Significance of Angiogenesis in Management and Prognosis of Breast Cancer

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Abstract: Introduction: Tumor angiogenesis is the main process responsible for the formation of new blood vessels that promote tumor growth and metastasis. The evaluation of both CD_{34}- CD_{105} showed the role of angiogenesis in the cancer proliferation and local spread, the angiogenesis level being maintained high even in the advanced stages of the disease. There was observed difference between the MVD assessed by (CD_{34}) and the MVD assessed by (CD_{105}), the study of this difference possibly leading to a better assessment of prognosis and adjusted therapies in the future. **Patients and Methods:** Endothelial cells are highlighted using a variety of endothelial markers. One of the best known marker is CD_{34}, pan endothelial marker; surface antigen. The most used immunohistochemical marker for identification of activated endothelial cells is CD105. Our study included 46 patients with breast cancer between 2010- 2013 who underwent breast surgery (CBS/ MRM) at Al- Zahraa University hospital department of surgery, Faculty of medicine, Cairo, Egypt. We used MVD quantification by highlighting the tumor bleed vessels with two different endothelial markers using the immune histochemical protocols. **Results:** The CD34 evaluation of MVD was 10 patients (approx.; 22.2%) with low vascular density and 36 patients (77.8) with high vascular density. In the same patients MVD for CD_{105} (endoglin); staining the intratumoral active endothelial cells was 16 patients (app 35.6%) showed low vascular density and 29 patients (approx. 64.4%) with high vascular density. There was a statistical significant correlation between high vascular density and the number of positive axillary lymph node (P= 0.054 for CD_{105} and P= 0.037 for CD_{34}), tumor size (P= 0.050 for CD_{105}) Mitotic index (MI) (p= 0.003 for CD_{34}; P= 0.002 for CD_{105}); ER and PR negativity (P= 0.014 for CD_{105}); and Her -2 neu overexpression (P= 0.026 for CD_{105}).

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Keyword: CD34, CD105, endoglin, breast cancer, Pan-endothelial marker, angiogenesis, ER, PR, Her- 2 neu, MI.

1. Introduction:

Tumor angiogenesis is the main process responsible for the formation of new blood vessels that promote tumor growth and metastasis. This process is driven by potent proangiogenic factors that are predominant in the tumor environment and are produced by both malignant cells and the host cells recruited to the tumor site. Tumor environment is characterized by the imbalance between proangiogenic and antiangiogenic factors, which drivers the constriction of numerous but structurally defective vessels.

These poorly perfused and abnormal vessels significantly contribute to the tumor pathology not only by supporting the expansion of the tumor mass but also by promoting chronic inflammation, enhancing thrombosis, impeding drug delivery and disseminating tumor cells. These problems associated with tumor vasculature continue to attract great attention of scientists and clinicians interested in advancing the understanding of tumor biology and development of new drugs. (1)

Quantification of tumoral tissue vascularization has become important to scientific research since Folkman's revolutionary idea that no tumoral tissue can grow more than 2mm without vascularization. (2)

Malignant cells contain an aberrant genome and divided at a high rate. (3) whereas endothelial cells are genetically stable maintaining a more normal growth rate. (4) thus in a growing tumor, malignant cells and endothelial cells grow at non-synchrenized rate, resulting in hypoxia and rapid expansion of the tumor population. (5)

Despite the scientific effort, there is still no standardized protocol to count and analyze neoangiogenesis. (6)

The most frequent method to assess angiogenesis is microvessel density (MVD) by visual count of stained blood vessels performed under high magnification light microscopy. (7)

2. Patients and Methods

Our study was conducted on 46 patients diagnosed with breast invasive carcinoma, two of

them have bilateral BC. Two types of surgery were done conservation breast surgery (CBS) and modified radical mastectomy (MRM). The study continued from 2010 – 2013 at Al- Zahraa hospital, department of surgery Faculty of Medicine University, Cairo, Egypt. The follow up period ranged from 3-24 months. For each patient we gathered additional information like medical pathologic records; morphological descriptions TNM classification, histologic grade, ER, PR, HER-2/ neu expression and various correlations were investigated statistically.

