

Impact of Breast Cancer Molecular Subtypes on the Incidence of Axillary lymph Nodes Metastases

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Abstract: Purpose: Breast cancer has at least four molecular subtypes with significant differences in prognosis and ALN involvement. This study aimed to investigate the impact of breast cancer molecular subtypes on the incidence of axillary lymph nodes metastases. **Methods:** Three hundred and twenty-nine female patients with invasive breast cancer were included in this study. Age at diagnosis, menopausal status, tumor size, type and grade, lymph node status and molecular subtypes were recorded. Four major molecular subtypes were classified, Luminal A; Luminal B, HER2+ and triple negative. **Results:** The mean age was 51.34 years old. Most of patients (86.9%) have Invasive ductal carcinoma. One hundred sixty four (49.5%) patients had node negative disease while 166 (50.5%) had node positive disease. Luminal A molecular subtype was recorded in 87 patients (26.4%), luminal B 163 patients (49.5%), HER-2+ 35 patients (10.6%) and TNBC 44 patients (13.5%). There are differences in ALN positivity by molecular subtypes, node positive disease was (11.5%) among luminal A patients, luminal B patients (66.9%), HER2+ (91.4%) and TNBC (34.1%). There were significant correlation between molecular subtypes and nodal status ($p=0.03$ for luminal B and <0.001 for luminal A, HER 2 positive and triple negative). **Conclusion:** luminal B and HER2+ve breast cancer subtypes were more likely to be associated with ALNM. Also, tumor size and a grade, LVI and ki67 were correlated with lymph node status. Further confirmatory studies are necessary to define factors that predict ALN metastases.

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1. Introduction

Breast cancer has been found to be the most common malignant tumor among females. It has many molecular, and pathological with different prognosis and therapy implications^[1]. Hormonal receptors have an independent for prognosis of case. ER expression was in average 80-90% of breast cancer, while PR expression was 70-80%^[1]. H.E.R-2/neu was 15-20% of breast cancer cases^[3,4].

Breast cancer is better represented by its combined receptor expression than by a single one used alone^[5,6]. Axillary lymph nodal (ALN) infiltration are correlated to overall survival and have association with staging, prognosis, and treatment of invasive breast cancer^[7-9]. Breast cancer has main 4 main subtypes, Luminal A, Luminal B, Her-2 positive and triple negative breast cancer. Molecular subtype (MST) has significant differences in prognosis^[10-11].

Triple negative breast cancer (TNBC) has aggressive clinical impact, with high metastatic rate compared to other subtypes, and characterized by a poor prognosis specially in case of decreased sensitivity to adjuvant chemotherapy^[11-13]. Some studies evaluated incidence of axillary node metastases in TNBC and they found that it is less frequent in this subtype.^[14,15]

Many studies have been investigated patients unlikely to benefit from ALN dissection, thus, the use of sentinel lymph node biopsy reduce the need of ALN dissection of missed metastasis. There are controversies about the relation between ALN status and molecular subtypes and role of LN involvement as an intrinsic characteristic^[16,16]. Aim of present study is to evaluate the association between ALN status and molecular subtype.

2. Patients and Methods:

Three hundred and twenty nine (329) breast cancer patients were included in this study. The study was conducted at Tanta University Hospital, clinical oncology department between January 2011 and January 2015. The clinical and pathological features, including age at diagnosis, menopausal status, tumor site, tumor size, histological type and grade, lymph node status and molecular subtypes were constructed.

Four molecular types were determined^[11]. Patients were classified as follows: Luminal A (ER+/PR+, HER2-, Ki67 < 14%); Luminal B (ER+/PR+, HER2+); HER2+ (ER-, PR-, HER2+); TNBC (ER-, PR-, HER2-). HER2 FISH+ or IHC 3+ was considered to be positive.

Statistical analysis:

All data were statistically analyzed using the Statistical version 21.0 (SPSS Inc., USA). Chi-square test was used for the correlation between clinical, pathological features and axillary lymph node status. Univariate and multivariate analyses was done using the logistic regression model with P value of less than 0.05 was considered to be statistically significant.

3. Results

A total of 329 patients with invasive breast carcinoma were included. The mean age is (50.43) years old. Invasive ductal carcinoma (86.9%) constitute majority of patients. Luminal A molecular subtype was recorded in 87 patients (26.4%), while luminal B, HER-2+ and TNBC were recorded in 163 patients (49.5%), 35 patients (10.6%) and 44 patients (13.5%) respectively.

