumor cells into the circulation and amplifying

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the paracrine effects exerted by endothelial cells on tumor cells(6).

Different histologic types of NSCLC have different potentials for systemic metastasis. Because of hematologic spread, adenocarcinoma has higher potential than epidermoid carcinoma(7). Macchiarini and coworkers have reported that in patients with T1N0M0 NSCLC; metastases after radical resection were associated with neovascularization of tumor tissue(8). Angiogenesis plays an important role in the initiation of metastasis. In the process of metastasis; primary tumor must have abnormal vascularity and cancer cell must travel through the circulation, reach the target organ, and induce angiogenesis for further growth(9). Teicher and associates suggested in their animal study that antiangiogenic therapy along with chemotherapy and/or radiotherapy could have improved treatment outcome in patients(10).

In this study we aimed to emphasize the intensity of angiogenesis, interms of microvessel density, along with types and differentiation of tumor.

Subject and Methods

Thirty eight patients (4 female, 34 male) with NSCLC, operated on in Sureyyapasa Chest and Cardiovascular Diseases Hospital in 1996, were included in the study. Patients were divided into 2 groups according to diagnosis. Group I consisted of 22 patients with epidermoid carcinoma where as 16 patients with adenocarcinoma were included in Group II. Groups were comparable with each other from the point of view of stage of disease, nodal involvement and size of tumor (Table 1). Patients ranged in age from 40 to 70 years (mean 52 years). Types of surgical resections done in patients are stated in Table 2.

Table 1. Comparison of patient groups

	Epidermoid ca	Adenoca	p
Size of tumor (cm)	5.34 (2-15)	3.88 (2-9)	NS
Stage I	8	4	NS
Stage II	3	4	NS
Stage IIIA	10	7	NS
Stage IIIB	1	1	NS
LN (-)	13	5	NS
LN (+) (N 1-3)	9	11	NS

ca: carcinoma; LN: lymph node; NS: nonsignificant

Table 2. Surgical procedures in all patients

Right hemithorax	n	Left hemithorax	n
Lower lobectomy	2	Upper lobectomy	8
Upper lobectomy	7	Lower lobectomy	1
Lower bilobectomy	4	Pneumonectomy	10
Middle lobectomy	1		
Pneumonectomy	5		

n: number of operated patients

The microvessels in tumors were demonstrated by staining endothelial cells immunohistochemically for factor VIII using streptoavidin-biotin, alkaline phosphatase complex technique (Biogenex, San Ramon, USA). Three to four microne paraffin embedded sections were mounted on poly-l-lysine coated slides, dewaxed, rehydrated and predigested with protease for 10 minutes at 37∞C. Protein block was applied for 10 minutes at room temperature and tapped off. Then monoclonal antibody to factor VIII antigen was applied for 2 hours at room temperature and washed in phosphate buffered solution (PBS). Then biotinylated antimouse antibody was applied for 20 minutes and washed in PBS followed by application of alkaline phosphatase conjugated streptoavidin-biotin complex for 20 minutes. After washing in PBS, the color was developed by incubating the slides with fast red tablet dissolved in naphthol phosphate intris buffer for 20 minutes. The tumor specimen was then counterstained with Mayer's solution which expressed a blue background. The intensity of angiogenesis was assesed by counting the stained microvessels under light microscopy (visual count). The microvessels in non-tumor parts were used as positive internal controls for assesing the quantity of staining for factor VIII.

Microvessels in non-necrotic area were counted on three 200x fields(20x objective with 10x ocular) randomly. The stained endothelial cells appeared as red linear fragments with internal lumens. The microvessel count was expressed as the average of the values of three readings.

Statistical analysis was done with SPSS software. The Mann-Whitney U Wilcoxon test was used to compare the microvessel counts in patients of both groups, in patients with or without nodal metastasis, and in patients with different stages of cancer (stage I-II versus stage III). Chi-square test was used to compare the other characteristics of both groups. Comparison of differentiation and tumor size, and differentiation and

microvessel counts, in group I patients were performed by Anova one-way analysis of variance.

