

Metastatic Potential in Node-Negative Breast Carcinoma: Role of nm23 and Angiogenesis

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ABSTRACT

Background: In node-negative breast carcinoma (NNBC), identification of patients who are at high risk of tumor recurrence and requiring adjuvant chemotherapy can't be identified by current clinicopathologic criteria and requires the development of additional prognostic parameters. **METHODS.** nm23, microvessel count (MVC) and estrogen receptor (ER) were detected immunohistochemically in tissue section of 43 NNBC. The patients were followed up for 5 years for evidence of tumor relapse. Post-operative radiotherapy was given to 24 patients. Out of the irradiated group 15 patients received chemotherapy and 3 patients received chemotherapy and hormonal therapy.

Results: Out of 43 NNBC, high nm23 was detected in 44.2% of cases and low nm23 was detected in 55.8%. A significant correlation was observed between nm23 level and tumor size as well as ER status of the patients. High MVC was encountered in 37.2% of cases and low MVC was detected in 62.8%. A significant correlation was observed between MVC and tumor size. A significant inverse correlation between nm23 level and MVC was observed. Follow-up of the patients for 5 years revealed relapse in 35.9%. The 5-year disease free survival (DFS) was 64.1%. nm23 level correlated significantly with DFS ($p=0.008$). The 5-year DFS was 84.2% for tumors with high nm23 level compared to 45% for tumors with low nm23 level. MVC level showed a high significant correlation with DFS ($p<0.001$). The 5-year DFS was 87.5% for tumors with low MVC compared to 26.7% for tumors with high MVC. Multivariate analysis proved that only MVC appeared to be independent prognostic variable for DFS ($p=0.04$). Using the combination of nm23 level and MVC, we could identify a high risk group of patients with both low nm23 and high MVC, and a low risk group of patients with both high nm23 and low MVC. The 5-year disease free survival was 25% and 93.7% respectively. The difference was statistically significant ($p<0.001$). In the high risk group of patients both radiotherapy and chemotherapy correlated significantly with relapse rate ($p=0.02$).

Conclusions: Both nm23 and MVC appear to contribute valuable additional prognostic information in NNBC. MVC proved to be the most important tumor factor predicting outcome. Using both nm23 and MVC together could be a guide for therapy, as we could identify a group

of patients who are at high risk of developing recurrence and who may benefit greatly from adjuvant treatment.

Key Words: Node-negative breast carcinoma - nm23 and angiogenesis.

INTRODUCTION

A significant heterogeneity in the clinical course of breast cancer has been observed even among patients with the same clinical or pathological stage [8]. A recent international consensus panel on the treatment of primary breast cancer underlined that in node -negative breast cancer, the most relevant factors for risk estimation remain tumor size, histologic and nuclear grade, steroid hormone receptor status, lymphatic and/or vascular invasion and age [10]. However, up to 30% of patients with node -negative breast cancer (NNBC) will get recurrence within 10 years of initial therapy [28]. Patients who are at high risk of tumor recurrence and requiring adjuvant chemotherapy can't be identified by current clinicopathologic criteria and require the development of additional prognostic parameters [20]. So, we need more selective and powerful prognostic factors which provide a more accurate reflection of the inherent biological aggressiveness of each tumor, so as patients at significant risk of recurrence could benefit from adjuvant therapy.

Stegg and co-workers [33] reported on nm23, as a tumor suppressor family of genes that inhibit metastasis. The human nm23 gene family consists of two genes, nm23-H1 and nm23-H2, with 88% homology [32]. The gene product was shown to have nucleoside diphosphate (NDP) kinases activity which are involved in microtubule association and G-protein regulation.

nm23 has been implicated in regulating basement protein deposition and restoring the normal phenotype to metastatic breast cells in culture. Transfection of the nm23-H1 gene suppresses the cytokine-stimulated motility of human breast carcinoma cells [15]. Several studies have emphasized the clinical significance of reduced nm23 expression and its correlation with onset of metastasis in various types of malignancy, namely breast cancer, melanoma and hepatocellular carcinoma [17,6,26].

Angiogenesis is a tightly regulated multi-step process that involves endothelial proliferation, migration and extracellular matrix remodeling. Folkman [7] has shown that tumor growth, progression and metastasis are accompanied by angiogenesis. Growth of tumor beyond 1-2 mm is dependent on tumor angiogenesis expressed as microvessel density (MVD) [27]. Angiogenesis appears to be necessary both early and late in the malignant process and hence, has the potential of being an important indicator of metastatic proclivity and long term outcome in breast cancer patients [36,14,22].

nm23 and angiogenesis are the phenotypic expression of different processes in the malignant progression. So, this study was designed to investigate nm23 expression and factor VIII related antigen in 43 NNBC cases, so as to reach a general consensus on their prognostic relevance. It would be of utmost interest to detect subgroups of NNBC patients at high risk for relapse to design an optimal treatment modality.

