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Original Article

Association between molecular subtypes and lymph node status in invasive breast cancer

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Abstract: Background: The predictors for the involvement of lymph node (LN) have been widely studied. But the implication of the molecular type has not been well studied. Using the database of our institution, we investigated this relation. Methods: Patients with T1 and T2 primary breast cancer without distant metastasis were included in our study from 2012 Jan to 2013 Dec. All patients undertook the resection of the primary and the axillary lymph nodes (ALNs). We collected the clinical data including age at diagnosis, the status of ER, PR and HER2, tumor size, nodal status, and histological type. The relationship between demographic, tumor characteristics and lymph node status was evaluated. Results: 814 patients were included in our study. The number and the percentage (in parentheses) of each type of breast cancer is as follows: Luminal A 230 (28.3%), Luminal Her2- 284 (34.9%), Luminal Her2+ 104 (12.8%), HER2+ 72 (8.8%), TNBC 124 (15.2%). On univariate and multivariate analysis, tumor size and tumor subtype show statistical significance with LN involvement. Using TNBC as a reference, both Luminal B type (Luminal HER2-, Luminal HER2+) shows significant higher probability of LN involvement. Conclusions: LN involvement is an intrinsic characteristic for molecular subtype of breast cancer. Triple positive and triple negative breast cancer accounts the most and least possibility of LN involvement.

Keywords: Breast cancer subtypes, axillary lymph node involvement

Introduction

Breast cancer is highly heterogeneous, conferring different progression, treatment and prognosis [1, 2]. Molecular type based on the high throughput technology has revolutionized the opinions and the treatment of breast cancer [3]. In addition, the traditional IHC based molecular type showed the highly consistency with the genetic expression, conferring the similar prognostic values [4]. So in our study, IHC based classification were taken.

Lymph node status is critical for the treatment of breast cancer. Axillary Lymph Node Dissection (ALND) is based on the sequential metastasis in lymphatic vessels and has been proven a success by the Halsted radical mastectomy compared with local excision. The use of Sentinel Node Biopsy (SNB) has shown perfect predictability, thus avoiding unnecessary ALND and the following morbidity. Novel clinical trials have challenged the recent opinions in axillary treating and two recent systemic reviews [5, 6] reach the new recommendations. ALND should not be appropriate for patients undertaking breast conserving surgery and whole-breast radiation with less than 3 metastatic axillary lymph nodes [5].

Despite of some relevant studies [7-10], the data of the relation between ALN status and molecular type is insufficient. And there are controversies about the role of LN involvement as an intrinsic characteristic. The aim of this study is to identify the relation between ALN status and molecular subtype.

Patients and methods

Inclusion and exclusion criteria of the study

A prospective database between 2012 Jan and 2013 Dec was reviewed for this study. All patients were included consecutively. Inclusion criteria included: 1. Primary tumor without dis-

Table 1. Demographic and tumor characteristics

Variable	Population (n = 814)
Age	54.1 ± 9.8
LN positive (%)	42.1 (343/814)
Histology subtype (%)	
IDC	87.1 (709/814)
ILC	2.9 (24/814)
Others	10.0 (81/814)
ER positive (%)	74.4 (606/814)
PR positive (%)	54.2 (441/814)
HER2 positive (%)	23.7 (193/814)
Molecular subtype (%)	
Luminal A	28.3 (230/814)
Luminal Her2-	34.9 (284/814)
Luminal Her2+	12.8 (104/814)
Her2+	8.8 (72/814)
TNBC	15.2 (124/814)
Tumor size	2.0 (1.4, 2.5)
T (%)	
T1	69.8 (568/814)
T2	30.2 (246/814)
N (%)	
N0	57.9 (471/814)
N1	26.4 (215/814)
N2	9.8 (80/814)
N3	5.9 (48/814)
Stage (%)	
1	44.2 (360/814)
2	40.0 (326/814)
3	15.7 (128/814)

tant metastases; 2. All patients underwent resection of primary cancer and ALNs for definitive LN staging; 3. All patients are female of Han nationality; 4. All patients were at T1 or T2 stage. Exclusion criteria included: 1. patients with recurrent tumor; 2. diagnosis of *In situ* breast cancer only. Clinicopathological data was collected as follows: age at diagnosis, pathological tumor size, ALN status, histological type and IHC biomarkers for molecular subtyping.

