

Determinants of lymph node status in women with breast cancer: A hospital based study from eastern India

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Background & objectives: Number of metastatic lymph nodes has a strong prognostic value in the course of breast cancer treatment, morbidity and mortality. This study was undertaken to determine the association between axillary lymph node metastasis and several variables such as age, tumour size, grade, lymphovascular invasion, oestrogen and progesterone receptor expression and HER2/neu status in patients with breast cancer.

Methods: In this study 426 (with complete information on study variables) patients with breast cancer on treatment during March 2010 to December 2013, were analyzed. TNM (tumour node metastasis) staging was evaluated. The histological grading of tumours was done according to modified Bloom-Richardson Grading System. The immunophenotype of the tumour was determined as the expression of oestrogen (ER) and progesterone (PR) receptors and HER2/neu status. Univariate and multivariate analyses were carried out to determine the independent predictors of metastatic lymph node.

Results: Among the studied patients, 44.36 per cent (189 of 426) of the patients had nodal metastases. Tumour histology, tumour grade, size and lympho-vascular invasion were related with node positivity. On univariate analysis, age, menopause, hormone receptor status did not relate with the node metastasis. Age, tumour grade, tumour size, lympho-vascular invasion and HER2/neu expression was likely to be associated with the number of lymph node metastasis.

Interpretation & conclusions: The lymph node status was associated with clinical stage, tumour grade, tumour histology and HER2/neu status. These factors may be used for better management of such patients.

Key words Breast cancer - lymph node - prognostic factors - tumour grade - tumour marker

Identification of clinically predictive and prognostic factors is considered as an important issue in treatment evaluation of breast cancer. There are a number of tumour-related features available to predict

the prognosis of breast cancer. The involvement of axillary lymph nodes (LNs) is the most important prognostic factor in operable primary breast cancer and is strongly associated with both disease-free and

overall survival¹. The nodal system constitutes a major part of the peripheral lymphoid tissues in mammals. Histologic evaluation of axillary lymph nodes which includes serial sectioning of paraffin tissue blocks and immunohistochemical (IHC) staining, aids in detection of metastases. Elderly women with breast cancer are known to be associated with an increased probability of getting nodal involvement². In another study it was proposed that tumours of elderly patients had more favourable biologic characteristics³ and had a decrease in LNs involvement⁴. Voordeckers *et al*⁵ have suggested the use of number of involved nodes. The objective of this study was to determine independent determinants like age, tumour chronology (tumour size), tumour biology (tumour grade, lymphovascular invasion and the hormone receptor status) and growth factor (HER2/neu status) of the lymph node status in breast cancer patients.

Material & Methods

Five hundred fourteen newly diagnosed patients with breast cancer along with their complete clinical information were selected for the study. All of them had undergone the surgical treatment at the department of Surgery, Netaji Subhas Chandra Bose Cancer Research Institute (NCRI), Kolkata, India, during March 2010 to December 2013. Those patients who were treated for local recurrence, with only a carcinoma *in situ*, who were receiving neo-adjuvant therapy and those suffering from primary metastatic disease, were excluded. The final sample included 426 women. In each case at the time of diagnosis, age, menopausal status, pathologic tumour size, histologic subtype (including grade), number of lymph nodes dissected and number of positive nodes (burden of node positivity), lymphovascular invasion (LVI), hormonal receptor status and the HER2 (human epidermal growth factor receptor 2)/neu receptor status of the surgical specimen, were assessed. Axillary sampling (AS) was done for all the cases. All samples were collected from patients after surgery and prior to chemotherapy.

The study was approved by the Ethical Committee of our Institute and written informed consent was obtained from all participants.

Histochemistry: All tissue specimens were fixed by keeping them in 10 per cent formalin overnight and paraffin wax embedded; 4 µm sections were cut and stained with hematoxylin and eosin (H&E). After fixing, microscopic examination of the specimen was done and findings were recorded. Size, quadrant and

focality were assessed accurately. Lymph nodes were retrieved, numbers were noted, and grossly uninvolved nodes were submitted in entirety for processing whereas sections of grossly involved nodes were taken. Specimens were processed and studied in detail to get the information about the tumour morphology according to WHO classification (<http://codes.iarc.fr/topography/C50#174>) and guidelines. Grading of tumours was done according to Bloom-Richardson Grading System⁶. Benign tumours were excluded from the study. LVI was assessed on HE stained slides, as defined by Rosen and Oberman⁷. Tumour cell emboli within endothelium-lined vascular spaces are seen on HE stained slides of the breast cancer⁸.