MATERIAL AND METHODS

Patients and tissue samples:

A retrospective study done at National Cancer Institute, Cairo University, during the years from 1993 to 1995. It included 43 NNBC patients, operated upon at the Department of Surgery, and received adjuvant treatment in the form of post-operative radiotherapy (PORT), chemotherapy, hormonal or combined treatment modality. Modified radical mastectomy was performed for 40 patients while only 3 patients of the studied group had conservative surgery in the form of sector mastectomy with axillary evacuation. All patients had regular follow up by clinical and radiological examination for 5 years

Specimens were subjected to gross and mi-

croscopic investigations. Four sections of 5µ thickness each, were cut from each paraffin block. One section was stained with Hx and Eosin stain for routine identification and histologic grading following modified criteria of Scarff-Bloom Richardson (SBR) nuclear grading [21]. The other three sections were stained with anti nm23 antibody, factor VIII related antigen and hormone receptor ER.

Immunohistochemistry:

Avidin-biotin complex immunoperoxidase method was performed for nm23, factor VIII and ER receptor detection. The three sets of sections were de-waxed in xylol, rehydrated through descending graded alcohols and then immersed in 1% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. All steps were carried out at room temperature. Sections were subsequently washed in phosphate buffer saline (PBS) and then 30% normal goat serum for 20 minutes to reduce non-specific antibody binding. The primary antibodies were applied at their specific concentrations. nm23 H1 (BioGenex) was diluted 1:100, factor VIII related antigen (DAKO) was diluted 1:50 and Estrogen Receptor (BioGenex) was diluted 1:100. Slides were incubated for 2 hours at room temperature in humid chambers. Following further washing in PBS, sections were incubated with secondary antibody (biotinylated goat anti-mouse IgG) for 30 minutes at a concentration of 1:40 (Kirkegaard & Perry Laboratories Inc), then with avidin-biotin peroxidase complex (Vector Laboratories) for 30 minutes. Immune complexes were visualized with DAB solution (100 mg DAB, Sigma + 50 µl of 30% H₂O₂ in 20 ml PBS +1ml of 8% NiCl). As a positive control, one section known to give strong staining was included in each run, whereas, for negative control, tris- buffered saline was used instead of primary antibodies. Sections were counterstained with light green (for nm23 and ER) and Meyer's hematoxylin (for factor VIII).

All slides were evaluated without previous knowledge of the clinicopathologic data. Scoring for nm23 based on the proportion of stained cells was used. A minimum score of 40% was essential to consider a case positive for nm23. High nm23 positivity was considered if all malignant cells were positive. Low nm23 positivity was considered in intermediate score values [34, 13].

To assess angiogenesis, vascularity was determined by the number of vessels per field counted in the area of highest vascular density 'hot spot' at X400 magnification (0.1452mm²). Microvessels in three hot spots were counted and averaged. Single endothelial cells, endothelial cell clusters and microvessels in the tumor were counted. Branching structures were counted as a single vessel. Low angiogenesis was identified as microvessel count (MVC) < 15 microvessel/ endothelial cell per field at X 400. Vascularity in necrotic areas was not scored. [14].

Statistical Analysis:

Data were summarized as means and percentages. Comparison between frequencies was done by Chi-squared test or Fischer's Exact test for small numbers. Five years disease free survival rates were calculated by the Kaplan-Meier method [18]. Patients with lost follow up (4 cases), were excluded. Comparison between disease free survival rates was done using Log Rank test. Multivariate analysis was done using Cox regression test [3].

RESULTS

The present study included 43 node-negative breast carcinoma cases (NNBC), aged from 33-75 years with a mean of 50.2 years. Forty two percent of cases were pre-menopausal women under the age of 50 years. Tumor size ranged from 0.5-5 cm with a mean of 2.7cm. Only 17 patients had T1 lesions (less than 2 cm in diameter) (39.5%), while 26 patients had T2 lesions (2-5 cm) (60.5%). Tumors less than 1 cm constituted only 4.7% of the studied group (2 cases). Forty-one cases were invasive duct carcinoma and two cases were invasive lobular carcinoma. Out of 41 invasive duct carcinoma cases studied, 7.3% were grade I (3 cases), 68.3% were grade II (28 cases) and 24.4% were grade III (10 cases). Twenty-three cases were ER positive (53.5%), whereas, 20 cases were ER negative (46.5%).