Results

Pathologic staging after the operation showed that 12 patients were in Stage I, 7 patients in Stage II, 17 patients in Stage IIIA and 2 patients in Stage IIIB. No evidence of nodal metastasis was found in 47% (18) of 38 patients. Metastases were found as N1 in 6 cases, N2 in 13 cases and N3 in 1case. Size of the tumors ranged in diameter from 2 to 15 cm.

The microvessel counts in adenocarcinoma were significantly higher than in epidermoid carcinoma (p=0.0006) (Fig. 1). In 20 cases with nodal metastasis, microvessel counts of tumors were higher, but not significant, than in cases without lymph node involvement (Fig. 2). Similarly angiogenesis was more

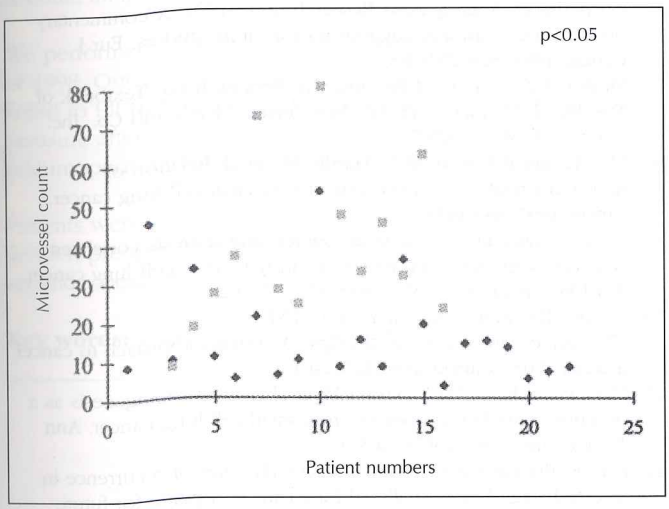

Fig. 1. Microvessel counts in adenocarcinoma and epidermoid carcinoma cases consicutively. Square, adenocarcinoma; triangle, epidermoid carcinoma.

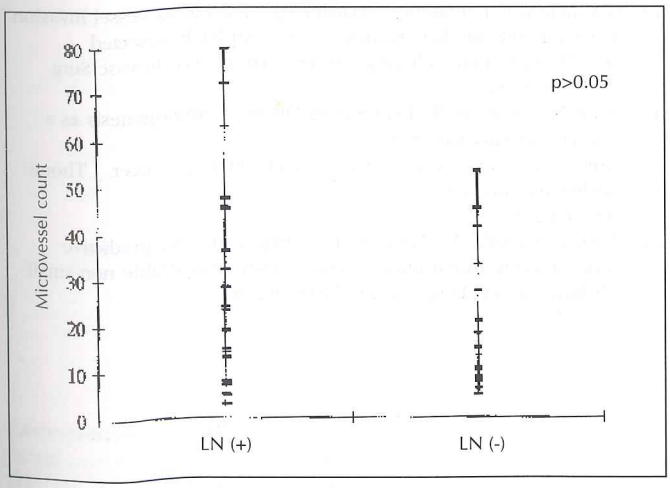

Fig. 2. Microvessel counts in consecutive patients with and without nodal involvement. LN, mymph node.

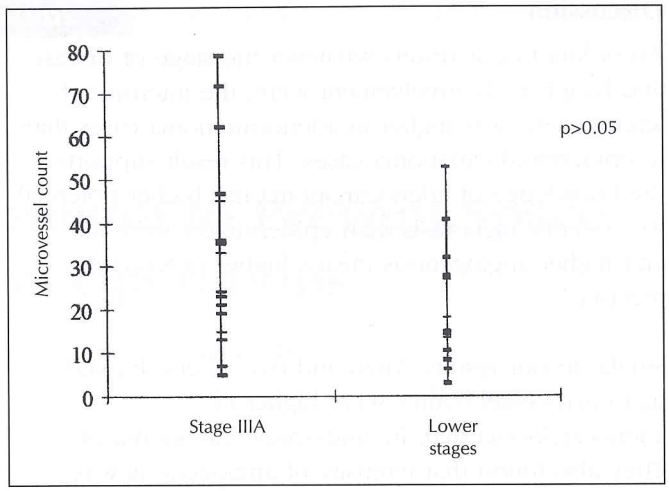

Fig. 3. Microvessel counts in patients with Stage IIIA disease and Stage I and II cases.

