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# The role of angiogenesis assessment in the prognosis of breast carcinoma and in the evaluation of the therapeutic effect of "shark care" drug as an angiogenesis inhibitor

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## ABSTRACT

There is still uncertainty about angiogenesis as a prognostic factor in breast cancer. The aim of this study was to evaluate the prognostic value of microvascular density (MVD) in breast carcinoma and soluble vascular cell adhesion molecule-1 (sVCAM-1), leptin, estradiol and total testosterone as angiogenic markers. Efficacy of shark care drug was assessed by patient's overall survival. In this study there were 30 premenopausal breast cancer patients (group II) and 15 controls (group I). Group II was subgrouped into 15 patients receiving chemotherapy alone (IIA) and 15 patients receiving chemotherapy + shark care drug (IIB). After modified radical mastectomy, microvessels were counted by staining tissues for factor VIII. For surrogate markers, enzyme-linked immunosorbant assay or radioimmunoassay kits were used. A high MVD was found only in areas of carcinoma. MVD and sVCAM-1 correlated significantly with each other and with lymph node involvement. After the follow up, all subgroup IIB patients were alive compared to 66.6% of subgroup IIA patients (p=0.02). A high MVD may be a poor prognostic marker of breast carcinoma and a target for antiangiogenic therapy. sVCAM-1 is useful for diagnosis and for monitoring response to therapy. Chemotherapy + shark care drug seem to ameliorate the outcome of these patients. [Turk J Cancer 2008;38(3):123-134]

## KEY WORDS:

Factor VIII-related antigen, angiogenesis, microvessel density, soluble VCAM-1, shark care drug

## INTRODUCTION

Experimental evidence suggests that the growth of a tumor beyond a certain size requires angiogenesis, which may also permit metastasis. The tumor growth dependency on angiogenesis makes the hypothesis of angiogenesis as a prognostic factor attractive (1).

Methods that quantify the degree of tumor angiogenesis have been shown to provide important prognostic information for patients with a variety of solid tumors. The common pathologic approach to assessing angiogenesis involves microscopic estimation of vascular density or microvessel density (MVD) on tissues probed for endothelial markers by immunohistochemistry. Several markers of blood vessel endothelium have been developed for routine use, including Factor VIII-related antigen (von Willebrand Factor or vWF). Studies have produced conflicting results regarding the capacity of microvessel quantifications in breast carcinomas to predict patient's outcome and the existence of metastasis (2-4).

Drugs inhibiting angiogenesis seem to offer treatment that is complementary to traditional chemotherapy. Shark care drug is a purified shark cartilage extract composed of collagen, U-995 and SCF2 proteins. The latter two proteins inhibit endothelial cell proliferation, endothelial cell migration, and matrix metalloproteinase activity in vitro as well as the formation of new blood vessels in the chorioallantoic membrane of chicken embryos, and tumor-induced angiogenesis in the cornea of rabbits (5-7). Surrogate markers of angiogenesis would facilitate the assessment of the response to angiogenesis inhibitors. Candidates for such biomarkers include soluble vascular cell adhesion molecule-1 (sVCAM-1), leptin, estradiol (E2) and testosterone. sVCAM-1 is a glycoprotein produced and secreted by activated endothelial cells. Leptin is a hormone produced by adipocytes and stimulates angiogenesis by estrogenic-dependent and non-estrogenic-dependent pathways. The estrogenic-dependent mechanism involves conversion of testosterone to estradiol which in turn stimulates the synthesis of vascular endothelial growth factor (VEGF) and its receptor. The non-estrogenic-dependent pathway involves activation of the transcription factor NF-κB which activates the expression of VCAM-1 (8,9).

CA 15-3 is a circulating breast cancer-associated antigen that is frequently used to follow up response of patients with breast cancer to chemotherapy and to detect recurrence of the disease (10).

In the present work, the clinical significance of tumor angiogenesis as a prognostic indicator in breast cancer patients was examined by counting MVD and correlating it with established prognostic parameters including nodal status, tumor size, tumor grade and estrogen (ER) and progesterone (PR) receptors status. The possibility of using sVCAM-1, leptin, estradiol and testosterone as surrogate markers of angiogenesis that help monitoring the response to shark care drug as well as in diagnosing breast cancer in addition to serum CA 15-3 was investigated. The efficacy of shark care drug as antiangiogenic adjuvant therapy was evaluated after a follow up period of 35 months.

## MATERIALS AND METHODS

This study included 45 premenopausal women divided into 2 groups;

Group I: 15 normal healthy premenopausal female volunteers included as control. Their ages varied from 25-41 years with a mean age of 33 years.

Group II: 30 premenopausal female patients with breast carcinoma of clinical stages II and III recently detected. Their ages ranged from 23-48 years with a mean age of 37 years. These patients were randomly selected new cases from the Departments of Surgery and Oncology of the Medical Research Institute, Alexandria University in the period from January 2003 to January 2006.