Criteria for the molecular subtype and other clinical data

Five molecular types were determined according to the current guideline [11]. The categorization were made as follows: Luminal A (ER+/PR+, HER2-, Ki67 < 14% or PR ≥ 20%); Luminal Her2- (ER+/PR+, HER2-, Ki67 ≥ 14% or PR < 20%); Luminal HER2+ (ER+/PR+, HER2+); HER2+ (ER-, PR-, HER2+); TNBC (ER-, PR-, HER2-). ER/PR was conceived to be positive, if the percentage of nuclear-staining cancer cells is no less than 1%. Both HER2 FISH (Fluorescence in situ hybridization) and IHC test were used for the confirmation of the status of HER2/neu. IHC 3+ or FISH+ was conceived to be positive of HER2 expression.

Lymph node was considered positive according to the HE staining and IHC test. Tumor size was calculated by the largest diameters pathologically. Categorization for Tumor size, nodal status, and staging were made by the American Joint Committee on Cancer (AJCC) TNM staging system for Breast cancer.

Statistical analysis

Continuous data was shown as the median [interquartile range (IQR)] or mean (SD), and the categorical data as the number (percentage). We categorized lymph node status negative and positive. Demographic and tumor characteristics were compared across categories of lymph node status using one-way ANOVA or rank sum test for continuous variables and Chi-square test for categorical variables. Univariate and multivariate logistic regression were applied to assess the influencing factors of lymph node metastases in breast cancer. Furthermore, a nomogram was depicted to show the result of multivariate logistic regression. A 2-sided $P < 0.05$ was considered statistically significant. We performed statistical analysis using the software of SAS.

Results

In our cohort, 814 patients are identified, 343 (42.1%) patients with ALNM (**Table 1**). The mean age of our cohort is 54.1 years old. The most histological type is invasive ductal type. The number and the percentage (in parentheses) of each type of breast cancer is as follows: luminal A 230 (28.3%), Luminal Her2- 284 (34.9%), luminal Her2+ 104 (12.8%), HER-2+ 72 (8.8%), TNBC 124 (15.2%).

As shown in **Table 2**, the tumor size is positively relevant with the LN positivity significantly. Based on the up-to-date AJCC classification, LN positivity in T1 and T2 is 36.6% and 54.9%

Table 2. Demographic and tumor characteristics by lymph node status

Variable	LN negative (n = 343)	LN positive (n = 471)	*P* value
Age	54.4 ± 9.9	53.7 ± 9.7	0.285
Histology subtype (%)			
IDC	56.7 (402/709)	43.3 (307/709)	0.217
ILC	66.7 (16/24)	33.3 (8/24)	
Others	65.4 (53/81)	34.6 (54/82)	
ER positive (%)	56.4.9 (342/606)	43.6 (264/606)	0.167
PR positive (%)	55.8 (246/441)	44.2 (195/606)	0.2
HER2 positive (%)	52.8 (102/193)	47.2 (91/196)	0.113
Molecular subtype (%)			
Luminal A	63.5 (146/230)	36.5 (84/234)	0.032
Luminal Her2-	53.2 (151/284)	46.8 (133/284)	
Luminal Her2+	51.0 (53/104)	49.0 (51/104)	
Her2+	55.6 (40/72)	44.4 (32/72)	
TNBC	65.3 (81/124)	34.7 (43/124)	
Tumor size	1.5 (1.2, 2.0)	2.0 (1.5, 2.5)	< 0.001
T (%)			
T1	63.4 (360/568)	36.6 (208/568)	< 0.001
T2	45.1 (111/246)	54.9 (135/246)	
Stage (%)			
1	100.0 (360/360)	0.0 (0/360)	< 0.001
2	34.0 (111/326)	62.7 (215/326)	
3	0.0 (0/128)	100.0 (128/128)	

respectively. The correlation between the frequency of LN metastasis and each T stage is shown in **Figure 1**. There are differences in LN positivity by molecular types, shown by the chi-square test. Furthermore, luminal Her2+ is deemed with the highest LN positivity (49.0%), followed by luminal Her2- (46.8), HER2+ (44.4%), Luminal A (36.5%) and TNBC (34.7%), which was shown in **Figure 2** in the form of frequency.