A total number of 24 patients, received radiation therapy. Three patients had conservative surgery and received radical course of radiotherapy to the breast, 50 Gy / 5 weeks/ 25 fractions (200 cGy / fraction), followed by boost to the site of primary lesion. The dose of the boost was in the range of 1000-1500 cGy / 1-1.5 weeks, 200 cGy / fraction given by electron

beam 12-15 MeV. Twenty-one patients with tumor size more than 4cm or high grade tumors received comprehensive post-operative radiotherapy for a dose of 4500 cGy / 4 weeks (225 cGy/ fraction). All patients were treated by Linear accelerator machine 6 MeV at source skin distance 100 cm. Adjuvant chemotherapy was given to 18 patients out of the irradiated group, which was CMF for 15 patients (cyclophosphamide 600 mg/m², methotrexate 40 g/m² and 5 FU 600 mg/m²), for a total number of 6 cycles administered I.V every 21 days, or CMF and hormonal treatment (tamoxifen 10 mg bid), for for 3 post-menopausal patients with ER positive tumors. Sixteen patients received concomitant chemoradiotherapy and only 2 patients started chemotherapy after the end of radiotherapy.

nm23, Tumor and Patient Characteristics: All tumors showed nm23 expression, the staining was cytoplasmic in 38 cases, both cytoplasmic and membranous in 5 cases. Nuclear staining could not be identified in our cases. Twenty-four cases were of low nm23 score (55.8%) and 19 cases (44.2%) were of high score (Figs. 1&2). A significant correlation was observed between nm23 level and tumor size ($p=0.029$). High nm23 level was detected in tumors less than 2cm, rather than in tumors larger than 2cm (58.8% and 34.6% respectively). There was a direct significant correlation between level of nm23 expression and estrogen receptor ($p=0.018$). Most of tumors showing high nm23 were more likely to be ER positive rather than ER negative (60.9% and 25% respectively). Although not statistically significant, there was a trend for low grade tumors to have high nm23 level. Sixty-seven % of grade I tumors had high nm23 level compared to 30% of grade III tumors. There was no statistical significant correlation between age and nm23 level (Table 1).

MVC, Tumor and Patient Characteristics: In 43 NNBC cases studied, the mean MVC was 19.7 per X400 field. Twenty-seven cases (62.8%) showed low MVC with a mean of 9.3 per X400 field and 16 cases (37.2%) showed high MVC with a mean of 27.5 per X400 field (Fig. 3). There was a significant correlation between MVC score and tumor size. Most of tumors less than 2cm showed low MVC (88.2%), whereas, 53.8% of tumors more than 2cm showed high MVC ($p=0.005$). No significant

correlation between age, tumor grade and ER status versus MVC was observed (Table 2).

A significant inverse correlation between nm23 level and MVC was observed. High nm23 was detected in 59.3% of tumors that showed low MVC, whereas low nm23 level was detected in 81.3% of tumors that showed high MVC ($p=0.012$) (Table 3).

Follow up of 39 available patients out of 43 cases studied for 5 years revealed that relapse was encountered in 35.9% of cases (13 patients had distant metastasis and only one patient had local recurrence constituting 7% of relapsed patients). Relapse rate by the end of the third year was only 12.8%. The 3-year disease free survival (DFS) was 87.2% and the 5-year DFS was 64.1%. A significant correlation was observed between tumor size and relapse rate ($p=0.02$). The 5-year DFS for tumors less than 2 cm was 87.5% compared to 47.8% for tumors more than 2 cm (Fig. 4). ER status of the patients was significantly correlated to relapse rate ($p=0.017$). No correlation was observed between relapse rate and histopathologic grade. Out of the 14 relapsed cases, 11 cases had low nm23 level and 3 cases had high nm23 level (Table 4). nm23 level correlated significantly with disease free survival ($p=0.008$). The 5-year DFS was 84.2% for tumors with high nm23 level compared to 45% for tumors with low nm23 level (Fig. 5). Regarding MVC, most of the relapsed patients showed high MVC (11/14) whereas, cases with low MVC constituted only 3 out of the 14 relapsed cases (Table 4). MVC level showed a high significant correlation with disease free survival ($p<0.001$). The 5-year DFS was 87.5% for tumors with low MVC compared to 26.7% for tumors with high MVC (Fig. 6). We performed a multivariate analysis including tumor size, nm23 level and MVC. Only MVC appeared to be an independent prognostic variable for DFS ($p=0.04$).