Group II was further subdivided into two subgroups:

-Subgroup IIA: 15 patients, 10 were of clinical stage II and 5 of clinical stage III. They received chemotherapy after surgery in the form of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) for clinical stage II and 5-fluorouracil, adriamycin and cyclophosphamide (FAC) for clinical stage III for 6 cycles (11).

-Subgroup IIB: 15 patients, 9 were of clinical stage II and 6 of clinical stage III. They received CMF or FAC for 6 cycles according to their clinical stage + shark care tablets (an antiangiogenic drug; arab co. for pharm and medicinal plant, MEPACO, Egypt), in a dose of 2 tablets/day, each tablet contains 740 mg of chondroitin-6-sulfate for a period as long as the chemotherapy was given.

Blood samples were collected once from group I, and in group II patients samples were obtained before surgery, 2 weeks after surgery and after 6 cycles of CMF or FAC with or without shark care drug. Serum samples were separated and stored at -80°C till used.

In each serum sample, sVCAM-1, leptin, estradiol, total testosterone, and CA15-3 were measured by the following:

-A ready to use human soluble VCAM-1(sVCAM-1) Enzyme-Linked Immunosorbant Assay (ELISA) kit was obtained from Biosource International, Inc. (USA) (12).

-Ready to use Leptin Enzyme Amplified Sensitivity Immunoassay (EASIA) kit was obtained from Biosource International, Inc. (USA) (13).

-Ready to use Estradiol (E2) (14) and Total Testosterone (15) radioimmunoassay (RIA) kits were obtained from Diagnostic Products Corporation (DPC; USA).

-A ready to use CA 15-3 Enzyme Immunoassay (EIA) kit was obtained from CanAg Diagnostics AB (Sweden) (16).

### Histopathology

Thirty cases of breast carcinoma were analyzed. All cases received uniform initial treatment in the form of

modified radical mastectomy after an informed written consent. Preoperative evaluation was done in the form of fine needle aspiration cytology to diagnose carcinoma as well as clinical, laboratory and X ray assessment to exclude any metastasis.

Pathologic parameters were obtained by examination of hematoxylin and eosin (H&E)-stained slides. The histological type of breast tumor was determined according to the WHO guidelines (17). Histological malignancy grading followed the grading system of Bloom and Richardson (18). Tumor size was measured as the largest diameter of the invasive carcinoma. Pathologic staging T1−T4 was performed according to the TNM Staging 2002 (19).

### Immunohistochemistry

Three 4-µm-thick sections from each formalin-fixed and paraffin-embedded tumor were mounted on coated slides. Paraffin sections were stained for:

-ER, PR (monoclonal, Novocastra, UK). Estrogen and progesterone receptor status were determined as positive when >10% of the tumor cells were positive (20).

-Rabbit polyclonal factor VIII-related antigen (FVIIIR-Ag) antibody using Spring Bioscience Company (UK) at a 1: 100 dilution to assess the MVD (4).

Epitope retrieval was performed before incubation with the primary antibody by microwave heating in citrate buffer (pH 6). The peroxidase-antiperoxidase technique was used and diaminobenzidine was used as the chromogen. Sections were counterstained lightly with hematoxylin.

Negative controls were produced by omitting the primary antibody and vascular endothelial cells were used as a positive internal control.

### Assessment of neovascularization

The quantitative vessel counts were performed according to the method described by Weidner et al. (21,22). Briefly, the entire tumor area was scanned using a x10 objective to select areas of most intense vascularization (hot spots). Three separate, non-overlapping fields were selected from these areas and all FVIIIR-Ag-stained microvessels were counted in each field. Counts were performed using a x20 objective; the field area was 0.74 $mm^2$. The microvessel count was scored by averaging the three field's counts.

An individual microvessel was defined as any brown-staining endothelial cells, or separate cluster of brown staining endothelial cells, clearly separated from adjacent clusters and background, with or without lumen, irrespective of size.

A further vessel count was undertaken with the intension of providing an 'internal control'. Control fields were selected so as to be situated entirely within healthy breast tissue and centered at a fixed distance of 3.3 mm from the nearest infiltrating tumor margin.

### Statistical analysis (23)

The Chi square test was used to determine the associations between the MVD with tumor size, clinical stage, histopathologic grade, ER, PR and axillary lymph node status. The Spearman correlation was used to correlate the various surrogate markers with the MVD as well as with the number of positive lymph nodes. Unpaired student's t test was used to compare the mean values of the various surrogate markers between the different groups. The paired student's t test was used to compare the mean values of the various surrogate markers within the same groups before and after surgery as well after 6 cycles of therapy. Survival of the two subgroups (IIA and IIB), after a follow up period of 35 months was compared using cumulative survival curves in order to evaluate the efficacy of combining the shark care drug to the treatment regimen. The Receiving Operating Characteristic (ROC) curve was used to compare the sensitivity of sVCAM-1 and CA 15-3 in diagnosing breast cancer. For all statistical analyses a p value $<0.05$ was considered significant.