Inclusion criteria:

1- Age: no age restriction.
2- Gender: males and females are included in our study.
3- Patients who have no history of previous breast sugery.
4- Patients without any history of chronic medical diseases.
5- Non pregnant female patients.
6- Size of the mass: less than 5 cm.
7- Laterality: unilateral or bilateral.
8- TNM staging: I-II.
9- Type of surgery: MRM and CBS.
10- Type of specimen: incisional, excisional and CNB.

Exclusion criteria

1- Unilateral or bilateral previous breast surgery.
2- Recurrent cases.
3- Stage III and IV metatases to distant sites (lung- liver- brain- intestine).
4- Pregnant patients.
5- Cases with chronic medical illness (HTN- IHD- Rh.D).
6- Cases subjected to neoadjuvant therapy.
7- Inflammatory carcinomatosis.
8- Carcinoma in situ.

Technique of preparation of specimens:

The tumor will be stained with immunostains CD_{34} and CD_{105} (endeglin) to measure the vascular density and the areas under active neovascularization (edge associated, intratumoral) respectively by evaluation of the already formed blood vessels through CD_{34} scoring and the newly formed blood vessels in the active growing tumor through CD_{105} scoring, correlating these findings with the tumor histologic type, grade size, lymphovascular invasion and the other clinicopathologic findings for selection of the proper treatment modality either surgical (conserving breast surgery or modified radical mastectomy) systemic, radiation, or endocrine therapy, with prediction of the possible prognosis according to the degree of the microvascular density (high or low).

Estimation of ER, PR and Her_2neu will be done for certain cases and it will be correlated with the vascular density of the tumor.

- Fixation

I. The tissue was put in 10% formalin, 40% formaldehyde, then tap water then in;
II. Buffered neutral formalin solution 40% formaldehyde, distilled water, sodium phosphate monobasic (4gm) and sodium phosphate dibasic (6.5gm).

- Processing of tissues

Paraffin embedding process was done as follow (Alcohol 80% 1-2hrs- Alcohol 95% -2 changes 1-2hrs each. Alcohol absolute -3 changes 1-2hrs each, xylene-2 changes 1-2hrs each, Melted paraffin-3 changes 1-2hrs each, then the tissue embedded in paraffin and cooled quickly).

- Immunohistochemistry

Paraffin sections on slides were pretreated for antigen retrieval, then with supersensitive monoclonal antibodies against CD_{34} and CD_{105} (Ultra vision detection kit, antipolyvalent, HRP/ DAB) with blockage of internal peroxidase activity. Streptavidin biotin peroxidase detection system was used, Utilizing DAB as a chromogenand hematoxylin as a counter stain: 1- Preparation of slides; 2-Deparaffinization and rehydration of the sections; 3-Antigen retrieval; 4-Application of primary antibody.

The antibodies against CD34 and CD105 used in the appropriate dilution, using PBS as a diluents and one to two drops of the diluted primary antibody were added on each section. The slides were left in a refrigerator overnight at $4^{o}C$. Slides were incubated for 5-10min with dual endogenous enzyme block; Excess dual endogenous enzyme block was dried around sections using paper tissue; The slides were incubated with the labeled polymer for 45 minutes; Slides were rinsed in PBS for 5 minutes; Each slide was dried around sections using paper tissue; Staining is completed by 5-10 minutes incubation with 3, 3'-diaminobenzidine (DAB) + substrate – chromogen which results in a brown-colored precipitate at the antigen site; Slides were washed in distilled water for 5 minutes; Counter stain: 1- Slides were immersed in mayor's hematoxilin for 1 minute; 2- Slides were washed in tap water; 3- Dehydration of the sections; 4- Slides were placed in 70%, 95% and then 100% alcohol each for 5 minutes; 5- Mounting procedures; 6-The cover slides were mounted using DPX.

- **Immunohistochemistry scoring & Evaluation**

We evaluated the immune expression of different markers using light Ziess Microscope (Germany) using X 400 power magnification in 10 microscopic fields. The value was calculated as the mean percentage of positive cells.