Using the combination of nm23 level and MVC, we classified our patients as; high risk group patients with both low nm23 and high MVC (12 cases), which had a more than 10 fold increase in the relapse rate as compared with low risk group of patients with both high nm23 and low MVC (16 cases). Relapse rate was 75% and 6.3%, respectively. The 5-year disease free survival was 25% for the high risk group of patients compared to 93.7% for the low risk

group of patients, the difference was highly significant ($p<0.001$) (Fig. 7). In the group of patients with high MVC, high nm23 patients had a better 5-year DFS than low nm23 (33.3% versus 25%) the difference did not reach a statistically significant value. However, among low nm23 group the 5-year DFS is significantly better if MVC is low compared with high MVC (75% versus 25%) ($p=0.001$) (Table 5).

The 5-year disease free survival was 71.4% for the irradiated group of patients compared to 55.6% for those patients who did not receive radiation therapy, with no statistical significant difference ($p=0.337$). On the other hand none of the irradiated group showed local recurrence. The 5-year disease free survival was 73.3% for the group of patients who received chemotherapy compared to 58.3% for those who did not, with no statistical significant difference ($p=0.496$). On the other hand, regarding the high risk group of patients (tumors exhibited both low nm23 level and high MVC), relapse was encountered in 75% of cases (9/12). In this group of patients all cases who did not receive post operative radiotherapy and chemotherapy had relapsed (7/7), whereas 40% of patients who received post operative radiotherapy and chemotherapy had relapsed (2/5 cases), the difference was statistically significant ($p=0.02$).

DISCUSSION

Understanding the metastatic progression of breast cancer offers opportunity to tailor therapy based on individual tumor characteristics rather than that of a group. For node negative breast cancer patients, the search for prognostic biomarkers is important both to identify those patients with occult metastasis and to spare chemotherapy treatment in those patients whose tumors have not developed the capacity for distant spread. Such tumor biomarkers have the potential to result in a significant reduction in unnecessary morbidity in those patients not needing chemotherapy and perhaps allow an increased intensity of therapy in those with occult disease that tends to recur.

nm23 is an anti-metastatic gene that was identified by differential screening of a murine melanoma cell line cDNA library with RNA from cell lines of differing metastatic potential. The expression was shown to be inversely related to metastatic potential with significantly higher expression in the cell clones with a low

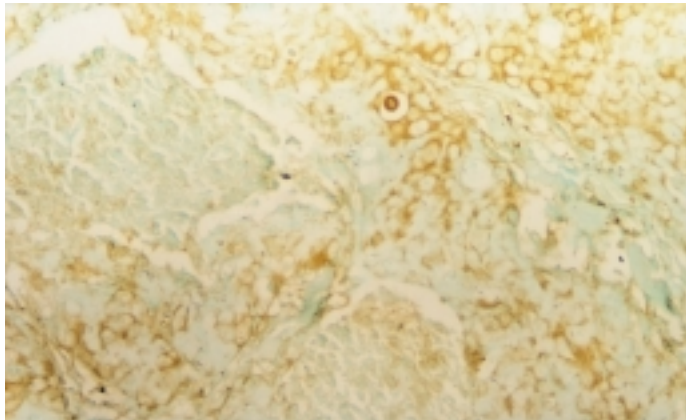


Fig. (1): Low nm23 positive staining in invasive duct carcinoma grade II showing mainly cytoplasmic staining of 40% of malignant cells (ABC immunoperoxidase-DAB chromogen X 250).

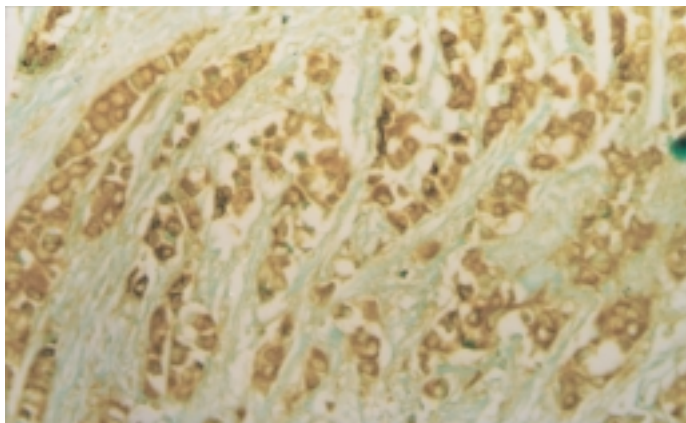


Fig. (2): High nm23 positive staining in invasive duct carcinoma grade II showing both cytoplasmic and membranous staining of all malignant cells (ABC immunoperoxidase-DAB chromogen X 400).