## RESULTS

The clinicopathologic parameters of the 30 patients (group II) with invasive breast carcinomas studied are summarized in table 1.

### Microvessel density (MVD)

Immunohistochemical staining for FVIIIR-Ag facilitated the enumeration of microvessels. The vascular hot spots were most frequently seen at the margins of the invasive carcinoma. In some cases, these hot spots were in the form of arborizing vessels that surround the tumor cells; in other cases, the hot spots were characterized only by the presence of numerous vessels (Figures 1&2).

**Table 1**
**Summary of the clinicopathological parameters of the 30 patients of group II**

| Parameters | Group II n=30 |
|---|---|
| Mean Age (range) | 37 (23-48) |
| Tumor size | |
| <2 | 6 |
| 2-5 | 17 |
| >5 | 7 |
| Lymph node involvement | |
| - | 6 |
| + | 24 |
| Clinical stage | |
| II | 19 |
| III | 11 |
| Tumor type | |
| IDC (NOS) | 20 |
| IDC+IS | 5 |
| IDC+mucoid differentiation | 1 |
| Atypical medullary carcinoma | 1 |
| Intracystic papillary carcinoma with microinvasion | 1 |
| ILC | 1 |
| Mixed ductal and lobular carcinoma | 1 |
| Pathological grade | |
| I | 4 |
| II | 19 |
| III | 5 |
| Estrogen receptor (ER) | |
| - | 6 |
| + | 24 |
| Progesterone receptor (PR) | |
| - | 11 |
| + | 19 |

IDC (NOS): Invasive ductal carcinoma, not otherwise specified; IDC+IS: Invasive ductal carcinoma with prominent in situ component; ILC: Invasive lobular carcinoma
No grading was performed for the case of invasive lobular carcinoma and the case of mixed ductal and lobular carcinoma

The mean±Standard Deviation (SD) MVD count for tumors was 66.06±22.5 vessels/x200 field (range 32−120). The counts in control areas ranged from 2-6 microvessels/x200 field with a mean±SD of 4±2 vessels/x200 field (Figure 3). The mean MVD was significantly higher in areas of carcinomas than in the control areas ($p<0.05$).

The mean microvessel count of 66 was used as a cutoff value for discriminating between low and highly vascularized tumors. Accordingly, 15 cases were highly vascularized and 15 were of low vascularity.

Nine out of 20 IDC (NOS) and 4/5 IDC+ IS had high vascularity. The case of mixed ductal and lobular carcinoma as well as the case of intracystic papillary carcinoma associated with microinvasion had high vascularity. The cases of IDC with mucoid differentiation and invasive lobular carcinoma, both were of low vascularity (Figures 4-6). Foci of ductal carcinoma in situ associated with invasive carcinoma showed a specific profile of vascular immunostaining; where a dense microvascular rim was

**Table 2**
**The association of MVD grade with the clinicopathologic parameters in the 30 patients of group II**

| Parameters | Low MVC n = 15 | High MVC n = 15 | $\chi^2$ test P value |
|---|---|---|---|
| Tumor size | | | |
| <2 | 3 | 3 | 1.82 |
| 2-5 | 7 | 10 | p=0.40 |
| >5 | 5 | 2 | |
| Lymph node involvement | | | |
| - | 6 | 0 | 7.5 |
| + | 9 | 15 | p=0.006 |
| Clinical stage | | | |
| II | 8 | 11 | 1.29 |
| III | 7 | 4 | p=0.26 |
| Pathological grade | | | |
| I | 1 | 3 | 2.85 |
| II | 9 | 10 | p=0.24 |
| III | 4 | 1 | |
| Estrogen receptor (ER) | | | |
| - | 3 | 3 | 0 |
| + | 12 | 12 | p=1 |
| Progesterone receptor (PR) | | | |
| - | 5 | 6 | 0.14 |
| + | 10 | 9 | p=0.71 |

**Table 3**
**The correlation of MVD and number of lymph nodes invaded with the serum markers studied before surgery in 30 group II patients**

| | sVCAM-1 (ng/ml) | Leptin (ng/ml) | E2 (pg/ml) | Testosterone ng/dl |
|---|---|---|---|---|
| MVD /×200 field | r=0.832* | r=0.318 | r=0.154 | r=0.244 |
| Number of lymph node involved | r=0.509* | r=-0.023 | r=-0.001 | r=0.107 |

r=Spearman correlation
*Significant correlation, $p<0.05$. Only sVCAM-1 significantly correlates positively

seen close to the ductal carcinoma in situ basement membranes in addition to stromal vascularity (Figures 7&8).

Three out of 4 grade I, 10/19 of grade II and 1/5 of grade III carcinomas had high vascularity. However, the case of atypical medullary carcinoma showed low vascularity (Figures 9&10).

Table 2 demonstrates the association between the clinicopathologic parameters and microvessel density (MVD) in breast cancer patients group.

Axillary node metastasis was significantly associated with high microvessel counts/x200 field, (p=0.006). No association was observed between the MVD and tumor size, histopathologic grade, clinical stage, and estrogen and progesterone receptors status.