1. CD_{34} evaluation and scoring

For CD_{34} the positive results were indicated as brown color in the cell membrane of endothelial cells of blood vessels. The slides were examined at 40- fold magnification. A positive vessel was defined for the identification of a vessel lumen with at least one positive stained endothelial cell. The stained vessels were counted in 3 consecutive fields from the representative tumor zone. The mean value was considered as microvessel density (MVD). (8).

2. CD_{105} evaluation and scoring

For CD_{105} the positive results were indicated as brown color in the cell membrane of endothelial cells of newly formed blood vessels. The slides were examined at 40- fold magnification. A positive vessel was defined for the identification of a vessel lumen with at least one positive stained endothelial cell. The stained vessels were counted in 3 consecutive fields from the representative tumor zone. The mean value was considered as microvessel density (MVD) (8).

Statistical analysis:

Statistical presentation and analysis of the present study was conducted, using the mean, standard error, student t- test, Chi-square, Linear Correlation Coefficient, Mann-Whitney, Receiver Operating Characteristic curve analysis and Analysis of variance [ANOVA] tests by statistical package for social sciences version 18 (SPSS V18).

Unpaired **Student t-test** was used to compare between two groups in quantitative data.

Mann-Whitney a nonparametric equivalent to the t test. Tests whether two independent samples are from the same population. It is more powerful than the median test since it uses the ranks of the cases. Requires an ordinal level of measurement. U is the number of times a value in the first group precedes a value in the second group, when values are sorted in ascending order.

Chi-square the hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship. Pearson chi-square and likelihood-ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2x2 tables.

Analysis of variance [ANOVA] tests: According to the computer program SPSS for Windows. ANOVA test was used for comparison among different times in the same group in quantitative data.

The interquartile range (IQR), also called the middle fifty, is a measure of statistical dispersion. The IQR is the 1st quartile to the 3rd quartile, these quartiles can be clearly seen on a box plot on the data. The median is the 2nd quartile.

Linear Correlation coefficient was used for detection of correlation between two quantitative variables in one group.

ROC-curve: Receiver Operating Characteristic curve analysis.

Sensitivity: Probability that the test results will be positive when the disease is present (true positive rate, expressed as a percentage).

Specificity: Probability that the test results will be negative when the disease is absent (true negative rate, expressed as a percentage).

PPV: Positive Predictive value (probability that the disease is present when the test is positive).

NPV: Negative Predictive value (probability that the disease is present when the test is negative).

Accuracy: the ratio of the true positive and true negative on all patients.

P value > 0.05 **NS** (Non significant).
≤ 0.05 **S** (Significant).
< 0.01 **HS** (Highly significant).

3. Results

Table (1): a) Tumor Laterality To MVD (CD34):

	MVD (CD34)		Total	P. value
	Low	High		
Right count (% within MVD (CD34)	7(70.0%)	21(60.0%)	28(62.2%)	0.610
Left count (% within MVD CD34)	3(30.0%)	12(31.4%)	15(31.1%)	
Bilateral: count (% within MVD CD34)	0(0.0%)	3(8.6%)	3(6.7%)	
Total count:	**10(100.0%)**	**36(100.0%)**	**46(100.0%)**	

Table (1): b) Tumor Laterality to MVD (CD105):

	MVD (CD105)		Total	P. value
	Low	High		
Right count (% within MVD (CD105)	12(75.0%)	16(55.2%)	28(62.2%)	**0.272**
Left count (% within MVD CD105)	4(25%)	11(34.5%)	15(31.1%)	
Bilateral: count (% within MVD CD105)	0(0%)	3(10.3%)	3(6.7%)	
Total count:	**16(100.0%)**	**30(100.0%)**	**46(100.0%)**	

Relation of MVD (CD34) and MVD (CD105) to the tumor site show no statistical significant difference. This means the vascularity of the tumor does not has specific site or side within the breast to affect its growth as the angiogenic factors which stimulating the angiogenesis not present only inside the tumor cells but also within the body fluids (serum, urine and ocular fluids).