Immunohistochemistry: Details of the immunohistochemistry (IHC) for oestrogen receptor (ER), progesterone receptor (PR) and HER2/neu were collected from histopathology records for each case. Commercially available antibodies for ER, PR and HER2/neu were used in the study. After tissue pre-treatment including steam antigen retrieval and protein block, slides were incubated with antibody as per instruction given in the commercial kit (Leica Microsystems, Wetzlar, Germany).

For the analysis of data, the patients were categorized in two ways. Firstly, the patients were categorized according to age group encompassing a period of ten years, starting with early age of cancer; group I: 25-35 yr; group II : >35-45 yr; group III: >45-55 yr; and group IV: >55 yr. Secondly, they were divided according to the presence of number of positive lymph nodes (pN1: 1-3; pN2: 4-9; pN3a :10-15 and pN3b: 16+) adapted from National Cancer Institute, stage information for breast cancer: <http://www.cancer.gov/types/breast/hp/breast-treatment-pdq#link/695toc>. Staging of tumour was recorded based on TNM (tumour node metastasis) staging system and AJCC Cancer Staging Manual⁹ of American Joint Committee on Cancer.

Statistical analysis: The association between the selected variables of tumour chronology and biology, and lymph node involvement was determined by using chi-square test. Multivariate logistic regression (MLR) analysis was carried out with the parameters found significantly associated in univariate analysis to determine the independent predictors of axillary node metastases. The area under the receiver operating characteristic (ROC) curve were calculated for independent predictors, area under the curve (AUC)=0.5 was taken as reference.

Results

Of the 426 patients, 332 (77.93%) underwent the modified radical mastectomy (MRM) and the remaining underwent breast conservative surgery (BCT). Average age of the patients was 48.4 yr (median=47, range 26-78 yr). Twenty five (5.87%), 147 (34.51%), 178 (41.78%) and 76 (17.84%) patients were categorized in groups I, II, III and IV, respectively. Of the 426 patients, 242 (56.8%) were found to be pre-menopausal and the remaining 184 (43.19%) were post-menopausal. LN metastasis was observed in 189 (44.37%) patients. The mean number of axillary lymph nodes was 8.67±0.31 (median 8; range 1-24). Most common TNM stage observed was stage II (n=260, 61.03%), followed by stage III (n=142, 33.3%) (Table I).

In our study, histological results revealed that the most common ductal carcinoma was prevalent among 303 (71.13%) patients; 34 patients (7.98%) were found with lobular carcinoma and the remaining 89 (20.89%) were found with other types of carcinoma. Ninety patients (21.13%) had grade 1 tumour whereas 220 (51.64%) and 116 (27.23%) patients had grades 2 and 3 tumours, respectively. T2 (47.89%, n=204) stage was the most common pathological T stage followed by T3 (42.49%, n=181) and T1 (9.62%, n=41) LVI was present in 69 (16.19%) patients. In patients who had no LVI,

38.97 per cent (n=136) had axillary node metastases, compared with 76.81 per cent (n=53) of patients where LVI was reported (Table II). Among these patients, 272 (63.85%) had ER positive tumours, and 238 (55.87%) had PR positive tumours; 95 (22.30%) patients were HER2/neu positive.

From ROC curve analysis (Figure) it was found that tumour grade (area: 0.553, CI: 0.497-0.609), tumour size (area: 0.583, CI: 0.527-0.639), histology (area: 0.434, CI: 0.378-0.489), and lympho-vascular invasion (area: 0.598, CI: 0.541-0.655) were associated with lymph node positivity. At univariate level all parameters except HER2/neu and menopausal status, were significantly associated with the metastatic lymph node. At multivariate level, manual backward selection methods was used to obtain the independent predictor based on *P* value of Wald statistics. Variables found to be significantly associated at univariate level, were inducted in the multiple logistic regression (MLR) with LN positivity as outcome (Table III). Based on the Wald statistics age, menopausal status, tumour grade, tumour size, LVI, tumour histology and HER2/neu were found to be independent predictors (*P*<0.001) of the outcome.

Discussion

The youngest patient in our study was 26 yr old. Previous studies have reported an association of lymph nodes with age in breast cancer patients^{10,11}. This age distribution of our patients was lower than that seen in western and Arab countries^{10,12}. No significant association was observed between different ages at breast cancer diagnosis and LNs metastases as reported earlier^{11,13}. But our data contradicted the result of Gajdos *et al*¹⁴ who studied 850 consecutive patients with T1 breast cancer and Olivotto *et al*¹⁵ from Canada who analysed 4312 women.

Since constitutional delay of menopause is prevalent in our country, a cut-off value of 51 years was used to identify the pre- and post-menopausal patients. We observed that node positivity was not related to menopausal status and premenopausal patients showed a non-significant trend towards a higher rate of lymph node metastases as also shown by Nouh *et al*¹⁶.