## Surrogate markers

*Angiogenic and prognostic utility of the surrogate markers*

Table 3 shows that the serum level of sVCAM-1 was significantly positively correlated with MVD and number of lymph nodes involved. However the other assayed parameters did not give any correlation with either MVD or lymph node involvement.

*Evaluation of the utility of the surrogate markers in the diagnosis and follow up of breast cancer patients*

The levels of sVCAM-1 and serum CA 15-3 before surgery were statistically significantly higher in the breast cancer patients group (group II) than in the control group (group I) (Table 4). Furthermore, both were significantly correlated with each other (r=0.434, $p<0.05$).

In group II, two weeks after surgery, sVCAM-1 decreased significantly compared with its level before surgery (Table 5).

After 6 cycles of chemotherapy alone (subgroup IIA) sVCAM-1 level showed a non-significant decrease. On the other hand, the subgroup IIB treated with 6 cycles of chemotherapy in combination with the shark care drug, sVCAM-1 showed a significant decrease compared to its level 2 weeks after surgery and to its level after 6 cycles of chemotherapy alone in subgroup IIA. Total testosterone

**Table 4**
**Mean (±SD) of all surrogate markers studied in the control and breast cancer patients groups before surgery**

| | sVCAM-1 (ng/ml) | Leptin (ng/ml) | E2 (pg/ml) | Testosterone (ng/dl) | CA 15-3 (IU/ml) |
|---|---|---|---|---|---|
| Control group (n=15) | 501.60±20.97 | 23.68±2.84 | 108.20±15.73 | 31.69±8.12 | 12.48±1.11 |
| Breast cancer patients group BS (n=30) | 1647.97±120.22* | 21.94±4.57 | 64.51±11.26 | 20.88±2.12 | 21.11±2.48* |

BS: before surgery
*Significance was compared with control group

**Table 5**
**Mean (±SD) of all surrogate markers studied in breast cancer patients (group II) before and after surgery**

| | sVCAM-1 (ng/ml) | Leptin (ng/ml) | E2 (pg/ml) | Testosterone (ng/dl) | CA 15-3 (IU/ml) |
|---|---|---|---|---|---|
| Breast cancer patients (n=30) | | | | | |
| BS | 1647.97±120.22 | 21.94±4.57 | 64.51±11.26 | 20.88±2.12 | 21.11±2.48 |
| 2W | 1132.19±101.65* | 21.48±3.34 | 58.97±8.65 | 23.20±2.12 | 19.35±2.24 |

BS: before surgery; 2W: 2 weeks after surgery
*Only sVCAM-1 was significantly decreased after surgery

level was lower in group II patients before surgery than in the controls. Furthermore, it significantly increased after 6 cycles of chemotherapy in combination with shark care drug (subgroup IIB) compared with its level 2 weeks after surgery and to its level after 6 cycles of chemotherapy in subgroup IIA (Table 6).

Variations in the levels of leptin and E2 were non-significant.

*sVCAM-1 and CA 15-3 as diagnostic markers*

ROC curves of sVCAM-1 and serum CA 15-3 were constructed using a set of cut-off points selected based on the levels of sVCAM-1 and serum CA 15-3 in the control group (Figure 11). Inspection of the two ROC curves was sufficient in such a way that the higher curve corresponds to the better diagnostic test. The area under the curve for sVCAM-1 was 99.1%, while the optimum cut off value

**Table 6**
**Mean±Standard Deviation (M±SD) of all surrogate markers studied in subgroups IIA and IIB**

| | sVCAM-1 (ng/ml) | Leptin (ng/ml) | E2 (pg/ml) | Testosterone (ng/dl) | CA 15-3 (IU/ml) |
|---|---|---|---|---|---|
| Breast cancer patients receiving chemotherapy alone (subgroup IIA) (n=15) | | | | | |
| 2w | 1283.09±99.64 | 19.85±4.11 | 56.07±11.43 | 22.59±2.50 | 16.73±2.51 |
| 6C | 1275.20±103.98 | 22.05±4.36 | 67.80±26.19 | 25.98±2.37 | 18.47±2.09 |
| Breast cancer patients receiving chemotherapy and shark care drug (subgroup IIB) (n=15) | | | | | |
| 2w | 981.29±172.11 | 23.11±5.39 | 61.87±12.24 | 24.68±3.54 | 21.97±3.66 |
| 6C | 479.63± 106.89*† | 25.91±6.74 | 58.72±12.18 | 35.45±3.28*† | 20.36±5.60 |

2W: 2 weeks after surgery; 6C: After 6 cycles of chemotherapy
*Significance was compared with breast cancer patients subgroup **(IIB)** after 2 weeks of surgery
†Significance was compared with breast cancer patients subgroup **(IIA)** after 6 cycles of chemotherapy
After 6 cycles of chemotherapy + shark care drug only sVCAM-1 was significantly lowered and testosterone was significantly higher than their levels in the same subgroup after 2 weeks of surgery and than subgroup IIA after 6 cycles of chemotherapy alone

was 678.1 ng/ml, at which the sensitivity was 93.3% and the specificity was 100%, with positive predictive value of 100% and negative predictive value of 88.2%. The area under the curve for serum CA 15-3 was 72.2%, while the optimum cut off value was 19 IU/ml, at which the sensitivity was 56.7% and the specificity was 100%, with positive predictive value of 100% and negative predictive value of 53.6%.