Table (2): a) Histologic Grade To MVD (CD34):

	MVD (CD34)		Total	P. value
	Low	High		
Grade II count (% within MVD (CD34))	10(100.0%)	34(97.1%)	44(97.8%)	1.000
Grade III count (% within MVD CD34)	0(0%)	2(2.9%)	2(2.2%)	
Total count:	**10(100.0%)**	**36(100.0%)**	**46(100.0%)**	

Table (2): b) Histologic Grade To MVD (CD105):

	MVD (CD105)		Total	P. value
	Low	High		
Grade II count (% within MVD (CD105))	16(100.0%)	28(96.6%)	44(97.8%)	**1.000**
Grade III count (% within MVD CD105)	0(0%)	2(3.4%)	2(2.2%)	
Total count:	**16(100.0%)**	**30(100.0%)**	**46(100.0%)**	

Relation of MVD (CD34) and MVD (CD105) to histologic grade in our study show no statistical significant difference.

Table (3): a) ER To MVD (CD34):

	MVD (CD34)		Total	P.value
	LOW	HIGH		
-ve count (%within MVD-CD34)	0(0%)	6(23.1%)	6(17.1%)	0.304
+ve count (%within MVD-CD34)	9(100.0%)	20(76.9%)	29(82.9%)	
Total count:	**9(100.0%)**	**26(100.0%)**	**35(100.0%)**	

Table (3): b) ER to MVD (CD105):

	MVD (CD105)		Total	P. value
	Low	High		
-ve: count (% within MVD (CD105)	0(0%)	6(30.0%)	6(17.1%)	**0.027**
+ve: count (% within MVD CD105)	15(100.0%)	14(70.0%)	29(82.9%)	
Total count:	**15(100.0%)**	**20(100.0%)**	**35(100.0%)**	

Although the relation of ER to MVD CD34 showed no statistical significant difference in our study yet the relation of ER to MVD CD105 showed statistical significant difference. In this relation patients with positive ER have low MVD CD105 indicating good prognosis.

Table (4): a) PrR to MVD (CD34):

	MVD (CD34)		Total	P. value
	Low	High		
-ve: count (% within MVD (CD34)	1(11.1%)	7(26.9%)	8(22.9%)	0.648
+ve: count (% within MVD CD34)	8(88.9%)	19(73.1%)	27(77.1%)	
Total count:	**9(100.0%)**	**26(100.0%)**	**35(100.0%)**	

Table (4): b) PrR MVD (CD105):

	MVD (CD105)		Total	P. value
	Low	High		
-ve: count (% within MVD (CD105)	1(6.7%)	7(35.0%)	8(22.9%)	**0.014**
+ve: count (% within MVD CD105)	14(93.3%)	13(65.0%)	27(77.1%)	
Total count:	**15(100.0%)**	**20(100.0%)**	**35(100.0%)**	

Although the relation of PrR to MVD CD34 shows no statistical significant difference, yet the opposite occurs with CD105. The tumors over expressing ER and PrR have lower vascular density in comparison with tumors having negative hormonal status, this means good prognosis associated with both of them.

Table (5): a) Her-2 to MVD (CD34):

	MVD (CD34)		Total	P. value
	Low	High		
-ve: count (% within MVD (CD34)	7(77.8%)	12(48.0%)	19(55.9%)	0.240
+ve: count (% within MVD CD34)	2(22.2%)	13(52.%)	15(44.1%)	
Total count:	**9(100.0%)**	**25(100.0%)**	**34(100.0%)**	

Relation of Her-2 to MVD (CD34) show no statistical significant difference.

Table (5): b) Her-2 to MVD (CD105):

	MVD (CD105)		Total	P. value
	Low	High		
-ve: count (% within MVD (CD105)	11(78.6%)	8(40.0%)	19(55.9%)	**0.026**
+ve: count (% within MVD CD105)	3(21.4%)	12(60.1%)	15(44.1%)	
Total count:	**14(100.0%)**	**20(100.0%)**	**34(100.0%)**	

There is significant statistical relationship between Her-2 neu over expression and high MVD CD105 indicating bad prognosis. ER, PrR and Her-2neu has a strong relationship with the noevascularity of the tumour, this appears in tumors with triple negative hormonal studies demonstrating high vascularity in comparison with others with positive ER and PrR and negative Her-2 nue.