For further prediction of the lymph nodal status, univariate and multivariate logistic regression models were used (**Table 3**). Tumor size shows a significant correlation with the ALN status. Compared with TNBC, both luminal B types show higher relevance with ALN status. Compared with the TNBC, the adjusted OR value is 1.993, 1.954, 1.666, 1.653, and 1.388 for luminal Her2+, luminal Her2-, HER2+ and luminal A respectively. A nomogram (**Figure 3**) calculates the risk factors of ALNM by tumor size and molecular subtype. The logistic regression model shows tumor size is the stronger indicator for LN positivity than molecular type.

Discussion

Our study shows the highest occurrence of LN metastases in triple positive breast cancer, and the lowest occurrence in TNBC. But for each biomarker used for subtying, no statistically significance was found. It shows a greater prognositic value of the combined phenotype. In addition, tumor size shows a positive correlation with the LN involvement.

The accurate prediction of LN status is a prerequisite for treatment decision. Principally, palpable LNs in patients with advanced breast cancer should be preceded with ALD without cytological or histological confirmation. For patients without any evidence of LN metastases, SNB should be performed to confirm the status of ALNs [5]. Accurate and convenient prediction of the LN status is critical for regional management of breast cancer.

The high throughput technology has brought a new breakthrough in the last decade, and valuable information has been provided [12]. Peru *et al* classified the breast cancer into subtypes by the genetic information for the first time [13]. However, due to the high economic outlay, the genetic classification has not been widely used clinically. Instead, IHC based molecular type shows high consistency with the genetically intrinsic type and highly cost-efficiency [14]. In our study, significant difference in the distribution of ALN positivity was found between different subtypes of breast cancer. It may suggest the different aggressiveness for different types. So LN involvement is an intrinsic characteristic of each molecular type. Definitely, the involvement of the LNs indicates a higher aggressiveness, more than a just later period of the tumor progression [15, 16]. The distribution of LN involvement in each molecular subtype is similar with the study of Van Calster *et al* [17] and

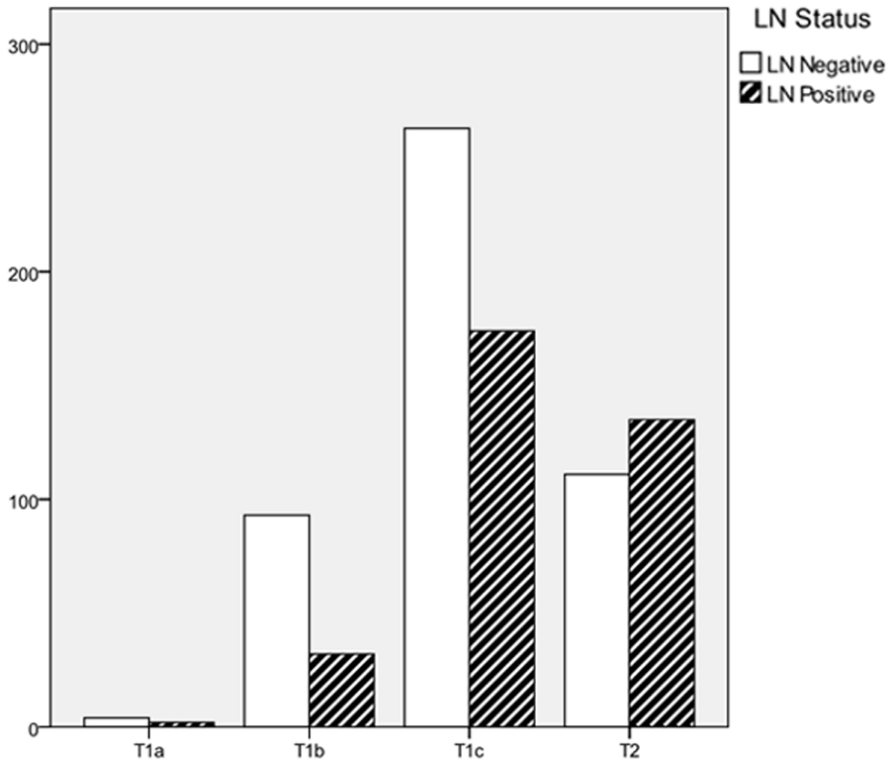

Figure 1. The frequency of lymph node positivity according to the T categorization.