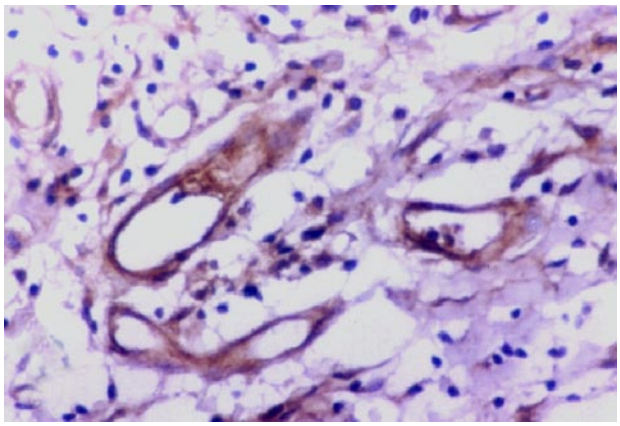


*Fig 1. Vascular hot spot with numerous vessels seen at the margin of an invasive ductal carcinoma (FVIIIR- Ag immunostaining, x400)*

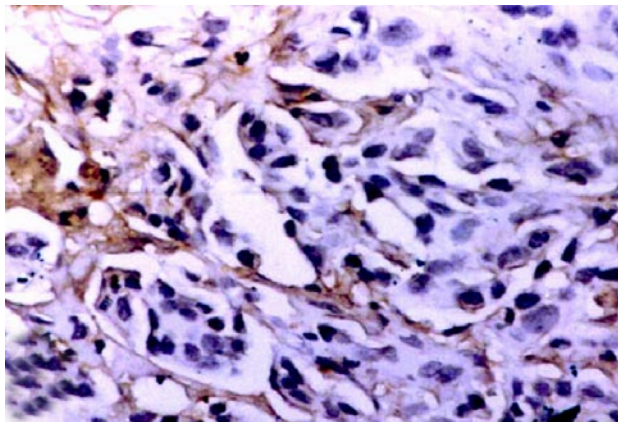


*Fig 2. Margin of an invasive ductal carcinoma grade II, showing a vascular hot spot with a tangle of arborizing vessels (FVIIIR-Ag immunostaining, x400)*

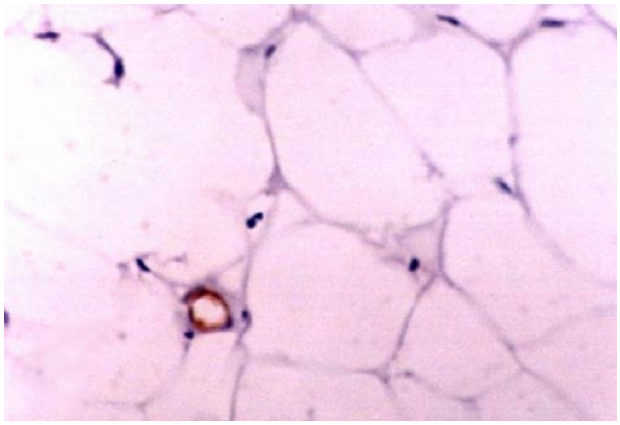


*Fig 3. Normal breast adipose tissue adjacent to invasive carcinoma, showing a single stained microvessel (FVIIIR- Ag immunostaining, x400)*

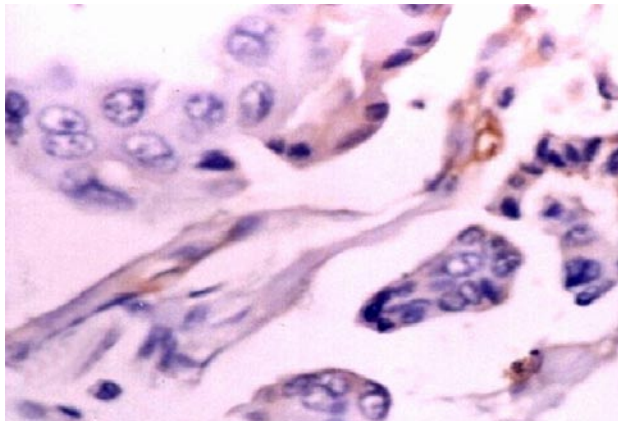
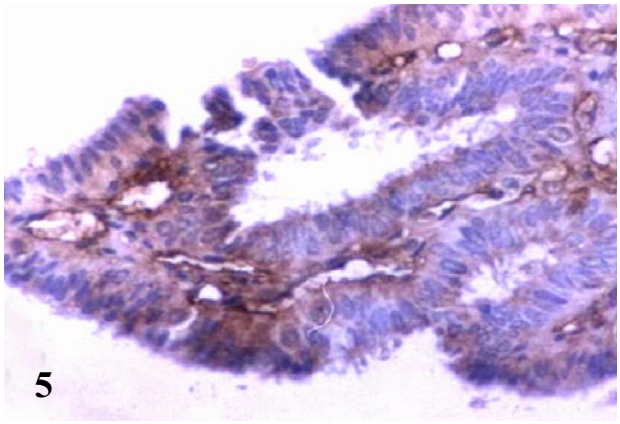