Tumour histology and grade appeared to play an important role in LN metastasis as described in many studies^{17,18}. We found a significant association between tumour histology and grade with nodal status.

Table I. TNM staging of tumours among the patients

Stage		No. of cases	Percentage
Stage I (n=24)			
	T1N0M0	24	5.63
Stage II (n=260)			
Stage II A (n=146)	T0N1M0	0	0
	T1N1M0	17	3.99
	T2N0M0	129	30.28
Stage II B (n=114)	T2N1M0	30	7.04
	T3N0M0	84	19.71
Stage III (n=142)			
Stage III A (n=127)	T2N2M0	45	10.56
	T3N1M0	63	14.78
	T3N2M0	19	4.46
Stage III B (n=15)	T4 any NM0	0	0
	Any T N3M0	15	3.52
Stage IV			
Distance metastatic		0	0

Table II. Relationships between axillary nodal metastasis and clinico-pathological factors

	Node Positive [#] N ₊ (n=189)				Node negative ^{##} N ₀ (n=237)	
	No. of nodes				Total (%)	N (%)
	1-3 (n=24)	4-9 (n=87)	10-15 (n=63)	16+ (n=15)		
Age group[‡](yr)						
25-35	7	6	0	1	14 (7.40)	11 (4.64)
>35-45	8	29	22	2	61 (32.28)	86 (36.29)
>45-55	6	37	35	9	87 (46.03)	91 (38.4)
>55	3	15	6	3	27 (14.29)	49 (20.68)
Menopause status						
Pre-menopause	18	55	31	9	113 (59.79)	129 (54.43)
Post menopause	6	32	32	6	76 (40.21)	108 (45.57)
Histology^{*‡}						
Ductal	14	69	49	9	141 (74.60)	162 (68.35)
Lobular	3	9	4	5	21 (11.11)	13 (5.49)
Other	7	9	10	1	27 (14.29)	62 (26.16)
Tumour grading^{*‡}						
1	8	12	10	1	31 (16.40)	59 (24.89)
2	12	34	39	6	91 (48.15)	129 (54.43)
3	4	41	14	8	67 (35.45)	49 (20.68)
Tumour size (Stage)^{*‡}						
T1	1	3	9	4	17 (8.99)	24 (10.13)
T2	15	28	25	7	75 (39.68)	129 (54.43)
T3	8	56	29	4	97 (51.33)	84 (35.44)
Lymphovascular invasion^{*‡}						
Present	3	19	25	6	53 (28.04)	16 (6.75)
Absent	21	68	38	9	136 (71.96)	213 (89.87)
Unspecified	---	---	---	---	0	8 (3.38)
Oestrogen receptor						
Positive	16	51	39	12	118 (62.43)	154 (64.98)
Negative	8	36	24	3	71 (37.57)	83 (35.02)
Progesterone receptor						
Positive	9	48	37	9	103 (54.5)	135 (56.96)
Negative	15	39	26	6	86 (45.50)	102 (43.04)
Her2/neu status^{*‡}						
Overexpressed	2	29	13	6	50 (26.45)	45 (18.99)
Not overexpressed	22	58	50	9	139 (73.55)	192 (81.01)

[#] N₊: No patients with involved metastatic lymph node; ^{##} N₀: patients without any involvement of lymph nodes
^{*}P<0.005 for node positive and node negative (χ^2 test)
[‡]: P≤0.02 for node positive subgroups (χ^2 test)

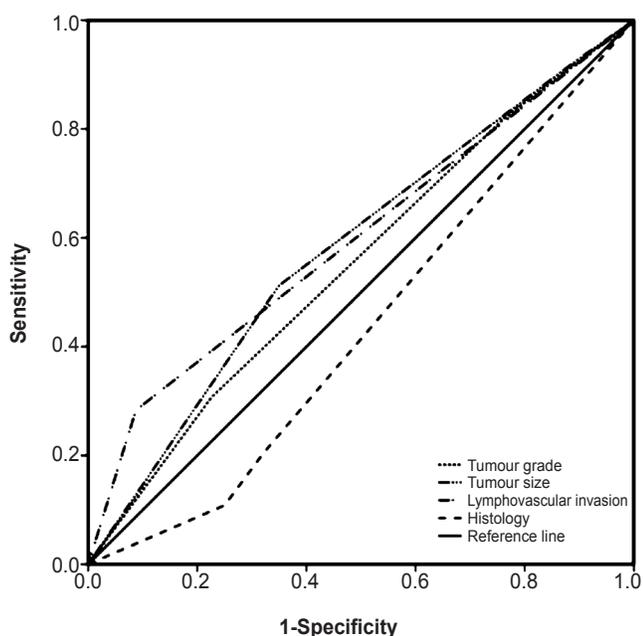


Figure. Receiver operating characteristic (ROC) curves for the prediction of the presence of lymph node metastasis by variables of tumour grade, Tumour size, lymphovascular invasion and tumour histology.