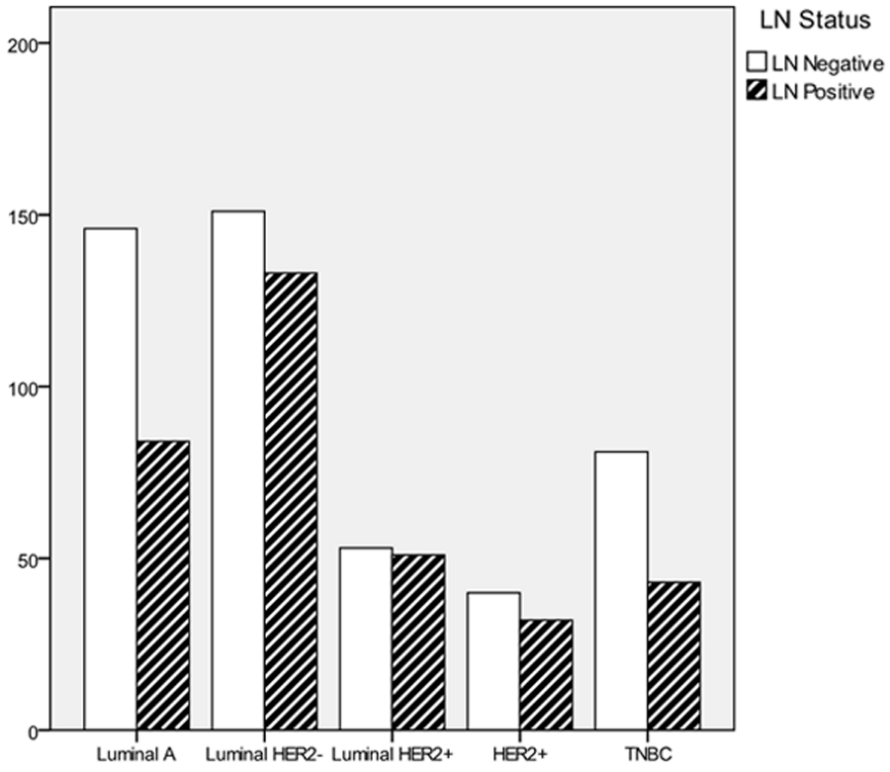

Figure 2. The frequency of lymph node positivity according to the molecular subtype.

Noguchi M *et al* [16]. For the differences of the relevant results, different inclusion criteria and different standard for categorization contributes a lot. The standard of positivity for ER is ranging from 1% to 10%. The patients in our study were all in T1 or T2 study, which may reduce the influence of the tumor size. However, tumor size which is still a powerful influencing factor.

Predictors used to identify the ALN status has been studied before [9, 15, 18-24]. Tumor size is the most valuable predictor for ALN status in patients with breast cancer, which confers different strategies for people in the different T stage [23]. Besides, potential predictors include lymphavascular invasion (LVI) [10, 15, 25], age at diagnosis [10], and so on.

There are several limits in our study. First, there is paucity of the potential predictors: histological grade, lymph vascular invasion (LVI), other biomarkers (Ki67, P53). Different standard were used to determine the tumor grade, so histological grade was not

Table 3. Univariate and multivariate logistic regression models for lymph node positive breast cancer

Covariate	Univariate		Multivariate	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age	0.992 (0.978-1.006)	0.285		
Histology subtype (versus IDC)				
ILC	0.655 (0.277-1.550)	0.335		
Others	0.692 (0.427-1.120)	0.134		
ER positive	1.260 (0.913-1.741)	0.16		
PR positive	1.205 (0.911-1.594)	0.191		
HER2 positive	1.306 (0.944-1.808)	0.107		
Molecular subtype (versus TNBC)				
Luminal A	1.084 (0.686-1.712)	0.73	1.388 (0.862-2.237)	0.178
Luminal Her2-	1.659 (1.072-2.569)	0.023	1.954 (1.241-3.075)	0.004
Luminal Her2+	1.813 (1.063-3.090)	0.029	1.993 (1.152-3.447)	0.014
Her2+	1.507 (0.832-2.729)	0.176	1.666 (0.906-3.064)	0.101
Tumor size	1.629 (1.372-1.933)	< 0.001	1.653 (1.387-1.970)	< 0.001
T (versus T1)				
T2	2.105 (1.554-2.852)	< 0.001		