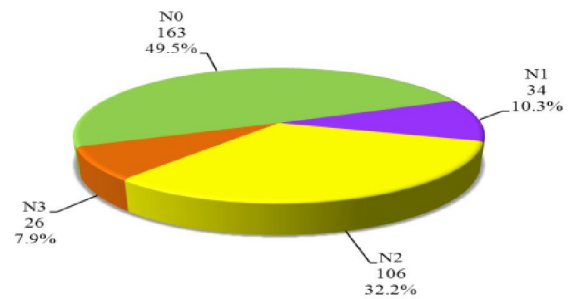
One hundred thirty nine patients were premenopausal (42.2%), 190 patients were postmenopausal (57.8%). Regarding tumor, T1 was represented in 18.2%, while T2 and T3 were recorded in 53.2% and 28.6% respectively. Grade I & II, representing 71.1% while grade III occurred in 28.9%.

Table 1, (Fig. 1) showed that 163 (49.5%) patients had node negative disease while 166 (50.5%)

had node positive disease. Nodal stage 2 was the most frequent (106 patients) followed by N1 (34 patients) and 26 patients had N3 stage.

Table (1): Distribution of the patients according to N stage (n = 329)

Stage N	No.	%
N0	163	49.5
N1	34	10.3
N2	106	32.2
N3	26	7.9
N1 + N2 + N3	166	50.5



(Fig. 1) Distribution of the patients according to N stage (n = 329)

Table (2): Relation between N stage and different parameters (n = 329)

	Total (n = 329)	N Stage		P
		N0 (n = 163)	(N1 + N2 + N3 (n = 166))	
Age				
≤50	144(43.8%)	50 (34.7%)	94 (65.3%)	<0.001*
>50	185(56.2%)	113(61.1%)	72 (38.9%)	
Sup type				
Luminal A	87(26.4%)	77(88.5%)	10 (11.5%)	<0.001*
Luminal B	163(49.5%)	54(33.1%)	109(66.9%)	0.03*
HER.2+	35(10.6%)	3(8.6%)	32(91.4%)	<0.001*
Triple -	44(13.5%)	29(65.9%)	15(34.1%)	<0.001*
Pathology				
Ductal	286(86.9%)	142(49.7%)	144(50.3%)	0.921
Lobular	43(13.1%)	21(48.8%)	22(51.2%)	
Menopause				
Pre	139(42.2%)	52(37.4%)	87 (62.6%)	<0.001*
Post	190(57.8%)	111(58.4%)	79 (41.6%)	
Tsize				
≤2	60 (18.2%)	47(78.3%)	13(21.7%)	<0.001*
>2- ≤5	175(53.2%)	103(58.9%)	72(41.1%)	
>5	94(28.6%)	13(13.8%)	81(86.2%)	
Grade				
G1 & 2	234(71.1%)	143(61.1%)	91(38.9%)	<0.001*
G3	95(28.9%)	20 (21.1%)	75(78.9%)	
LVI				
Non	206(62.6%)	142(68.9%)	64(31.1%)	<0.001*
Yes	123(37.4%)	21(17.1%)	102(82.9%)	
Ki67				
Low	103(31.3%)	92(89.3%)	11(10.7%)	<0.001*
High	226(68.7%)	71(31.4%)	155(68.6%)	

Table (3): Univariate analysis logistic regression for factor affecting stage.

	Sig.	Exp (B)	95% Confidence Interval for Exp (B)	
			Lower Bound	Upper Bound
age	.642	1.176	.593	2.331
menopaus	.268	1.473	.742	2.925
Tumor size	.027	.534	.305	.932
LVI	<.001	.140	.061	.322
ki67	.067	.155	.018	1.312
pathology	.457	.713	.293	1.737
grade	.389	.718	.338	1.525
Luminal A	.957	.939	.098	8.977
Luminal B	.032	.376	.154	.918
HER-2	.038	5.304	1.202	23.406
Triple -ve	.157	.326	.069	1.539

Table (4): Multivariate analysis logistic regression for factors affecting stage.

	Sig.	HR	95% C.I. for EXP (B)	
			Lower	Upper
suptype (1)	.905	1.146	.122	10.814
suptype (2)	.030	2.690	1.103	6.561
suptype (3)	.015	2.081	1.245	3.479
suptype (4)	.144	3.154	.676	14.708
T	.033	1.824	1.051	3.167
LVI	<.001	6.998	3.050	16.052

Table 2 showed that there are differences in LN positivity by molecular subtypes, (11.5%) of luminal A patients had N positive disease, (66.9%) of luminal B patients, HER2+ (91.4%) and TNBC (34.1%). There were significant correlation between molecular subtypes and nodal status ($p=0.03$ for luminal B and <0.001 for HER 2 in favors of node positivity and <0.001 for both luminal A and triple negative disease in favors of node negativity).