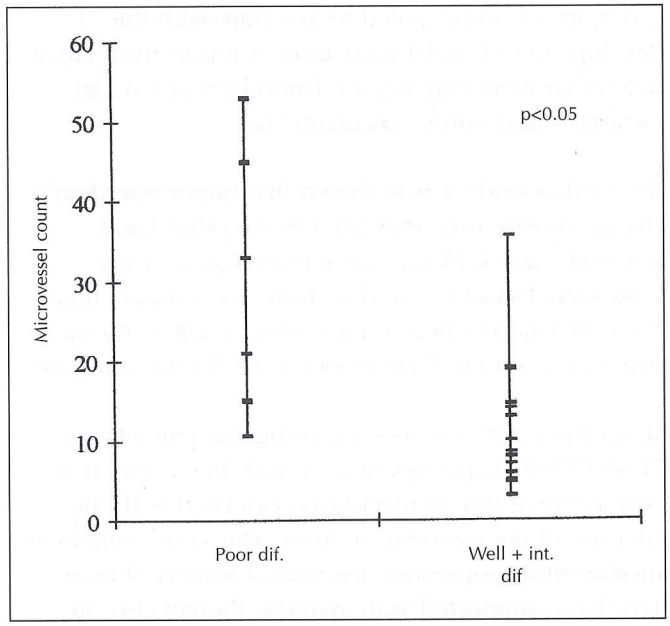

Fig. 4. Microvessel counts according to differentation of epidermoid carcinoma. Poor dif., differentiated tumor; Well + int. dif., well and intermadiate differentiated tumor.

intense in patients with Stage IIIA disease when compared with the other lower stages, but there was not statistical significance (Fig. 3). We couldn't find a correlation between microvessel count and tumor size.

Histopathologic examination of epidermoid carcinoma specimens showed good differentiation in 3 cases, intermediate in 13 cases and poor differentiation in 6 cases. Microvessel counts were significantly higher in poor differentiated tumor specimens than in well and intermediate differentiated tumors (p=0.0064) (Fig. 4).

There was not correlation between tumor size and differentiation of tumor in epidermoid carcinoma.

Discussion

According to our results whatever the stage of disease and lymph node involvement were, the intensity of angiogenesis was higher in adenocarcinoma cases than in epidermoid carcinoma cases. This result supports the knowledge of adenocarcinoma has higher potential for systemic metastasis than epidermoid carcinoma, and higher angiogenesis means higher potential for metastasis.

Similar to our results, Yuan and coworkers showed that microvessel counts were higher in adenocarcinoma than in epidermoid carcinoma (9). They also found that intensity of angiogenesis was significantly high in advanced stage (stage IIIA-B) and in presence of nodal involvement. Although we found similarly high intensity in these specimens microvessel count, in our study, failed to associate with the development of nodal metastasis. A report from Japan stated that there was not a relation between nodal metastasis and tumor vascularity(11).

In another study it was shown that tumor size didn't change disease outcome(12). On the other hand, however, size of tumor was reported as a strong prognostic factor(13). In this study, we couldn't find any correlation between microvessel counts and tumor size which is an indirect evidence of disease outcome.

Macchiarini and coworkers investigated patients with T1N0M0 NSCLC postoperatively and they found that postoperative distant metastasis correlated with the intensity of neovascularization(8). The same authors in another study suggested that radical surgery should have been supported with systemic therapy(14). In epidermoid carcinoma as well as adenocarcinoma cases, radical surgery alone could not be satisfactory and adjuvant therapy should be taken into consideration. In our study, poor differentiated epidermoid carcinoma showed intense angiogenesis which supports previous data that even in early stages, epidermoid carcinoma should be evaluated further by taking into consideration its differentiation. It was claimed in several reports that microvessel quantitation might define patients with stage I NSCLC who could benefit from adjuvant therapy after surgical resection (15, 16). Indeed, more studies with a large number of patients are needed to observe the relation between angiogenesis and the necessity of systemic therapy and in these studies survival takes of patient groups should be compared.

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