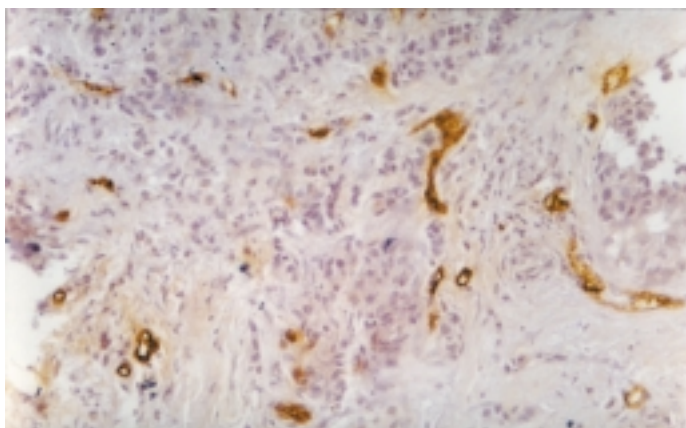


Fig. (3): High MVC in invasive duct carcinoma grade II, score 25 / X 400 field (ABC immunoperoxidase - DAB chromogen X 250).

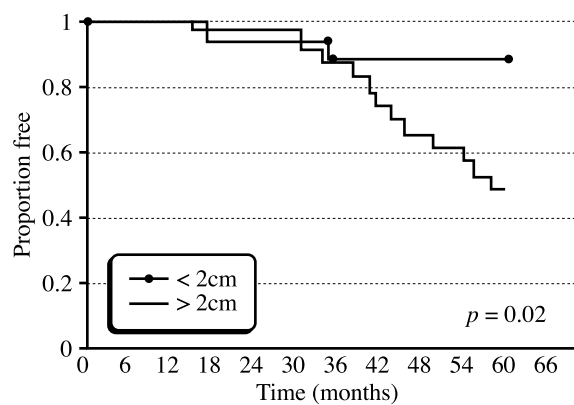


Fig. (4): The disease free survival curve for 39 patients of NNBC. The 5-year DFS rate was 87.5% for tumors < 2cm compared to 47.8% for tumors \geq 2cm (statistically significant difference).

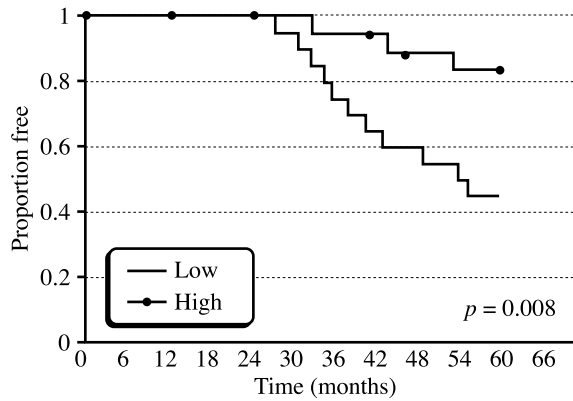


Fig. (5): The disease free survival curve for 39 patients of NNBC categorized according to nm23 level. The 5-year DFS rate was 84.2 % for tumors with high nm23 compared to 45% for tumors with low nm23 (statistically significant difference).

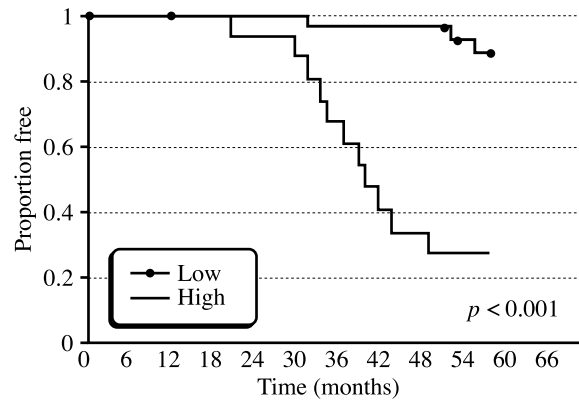


Fig. (6): The disease free survival curve for 39 patients of NNBC categorized according to MVC. The 5-year DFS rate was 26.7 % for tumors with high MVC compared to 87.5% for tumors with low MVC (statistically significant difference).