*Fig 4. Invasive ductal carcinoma with mucoid differentiation showing low vascularity (FVIIIR- Ag immunostaining, x400)*



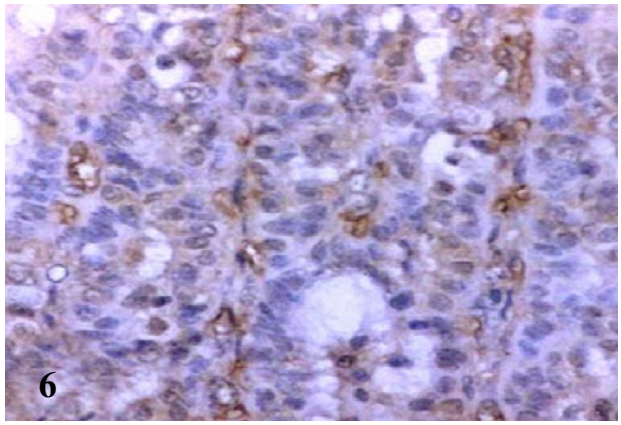
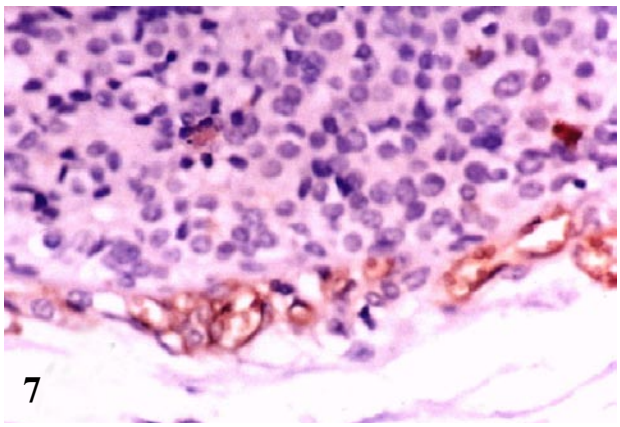




*Fig 5&6: Intracystic papillary carcinoma with microinvasion showing highly vascularized papillae core and stroma (FVIIIR- Ag immunostaining, x400)*



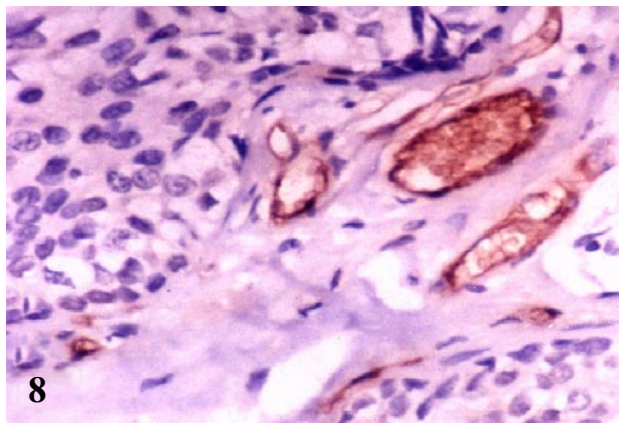




*Fig 7&8. Foci of ductal carcinoma in situ solid variant showing a dense microvascular rim adjacent to the basement membrane in addition to prominent stromal vascularity (FVIIIR- Ag immunostaining, x400)*

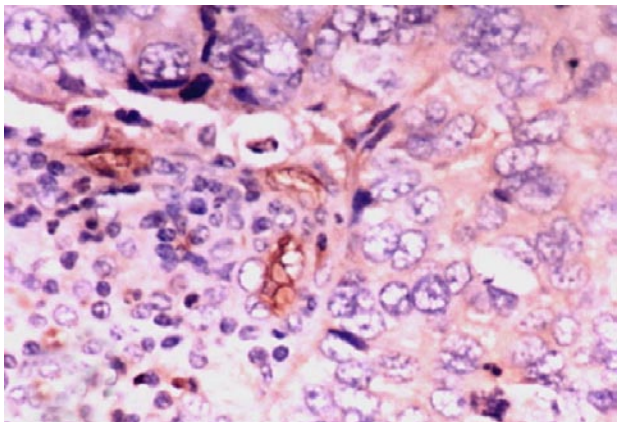


*Fig 9. Atypical medullary carcinoma showing low vascularity (FVIIIR- Ag immunostaining, x400)*