Table (6): a) Type of surgery MVD CD34:

	MVD (CD34)		Total	P. value
	Low	High		
CBS: count (% within MVD (CD34)	6(60.0%)	13(39.4%)	19(44.2%)	0.295
MRM: count (% within MVD CD34)	4(40.0%)	20(60.6%)	24(55.8%)	
Total count:	**10(100.0%)**	**33(100.0%)**	**43(100.0%)**	

Relation of the type surgery to MVD CD34 show no statistical significant difference.

Table (6): b) Type of surgery MVD (CD105):

	MVD (CD105)		Total	P. value
	Low	High		
CBS: count (% within MVD (CD105)	11(68.8%)	8(29.6%)	19(44.2%)	**0.013**
MRM: count (% within MVD CD105)	5(31.3%)	19(70.4%)	24(55.8%)	
Total count:	**16(100.0%)**	**27(100.0%)**	**43(100.0%)**	

The relation of MVDCD105 to MRM showed statistical significant differences this means accurrate assessment of the vascularity of the tumor needs large suface area of the cut section. Also patients with high MVD CD105 underwent MRM should be followed up at regular short intervals as the probability of recurrences in these cases are high in comparison to others with low vascularity. The relation of MVD CD105 to CBS show statistical significant difference this means the probability of recurrence in these case is high so we recommend to enumerate the MVD of the tumor in the criteria of indications for CBS.

Table (7): a) Mitotic Index To MVD (CD34):

MVD (CD34	Tumor length	Tumor Width	Mitotic index
1.00 N	10.00	10.00	10.00
Mean	2.49	1.85	3.60
Std Deviation	.98	1.12	0.58
Median	2.25	1.65	3.00
Minimum	1.50	.20	1.00
Maximum	4.70	1.00	7.00
2.0 N	**35.00**	**35.00**	**35.00**
Mean	**3.25**	**2.56**	**5.91**
Std Deviation	**2.15**	**1.89**	**2.09**
Median	**3.00**	**2.50**	**6.00**
Minimum	**.20**	**.20**	**1.00**
Maximum	**12.00**	**10.00**	**10.00**

Table (7): b) Test Statistics

	Tumour length	Tumour Width	Mitotic index
Man- Whitney U	**127.500**	**133.500**	**67.000**
Z	**-1.300**	**-1.138**	**-2.984**
Asymp. Sig (2-tailed)	**.193**	**.255**	**.003**

1.0 indicating low vascularity
2.0 indicating high vascularity

Although relation of MVD CD34 totumour size shows no statistical significant difference yet MVD CD34 to MI shows significant difference (P,003).

Table (8): a) Mitotic Index To MVD (CD105):

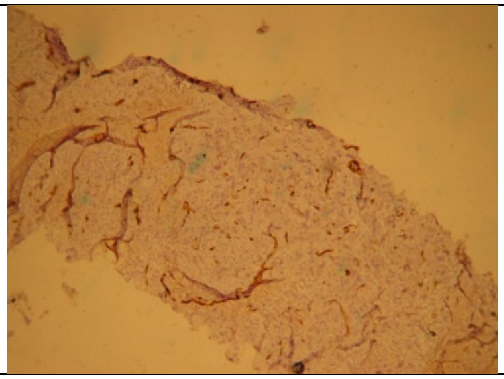
MVD (CD105	Tumour length	Tumour Width	Mitotic index
1.00 N	16.00	16.00	**16.00**
Mean	2.32	1.71	**4.06**
Std Deviation	1.25	1.19	**1.88**
Median	2.00	1.50	**3.00**
Minimum	.20	.20	**1.00**
Maximum	4.70	4.00	**8.00**
2.00 N	**29.00**	**29.00**	**29.00**
Mean	**3.51**	**2.78**	**6.14**
Std Deviation	**2.17**	**1.92**	**2.03**
Median	**3.20**	**2.80**	**6.00**
Minimum	**.50**	**.50**	**1.00**
Maximum	**12.00**	**10.00**	**10.00**