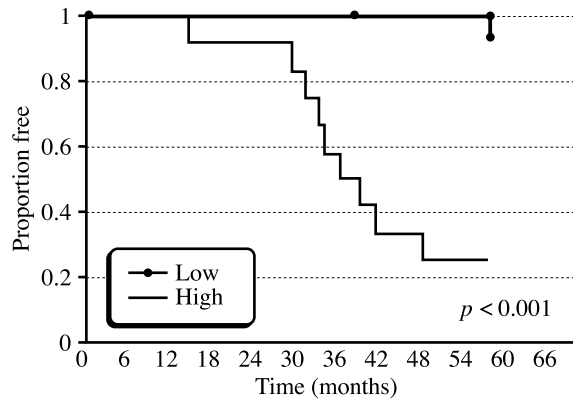


Fig. (7): The disease free survival curve for the high risk versus low risk groups of patients with NNBC. The 5-year DFS rate was 25 % for the high risk group compared to 93.7% for the low risk patients (statistically significant difference).

Table (1): Relation of nm23 level to various clinico-pathologic variants.

Variable	Low nm23	%	High nm23	%	Total	p value
<i>Age:</i>						
< 50	10	55.6	8	44.4	18	0.977
≥ 50	14	56	11	44	25	
<i>Tumor size:</i>						
< 2 cm	6	41.2	11	58.8	17	0.029
≥ 2cm	18	65.4	8	34.6	26	
<i>Histologic type:</i>						
IDC						
I	1	33.3	2	66.7	3	0.509
II	16	57.1	12	42.9	28	
III	7	70	3	30	10	
ILC						
	-	-	2	100	2	
<i>ER:</i>						
+ve	9	39.1	14	60.9	23	0.018
-ve	15	75	5	25	20	
Total	24		19		43	

Table (2): Relation of MVC to various clinico-pathologic variants.

Variable	Low MVC	%	High MVC	%	Total	p value
<i>Age:</i>						1
< 50	11	61.1	7	38.9	18	
≥ 50	16	64	9	36	25	
<i>Tumor size:</i>						0.005
< 2 cm	15	88.2	2	11.8	17	
≥ 2cm	12	46.2	14	53.8	26	
<i>Histologic type:</i>						0.598
IDC					41	
I	2	66.7	1	33.3	3	
II	19	67.9	9	32.1	28	
III	5	50	5	50	10	
ILC	1	50	1	50	2	
<i>ER:</i>						0.361
+ve	16	69.6	7	30.4	23	
-ve	11	55	9	45	20	
Total	27		16		43	

Table (3): Correlation between nm23 level and MVC.

	MVC				Total
	Low	%	High	%	
Nm23					
Low	11/27	40.7	13/16	81.3	24
High	16/27	59.3	3/16	18.8	19
Total	27		16		43

p value = 0.012

Table (4): Correlation of relapse rate to various clinico-pathologic variants.

Variable	Relapsed	%	Non-relapsed	%	Total	p value
<i>Age:</i>						1
< 50	8	47.1	9	52.9	17	
≥ 50	6	27.3	16	72.7	22	
<i>Tumor size:</i>						0.02
< 2 cm	2	12.5	14	87.5	16	
≥ 2cm	12	52.2	11	47.8	23	
<i>Histologic type:</i>						0.598
IDC					38	
I	1	50	1	50	2	
II	7	26.9	19	73.1	26	
III	6	60	4	40	10	
ILC			1	100	1	
<i>ER:</i>						0.017
+ve	4	18.2	18	81.8	22	
-ve	10	58.8	7	41.2	17	
<i>MVC:</i>						<0.001
High	11	73.3	4	26.7	15	
Low	3	12.5	21	87.5	24	
<i>nm23:</i>						0.008
High	3	15.8	16	84.2	19	
Low	11	55	9	45	20	
<i>Radiotherapy:</i>						0.337
+ve	6	28.6	15	71.4	21	
-ve	8	44.4	10	55.6	18	
<i>Chemotherapy:</i>						0.496
+ve	4	26.7	11	73.3	15	
-ve	10	41.7	14	58.3	24	
Total	14		25		39	

Table (5): Categorization of patients according to nm23 level and MVC in relation to relapse rate.

Groups of patients	Relapsed	%	Non-relapsed	%	Total
1- Low nm23 & High MVC	9	75	3	25	12
2- High nm23 & High MVC	2	66.7	1	33.3	3
3- Low nm23 & Low MVC	2	25	6	75	8
4- High nm23 & Low MVC	1	6.3	15	93.7	16
Total	14		25		39

Group 1 versus 4 (statistically significant $p < 0.001$).