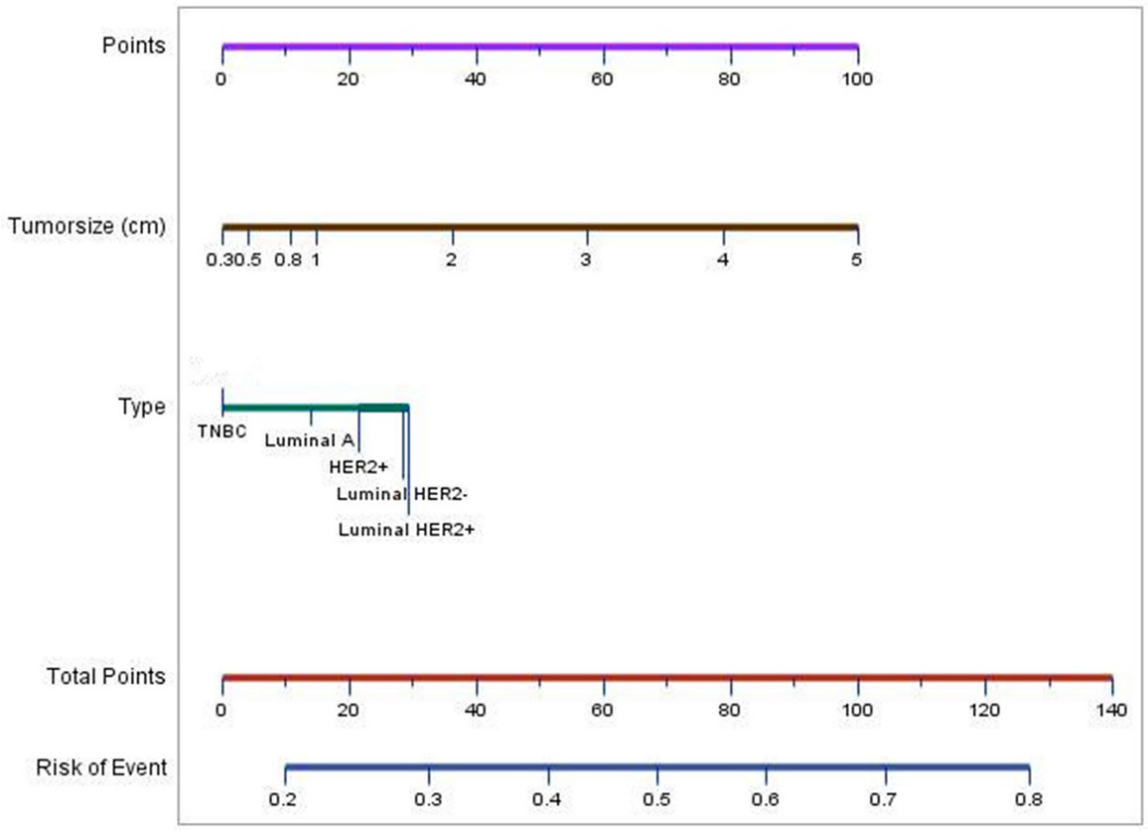

Figure 3. Nomogram to calculate the possibility of ALNM in breast carcinoma. To calculate the risk of ALNM, first, identify the points of tumor size and type in the first line respectively; second, combine the two points in the line of total points; last, identify the risk of ALNM in the lowest line.

assessed in our study. Ki67 were not assessed in all of our patients, so the categorization of molecular types was made by the PR in those patients who did not receive the Ki67 assessment. So a comprehensive regression model was not reached. 2 the study was not conducted in a prospective way, which may introduce unexpected bias.

In our study, LN involvement is an intrinsic characteristic for molecular subtype of breast cancer. Triple positive and triple negative breast

cancer accounts the most and least possibility of LN involvement. But new combined biomarkers or new convenient technology is needed to predict the LN status correctly in the future.

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Disclosure of conflict of interest

None.

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