Table (8): b) Test Statistics

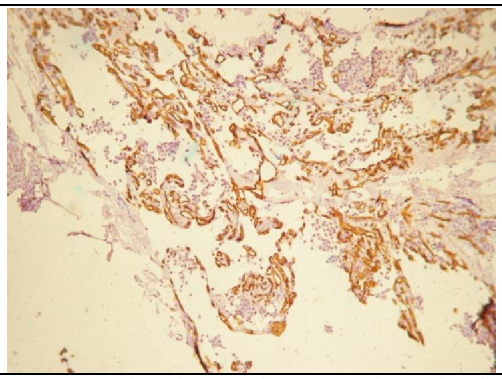
	Tumour length	Tumour Width	Mitotic index
Man- Whitney U	**1.9.000**	**142.000**	**102.000**
Z	**-2.211**	**-2.144**	**-3.119**
Asymp. Sig (2-tailed)	**.027**	**.032**	**.002**

1.0 indicating low vascularity
2.0 indicating high vascularity

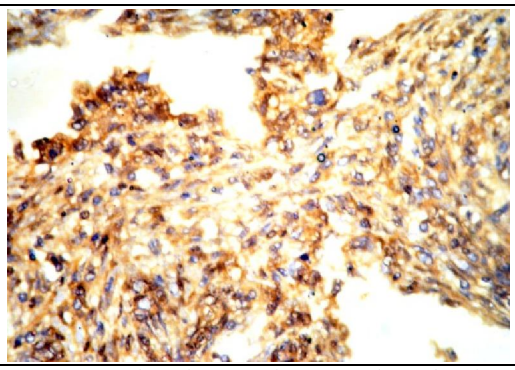
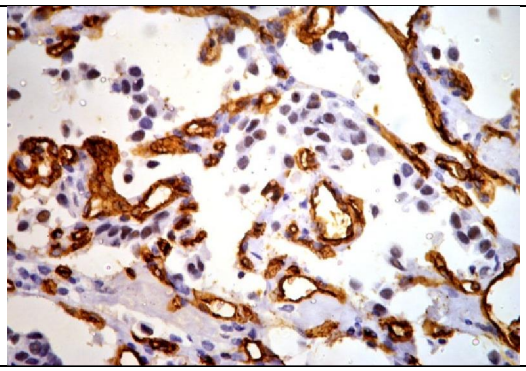
As shown in the table there is significant relationship between the tumour size and microvascular density and mitotic index, as the Tumour may reach to a large size if angiogenesis is high, also high vascular density is usually associated with high histologic grade irrespective of the histologic type, with increased neovascularization microinvasion may be expected as seen in patients with negative nodal status on whom recurrences were recorded in other studies. P.value = 0.05.



Study's case core biopsy showing IDC with low vascularity (CD105)



Study's case, Invasive duct carcinoma, grade 2, showing high MVD expressed in CD105; Immunohistochemistry staining x50

	
Study's case,Invasive duct carcinoma,grade 2,showing high MVD expressed in CD34;Immunohistochemistry staining x100.	Study's case,Invasive duct carcinoma,grade 2,showing high MVD expressed in CD34;Immunohistochemistry staining x100.

4. Discussion

In our study we also demonstrated an increased MVD in about 77.8% of the cases whereas low MVD was observed in about 22.2%, so we encourage early detection of BC through international application of the screening programs which will have more psychological, social and economic benefits in the management of BC patients beside its implication on the prognosis and outcome of the disease. There is direct relation between the type of surgery and the ability to assess the vascular density of the tumor where smaller lumpectomized specimens had lower vascularity this means vascular density cannot be assessed with any reliability on core biopsy as it is infrequently seen due to smaller surface area of the sections examined so in order to obtain accurate and conclusive results sizable biopsy for example incisional or excisional one should be taken. The present study also showed low MVD (CD105) in patients with ER receptor positive, with good prognosis in both of them, in comparison to patients with Her-2 nue over expression in whome the vascular density measured through endoglin staining (CD105) was higher this may pointed to the greater likelihood of metastatic potential even in node negative patients this agree with the demonstration of ***Ebos, (9);*** Her-2 neu oncogene promotes angiogenesis through upregulation of VEGFA, which is the key modulator of angiogenesis and is highly expressed in cancerous tissue and correlates with its more aggressive features. (10)