Group 1 versus 3 (statistically significant $p = 0.001$).

Group 1 versus 2 (not significant).

ability to metastasize [33]. Several studies were reported on the correlation between nm23 expression, tumor pathological features and outcome in the patients with breast carcinoma. In a series of 71 patients of whom 26 were node-negative and had a follow up of 4 years, a positive correlation between mRNA expression and outcome was recorded [16]. Using antibody to nm23 protein, several investigators found correlation with outcome, incidence of metastasis, nodal involvement or histologic grade. Barnes et al. [1] in a study of 39 patients, were the first to demonstrate that low nm23 protein expression is associated with decreased survival of breast cancer patients. Royds et al. [30] in an immunohistochemical study using a simplified scoring system in which any lack of nm23 staining was scored as low nm23, showed a correlation between nm23 expression, tumor grade and the likelihood of nodal involvement.

In our study 55.8% of 43 NNBC patients showed low nm23 level. A similar finding was reported by Heimann et al. [13,15], who reported low nm23 in 56% of NNBC patients. A higher figure of 68.6% was reported by Tokunaga et al. [34] as they detected nm23 mRNA by immunoblotting technique. In this work, a significant correlation was observed between nm23 level and tumor size. High nm23 was detected in 59% of tumors < 2 cm, and low nm23 was detected in 65% of larger tumors (2-5cm). Similar findings were reported by Heimann et al. [13]. As tumors grow in size, they progress in the malignant cascade [19], accordingly the increase of the proportion of low nm23 with increasing size is consistent with this progression in the malignant process. In our study there was no significant correlation between nm23 level and tumor grade in contrary to the data reported by Heimann et al. [13] and Royds et al. [30]. This

difference could be attributed to the low incidence of low grade tumors included in this study (7%) compared to other studies. A statistically significant correlation was detected between nm23 level and ER status of the patients (61% of ER positive cases showed high nm23), the same observation was reported by Sawan et al. [31]. There was no statistical significant correlation between age and nm23 level. This is in concordance with the result reported by Hirayama et al. [17].

Angiogenesis is an important step in tumor progression and metastasis [7]. It is likely an early event in tumor development [11]. Neovascularization is a necessary early step for the progression to metastatic competence [12]. Angiogenesis is necessary, both early and late in the malignant process. Growth of tumors beyond 1-2 mm cubic depends on angiogenesis, and tumor spread beyond the primary site depends on access to the vasculature [7]. Tumor cells rarely shed into the circulation before the primary tumor is vascularized, and micrometastases cannot grow to a detectable size until after they have become vascularized. Thus, angiogenesis is necessary at the beginning as well as at the end of the metastatic cascade [36].

The angiogenic activity is mediated by specific angiogenic molecules released from the tumor cells per se or from cells recruited to the area (macrophages and mast cells) [5]. It is now known that, in solid tumors, there is a prevascular phase during which little or no angiogenic activity is released by the tumor cells and that, despite their proliferative capacity, such tumor cells can not expand the tumor population beyond a few cubic millimeters [36]. The onset of angiogenic activity does not require that all tumor cells become angiogenic. In fact tumors

appear to be heterogeneous for angiogenic activity. Consequently, it is imperative to determine microvessel density in the areas of the most intense neovascularity (hotspot) [36]. Different median values have been reported in different studies on breast cancer. In an overall analysis of published data, the highest concentration of about 100 microvessels per mm² was reported when node-positive breast carcinoma was included in the study [35,25].

In our study the mean MVC was 19.7 per X400 field. The cutoff was defined to be less than 15 as it corresponds to the MVC of the 25th percentile of the tumors [14]. Low MVC with a mean of 9.3 per X400 field constituted 62.8% and high MVC with a mean of 27.5 per X 400 field constituted 37.2% of our cases. Similar findings were reported by Obermair et al. [27] who reported a percentage of (62% and 38% respectively). Hieman and his colleges [14] reported a slightly higher figures of the mean MVC (22.4 per X400 field) and high MVC was encountered in 72% of their cases. This difference might be attributed to the difference in primary antibody used to assess angiogenesis (CD34). In our study a significant correlation between MVC score and tumor size was detected. Most of tumors less than 2 cm showed low MVC (88.2%), whereas, 53.8% of tumors more than 2cm showed high MVC. This goes in concordance with Linderholm et al. [23] and Medri et al. [24]. There was no significant correlation between MVC and age, tumor grade or ER status. The same observation was reported by Medri et al. [24].