Tumour size is an important determinant factor in LN metastasis. Postacı *et al*¹⁹ reported a close relationship between tumour size and axillary lymph node involvement. The risk of axillary lymph node metastasis increases as tumour size increases which suggests that nodal metastasis is indicative of tumour chronology²⁰. T stage was significantly associated with

nodal positivity. Larger tumour size was found to be an independent predictor of node positive disease in our study, concurring with data from several other centers^{21,22}.

In patients with axillary lymph node metastasis, presence of LVI plays as an independent significant prognostic factor. LVI was found to be significantly associated with the axillary LNs in our study. Rezaianzadeh *et al*²³ showed that in breast cancer the prognostic value of lymphovascular invasion was significantly concomitant with metastatic axillary lymph nodes. Hence LVI plays a crucial role in the therapeutic protocol of breast cancer patients and is used as a decision making tool in the adjuvant chemotherapy.

The predictive role of sex hormone receptor status in previous investigations has been controversial, with some studies showing no value for ER or PR status^{14,21} and others pointing to lower risk of axillary lymph node metastases in tumours negative for either receptor²⁴ or for PR only²⁵. In our study, hormone receptor status was not found to be related to the LN metastasis. Ravdin *et al*²⁶ reported that PR concentrations were associated independently with an increased risk of axillary lymph node metastases.

On univariate analysis it was observed that tumour histology, tumour stage, presence of LVI, ER status, PR status and higher grade tumours were significantly associated with a higher risk of nodal metastases.

Table III. Results of the univariate and multivariate analysis of all variables

Independent variable	Univariate analysis (Age adjusted)				Multivariate Analysis			
	P value	Odds ratio	95% CI		P value of Wald Statistics	Odds ratio	95% CI	
			Lower	Upper			Lower	Upper
Age					<0.001	0.325	0.178	0.595
Menopause status	0.788	0.923	0.513	1.659	0.022	0.223	0.0621	0.804
Histology	0.003	0.585	0.409	0.838	<0.001	0.247	0.143	0.426
Tumour grade	<0.001	4.694	2.799	7.870	<0.001	5.140	2.544	10.385
Tumour size	<0.001	5.809	3.383	9.977	<0.001	14.675	6.487	33.195
Lympho-vascular invasion	0.013	1.773	1.129	2.784	<0.001	3.580	1.991	6.435
ER	0.016	0.484	0.269	0.874	0.850	1.135	0.304	4.237
PR	0.041	0.553	0.314	0.975	0.084	0.327	0.0920	1.163
HER2/neu	0.756	1.094	0.620	1.931	<0.001	5.296	2.268	12.368

Model statistics: Chi square 451.838 ($P=0.103$) Hosmer-Lemeshow statistics 29.593 ($P<0.001$)
 ER, oestrogen receptor; PR, progesterone receptor

However, on multivariate analysis, age, menopausal status, tumour grade, tumour size, LVI, tumour histology and HER2/neu were independent predictors based on the logistic regression. These findings though supported the study of Harden *et al*²⁷ following multivariate analysis but contradicted the report that tumour grade was not associated with positive LN.

In our study, T2N0M0 stage was most common in 30.28 per cent patients. Nigam *et al*²⁸ studies 328 patients with breast cancer in west Delhi and found T2N0M0 stage in 19.5 per cent cases.

Nisa *et al*²⁹ have shown a positive relationship between ER, PR and HER2/neu and axillary lymph node metastases. Our study showed an inverse relationship. Most importantly, we found HER2/neu status to be associated with the number of positive lymph node.

Though our study revealed significant association of lymph node with other pathological factors, it had few limitations like small sample size, retrospective nature and it was conducted at a single institution.

In conclusion, our study showed that the best predictive variables to axillary lymph node involvement in breast cancer were tumour histology, tumour grade, tumour stage and lympho-vascular invasion. Neither hormonal receptors (ER/PR) nor tumour markers (HER2/neu) were good determinants for the outcome in the studied sample. Further, our data indicated that the axillary lymph node status was not only a reflection of the chronological age of a tumour, but also of tumour biology. The current data may be used to tailor the management protocol of patients with breast carcinoma to accurately aim the diagnostic and therapeutic procedures, thus improving the quality of life of the patients. Future prospective studies have to be conducted to confirm and add to these findings.

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Conflicts of Interest: None.

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