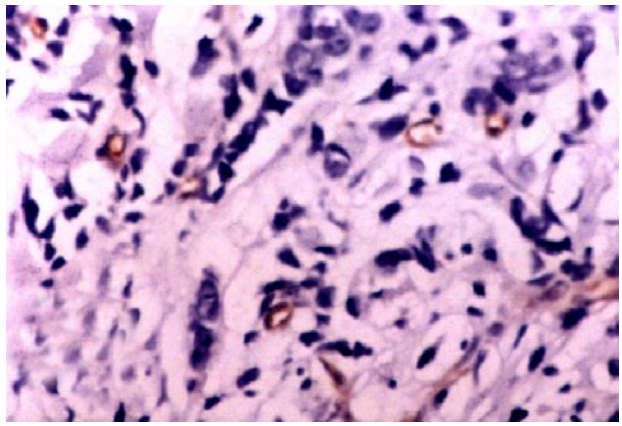


*Fig 10. Invasive ductal carcinoma grade II showing high vascularity (FVIIIR- Ag immunostaining, x400)*

*Evaluation of shark care drug as adjuvant therapy in breast cancer patients*

Figure 12 shows that at the end of the follow up period of 35 months, the overall survival of subgroup IIB patients (treated with chemotherapy + shark care drug) was 100%. It was significantly better compared to that of subgroup IIA (treated with chemotherapy alone) 66.6% ($p<0.02$).

**DISCUSSION**

The ability of tumors to induce a vascular stroma is a critical requirement for tumor progression at all stages of breast cancer development. Measurements of angiogenesis may have clinical utility in the evaluation of breast cancer, particularly for estimation of metastatic risk. Investigators have proposed also that MVD might also have predictive value with regard to benefit from adjuvant chemotherapy, or by specific antiangiogenic drugs (3,4,24).

In the present study, primary breast cancer tumor tissue sections were immunostained with antibody to factor VIII-related antigen to facilitate counting of MVD. Compared to adjacent normal lobules (4±2 vessels/x200 field), carcinoma exhibited a significantly greater density of vWF positive vessels (66.06±22.5/x200 field) confirming that the tumor vascular counts obtained are at least to some extent due to neovascularization and that angiogenesis is important for tumor growth and progression. On the other hand, the mean MVD used in the present work as a cut off value discriminating between tumors of low and high vascularity was more or less similar to those reported in the literature (25,26).

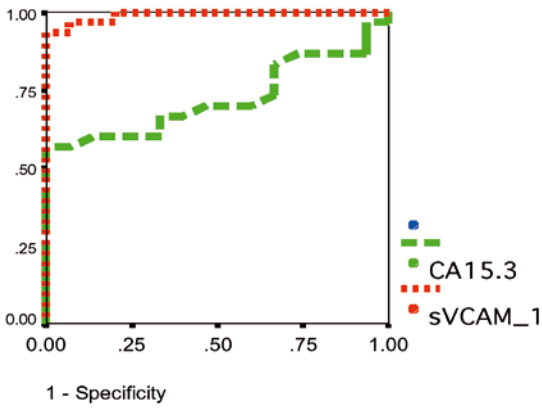



*Fig 11. ROC curves comparing the accuracy of serum sVCAM-1 and CA 15-3 in discriminating between breast cancer cases and control*

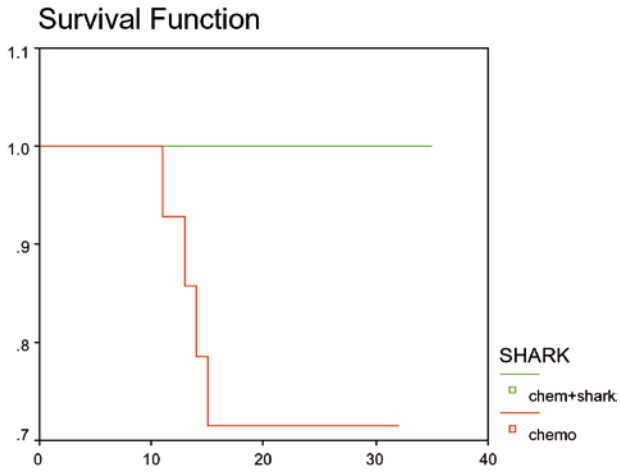



*Fig 12. Overall survival curve for breast cancer patients treated with chemotherapy with and without shark care drug*
*All the patients who survived the 3 years were all disease free*

A dense microvascular rim adjacent to the basement membranes of DCIS associated with invasive ductal carcinoma was seen in the present work. This finding is consistent with previous publications which suggested that periductal vascularization may be caused by the direct release of angiogenic factors by neoplastic cells and could be important in determining the transformation from in situ to invasive disease (27,28).

The high vascularity observed in the case of intracystic papillary carcinoma included in this study could be explained by the presence of co-existent foci microinvasion. The low vascularity observed in the single case of invasive lobular carcinoma studied in the current work is in line with the results reported by others (1,29).