CD34 and CD105 are relatively specific markers for tumor vascular endothelium, were used to stain the microvessels in our study by which we found that MVD was related to lymphatic metastasis (as shown by axillary lymph node invasion, clinical staging, tumor size and hormonal status of the breast cancer. The study further confirmed that microvessel formation and high histologic grades are observed in advanced stages III and IV in whome the poor prognosis well known. Our study support the results demonstrated by Yang., in which MVD is closely related to tumor invasive ability, metastasis, and prognosis. (11)

The endoglin (CD105), a relatively specific marker for tumor neovascular endothelial cells, was used to stain microvessels in our study by which we found that MVD is closely related to lymphatic metastasis, clinical staging and tumor size of the breast carcinoma; a uniqe findings not observed with CD34 which stain the already formed blood vessels on them the tumor attain its clinic-pathologic features that appears at diagnosis. Folkman, put forward theory that tumor growth and metastasis depended on neovascularization which provides a plenty of nutriments to cancer cells. The more blood vessels in tumor tissues are the higher incidence for tumor cells to enter the circulation. MVD in breast cancer tissues is closely related to distant metastasis and survival time, having been confirmed in similar studies on stomach cancer, melanoma, non small cell lung cancer, and prostate cancer. (12)

Toi et al., (13) said in breast cancer, VEGF production correlates with early relapse and ***Rykala et al., (14)*** added, it appears that tumors that produce multiple angiogenic factors show increased rates of primary tumor expansion. So in our study we showed a direct corelation between the vascular desity (number of vessel per high power field) and the likelihood of metastasis in human breast cancer patient the implication of this study is that vascular density can function as indeperdent prognostic variable in breast cancer. Thus, tumor with increased vascular density is indicative of increased metastatic potential. As Her-2nue (activated oncogene) cause genetic induction of angiogenesis through upregulation of VEGF on the vascular endothelial cell and the results of our study demonstrate overexpression of Her-2nue in highly vascular tumors so the combined use of

Herciptin (Trastuzumab) and anti-VEGF drug can be more effective than either drug used alone.

During the follow up period we discovered four cases with recurrences of their tumor in whom the mean vascular denisity was high for both CD34 and CD105 so modified radical mastectomy was done. These data signify our results in which regardless of the number of the lymph node involvement or the tumor size if the primary tumor has high vascularity the cases are vulnerable to recurrences as shown in the follow up results. The quantification of tumoral angiogenesis can allow the division of patients according to the type of treatment and can also permit the management selection in the long run for the individuals with low tumoralmicrovascular density. Seriate determination of protein CD34 every 6 months can play the part of a cancer progression or regression marker and stands for a new tool in assessing the prognosis of these tumors. (15)

Finally, the real importance of blood microvessel density (MVD) is still controversial. Most of the available data have the same degree of discrepancy related to the significant correlation between high MVD and cancer metastasis with poor patient outcome.

Conclusion

The assessment of both CD34- CD105 showed that role played by angiogenesis in the cancer proliferation and local spread, the angiogenesis level being maintained high even in the advanced stages of the disease. There is difference between MVD measured using CD34 and MVD measured using CD105 which stain the neoendothelial cells in activity dividing tumor in our study and the study of this difference might lead in the future to a better assessment of prognosis and adjusted therapies.

Recommendations:

From our study we can recommend the following: 1-Incorporating the Endoglin in the prognostic panel of BC. 2-Measurement of the vascularity of the tumor should be taken in mind as a one of the steps in the management of BC because the anti angiogenic therapy in the near future will be effective treatment modality limiting the spreading of the tumor. 3- For patients undergoing CBS assessment of MVD of importance as the recurrences of tumors with high MVD is observed.

Conflict of interest

The authers declare that they have no conflict of interests the work is part of MD thesis of Dr. Hoda El Sayed Khodair the authers declared that no financial support was provided to the current scientific work or any of its coauthers.

Auther contribution

All authers have contributed equally in preparing this manuscript and thus share the first authorship.

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