In this work, a significant inverse correlation between nm23 level and MVC was observed. High nm23 which was detected in 59% of tumors showed low MVC, whereas, low nm23 level detected in 81% of tumors showed high MVC. The same observation was detected by Heimann et al. [13].

In the present study follow up of 39 patient for 5 years revealed that relapse was encountered in 35.9%. Relapse rate by the end of third year was only 12.8%. The majority of relapses were due to distant metastasis (13 patients had distant metastasis and one patient had local recurrence). The 3- year disease free survival (DFS) was 87.2% and the 5year-DFS was 64.1%. Higher figures were reported by Gasparini et al. [9], who reported 5-year DFS of 82%.

Medri et al. [24] reported 5-year DFS of 75% and Heimann et al. [15] reported 14 -year DFS of 68%. The reason for this difference could be attributed to the following facts: 41.9% of our patients were pre-menopausal women, larger size of tumors included in our study (60.5% of tumors were 2-5 cm), lower incidence of grade I tumors (7%) and 44.2% of our patients did not receive any adjuvant treatment. ELBolkainy [4] stated that breast cancer in Egyptian patients is biologically more aggressive than that encountered in the west and that could be explained by the predominance of pre-menopausal patients and the late presentation of patients at an advanced stage. In our study tumor size as well as ER status proved to be an informative parameters to predict biologic behavior of node-negative breast carcinoma. Tumor size was found to affect disease free survival rates (12.5% of patients with tumors < 2cm relapsed in comparison to 52.2% of patients with tumors > 2cm). Several studies have correlated tumor size with survival in node-negative breast cancer [15, 24,2].

Our study has established the role of nm23 and MVC as prognostic indicators in node negative breast cancer. nm23 level correlated significantly with disease free survival. The outcome was significantly better in the patients with high nm23 scores than those with low nm23 scores. The 5-year DFS was 84% in the patients with high nm23 as compared with 45% in low nm23 tumors. The same findings were reported by Heimann et al. [13]. In our cases, the extent of tumor angiogenesis also had a highly significant prognostic value. The 5-year DFS was 88% in patients with low MVC as compared to 27% in tumors with high MVC. The same findings were reported by Heimann et al. [14,12,15], Medri et al. [24] and Coradini et al. [2].

In this work, using the combination of both nm23 level and MVC, we could identify a high risk group of NNBC, as it was found that patients with both low nm23 and high MVC had more than 10 fold increase in the relapse rate compared with those having both high nm23 and low MVC. Relapse rate was 75% versus 6.3%. The 5-year disease free survival was 25% for the high risk group of patients compared to 93.7% for the low risk group of patients. The difference was statistically highly significant. In our study multivariate analysis proved that

MVC is the only independent prognostic factor. Regarding the group of patients with high MVC, in whom the prognosis was poor (5-year DFS was 26.7%), we wanted to determine whether nm23 had an additional prognostic value. In the group of patients with high MVC, high nm23 patients had a better 5-year DFS than low nm23 (33% versus 25%); the difference did not reach a statistically significant value. However, among low nm23 group the 5-year DFS is significantly better if MVC is low compared with high MVC (75% versus 25%).

In our study both radiotherapy and chemotherapy had no significant impact on 5-year DFS. However the incidence of local recurrence was only 7% of the relapsed cases. This goes in concordance with Ragaz et al. [29], who stated that adjuvant post-operative radiotherapy and chemotherapy showed a decreased incidence of loco-regional relapse which could be explained by the marked improvement of the quality of radiotherapy given as well as the wide use of systemic adjuvant treatment in indicated patients. However, in the high risk group of patients, 40% of patients that received post-operative radiotherapy and chemotherapy had relapsed, whereas, all cases who did not receive post operative radiotherapy and chemotherapy had relapsed, the difference was statistically significant. This goes in concordance with Quiet et al. [28] who stated that the use of adjuvant chemotherapy in node-negative breast cancer had been shown to improve the short-term (5-year) disease-specific survival rate.

In conclusion, both nm23 level and MVC appear to contribute valuable additional prognostic information in node-negative breast cancer patients. MVC proved to be the most important tumor factor predicting outcome. Furthermore, evaluation of nm23 level and MVC together proved to be a better prognostic indicator in NNBC. So, using both of them together could be a guide for therapy, as depending on nm23 level and MVC we could identify a group of patients who are at high risk of developing recurrence and who may benefit greatly from adjuvant treatment.

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