In the same context, higher tumor grade is significantly associated with ALN metastases, ($p<0.001$), 34.9% of grade I & II tumors had ALN deposits compared to 78.9% of grade III tumors. There is a significant higher rate of ALN involvement among young age patients ($p<0.001$), (65.3%) of patients who had ALN metastases were younger than 50 years old compared with (38.9%) in patients older than 50 years old (Table 2).

Correlation between LVI and ALN involvement showed significant relation ($p<0.001$), (82.9%) of patients with LVI had ALN involvement compared to (31.1%) in patients without LVI. Also there is a significant higher rate of ALN involvement among patients with high ki67 ($p<0.001$), (68.6%) of patients with high ki67 expression had ALN metastases (Table 2). There was no association between lymph node metastases and tumor pathology.

Table (3) showed univariate logistic regression models. Tumor size, molecular subtypes and LVI showed a significant correlation with the ALN status. In multivariate logistic regression analysis, tumor size ($P=0.03$), luminal B subtype ($P=0.03$), HER-2+

subtype ($P=0.02$) and LVI ($P<0.001$) were independent factors in correlation with the ALN status. Table (4)

4. Discussion

Breast cancer is a complex, heterogeneous disease at the molecular level^[17]. The immunohistochemistry (IHC) using gene expression microarrays: the strongest factors correlated to overall survival in breast cancer patients, and as such, it has been a major determinant in therapeutic decision making.^[20]

This study evaluated 329 patients with invasive breast carcinoma, mean age (50.43) years old. The most histological type is invasive ductal carcinoma (86.9%). Luminal A in 87 patients (26.4%), Luminal B in 163 (49.5%), HER-2+ in 35 (10.6%), and TNBC in 44 (13.4%) patients.

This study showed that, 163 (49.5%) patients had node negative disease while 166 (50.5%) patients had node positive disease. Nodal stage N2 was the most frequent (106 patients) followed by N1 (34 patients) and 26 patients had N3 stage. The tumor size, grade, menopausal status, LVI, and Ki67 expression were significantly correlated the LN positivity. There are differences in LN positivity by molecular subtypes, 10/87 luminal A patients had N positive disease (11.5%), luminal B 109/163 patients (66.9%), HER2+ 32/35 (91.4%) and TNBC 15/44 (34.1%). There were significant correlation between molecular subtypes and nodal status ($p=0.03$ for luminal B and <0.001 for luminal A, HER 2 and triple negative).

In our study there was a lower risk of axillary lymph nodal (ALN) involvement in luminal A breast and triple negative cancer patients and increased frequency of LN metastasis in luminal B and HER2+breast cancer patients. Dent et al^[20] showed that patients with Triple negative breast cancer has less lymph node metastasis but is more aggressively. It maybe due to hematogenous spread or lack of targetable treatment.

Chengshuai Si et al^[21] study showed that tumor size and tumor subtype show statistical significance with LN involvement. Luminal B type showed significant higher probability of LN involvement, Triple positive and triple negative breast cancer accounts the most and least possibility of LN involvement.

Emi Yoshihara et al^[22] study the incidence of ALNM with the presence of LVI ($P < 0.001$), larger tumor size ($P < 0.001$), higher histologic grade ($P < 0.0001$) and no effect of age. Elsayed M Ali et al^[23] study evaluated 258 patients with invasive breast carcinomas, ER and PR expression were demonstrated in 78.7% and 76.4%, respectively and over-expression of HER-2/neu was detected in 13.2% of cases. There was a strong correlation between tumor size and tumor grade with lymph node involvement ($p = 0.0001$ and 0.024 , respectively). Triple positive breast cancer is more likely to have axillary lymph node metastasis and ER+/PR+/HER-2- (PPN) is the most protected group ($p < 0.001$).

Patani NR, et al^[24] study evaluated 590 patients with mean age 52 years. Positive ALNs were found in 302 patients (51%). Five factors were significantly associated with ALN metastasis; younger age, lower mammographic density, higher BI-RADS category, larger tumor size, and presence of lymphovascular invasion. Tufale et al^[25] evaluated the correlation of various clinicopathological variables with axillary nodal involvement in T1 breast cancer. tumor size, LVI, histological grade, tumor palpability & ER/POR/Her2 receptor profile were found to be significantly associated with axillary lymph node involvement (ALONI) and also found that age of the patient, family history and histological type of tumor were not significantly correlated with ALONI.

Conclusion:

Analysis of breast cancer subtypes is important, because it provides valuable prognostic and predictive information's. our results showed that, luminal B, HER2/neu positive, tumor size and LVI are independent prognostic factors for ALN metastases. Further confirmatory studies are necessary to define factors that predict ALN metastases.

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