An important finding of the present work is that a high MVD count was significantly associated with axillary lymph node metastases. This is confirmed by other investigators and is supported by a recent study demonstrating the correlation of MVD with metastasis associated antigen 1 (MTA1), and implies that if a tumor has the ability to metastasize, an increasing vascular component would progressively facilitate this process (30-35). Taking in consideration the importance of axillary lymph node status in predicting breast cancer patient's overall survival, it can be said that MVD may be a useful additional prognostic factor that, when used in combination with more established parameters, can help in appropriate patient management, especially in stratifying patients for antiangiogenic treatment (2). Others found no significant relationship between microvessel density and lymph node metastases; this could be attributed to the fact that they performed their study solely on cases of invasive lobular carcinoma or their use of a different antibody and technique of counting (29,36).

The other clinicopathologic parameters studied in the present work, including clinical stage, tumor size, tumor grade, and estrogen and progesterone receptors status, did not correlate with the MVD. Several previous researches were confronted with similar results, however others have found a correlation between the MVD and advanced stage, large tumor size, high grade, and negative estrogen receptor status; differences in methodologies, as well as heterogeneity in the selection of study population and applied techniques might play a role in these discrepancies (1,3,26,29,30, 37-40).

Anti-angiogenesis is one of the newest weapons for fighting breast cancer (41-43). One of the targets of this study was to evaluate the antiangiogenic efficacy of the shark care drug in breast cancer patients. In fact It was found that patients treated with the shark care drug + chemotherapy (subgroup IIB) had a significantly better overall survival than patients receiving chemotherapy alone (subgroup IIA).

To monitor the response to new specific antiangiogenic drugs, reliable predictive markers of angiogenesis are required (36). MVD provides a snapshot of angiogenesis that cannot be repeated once the primary tumor has been removed. As a result of these criteria, we assayed serum sVCAM-1, leptin, E2 and total testosterone as angiogenic and prognostic biomarkers by correlating their levels with

tumor MVD and lymph node metastasis. In addition the variations in their levels after surgery and after 6 cycles of chemotherapy with or without shark care drug were also analyzed.

In accord with Byrne et al. (44) sVCAM-1 levels before surgery were significantly higher in breast cancer patients (group II) than in the healthy controls (group I) and correlated significantly also with the MVD and lymph node metastasis. The levels of sVCAM-1 decreased significantly 2 weeks postoperatively. Treatment by chemotherapy alone did not influence the sVCAM-1 levels; in contrast, chemotherapy in conjunction with shark care drug significantly lowered the levels of sVCAM-1. Therefore, it seems that sVCAM-1 is a reliable surrogate marker of angiogenesis that can be used as a diagnostic and prognostic marker as well as a rapid method to assess antiangiogenic drugs during the follow up of breast cancer patients.

The current work demonstrated that the levels of sVCAM-1 and serum CA 15-3 before surgery correlated significantly with each other and with lymph node involvement, and were significantly higher in breast cancer patients than in the control group, so both of them could be helpful in diagnosing breast cancer. Using the ROC curve we compared the diagnostic sensitivity of sVCAM-1, as a new breast cancer marker, versus that of CA 15-3 as an older one. The sensitivity of sVCAM-1 was found to be higher than that of CA 15-3. Hence, it could be said that sVCAM-1 is superior to CA 15-3 in diagnosing breast cancer in premenopausal women. Others stated that combining measurement of markers from two different tumor cell compartments (CA 15-3 as an epithelial marker with sVCAM-1 as an endothelial marker) will likely improve early assessment of breast cancer response to therapy (44).

As regards Leptin, E2 and total testosterone, the present study failed to detect any correlation between them and MVD or lymph node involvement, thus, eliminating their possible use as angiogenic or prognostic markers in breast cancer patients. On the contrary, total testosterone levels were lower in patients than controls and increased significantly in response to antiangiogenic drug (subgroup IIB compared to subgroup IIA); therefore it seems to act as an angiogenesis inhibitor.

It can be concluded that, a high MVD in a case of invasive breast carcinoma may be a predictor of metastatic disease in axillary lymph nodes. In addition, MVD leads to more insights, not only in improved prediction of prognosis but also in determining whether those patients would benefit or not from adding antiangiogenic drugs to the currently established adjuvant therapies.

sVCAM-1 is a simple, fast, sensitive, specific and a dynamic surrogate angiogenic marker that is superior to CA 15-3 in diagnosing breast cancer and monitoring the response to surgery and chemotherapy with the antiangiogenic shark care drug.

Serum leptin and E2 have no role as angiogenic, diagnostic, follow up, nor prognostic markers in premenopausal females with breast cancer.

Serum total testosterone may be an angiogenesis inhibitor, an observation that needs to be confirmed by future studies on larger number of cases.

The findings of the present work also suggest including the shark care drug among the future treatment strategies of breast cancer patients, after confirmation of these results by future studies on a larger number of patients.

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