

RESEARCH ARTICLE

The Pre-Treatment Neutrophil-Lymphocyte Ratio: a Useful Tool in Predicting Non-Sentinel Lymph Node Metastasis in Breast Cancer Cases

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Abstract

Background: The sentinel lymph node (SLN) biopsy is a highly accurate predictor of overall axillary nodal status in early breast cancer patients. There is however, still a debate on which patients with a positive SLN can benefit from axillary lymph node dissection (ALND). Numerous studies have been designed to identify variables that are predictive of non-SLN metastasis to avoid a complete ALND. The aim of this study was to determine whether the pre-treatment neutrophil-lymphocyte ratio (NLR) can be a predictive factor of non-SLN metastasis in early breast cancer patients. **Materials and Methods:** The records of 214 consecutive patients with cT1-3N0 invasive breast cancer who had undergone intraoperative SLN evaluation at Songklanagarind Hospital between the 1st of March 2011 and the 30th of May 2016 were examined. Data on patient demographics, tumor variables and NLR were collected and factors for non-SLN metastasis were analyzed using multivariate logistic regression. The power of the NLR was quantified with receiver operating characteristics (ROC) curves as measured by the areas under curves (AUC). **Results:** Multivariate analysis established presence of lymphovascular invasion (OR 8.4, 95%CI 2.3-31.3, $p=0.002$), macrometastasis (OR 6.6, 95%CI 1.8-24.7, $p=0.005$), and NLR (OR 2.3, 95%CI 1.1-4.8, $p=0.033$) as predictive factors of non-SLN metastasis with statistical significance. The AUC for NLR was 0.7 (95%CI 0.6-0.8) with an optimal cut-off of 2.6 giving a sensitivity of 62%, a specificity of 83.8%, a positive predictive value of 77.3% and a negative predictive value of 70.5%. **Conclusion:** Pre-treatment NLR is a useful diagnostic aid for predicting additional non-SLN metastasis.

Keywords: Neutrophil-lymphocyte ratio - NLR - breast cancer - non sentinel lymph node

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Introduction

The axillary nodal status remains the most important prognostic factor in breast cancer patients (Fisher et al., 1983; Weaver et al., 2011). Sentinel lymph node (SLN) biopsy is a highly accurate predictor of overall axillary nodal status, which has become the standard method in early breast cancer patients, who are clinically negative for lymph node (Lyman et al., 2005). There is still a debate on which patients with a positive SLN may still benefit from axillary lymph node dissection (ALND) because the ACOSOG Z0011 trial found that an ALND can be safely omitted with some patients with a SLN metastasis, as it does not show differences in the locoregional recurrence rates nor in the survival rate (Giuliano et al., 2011) and the incidences of non-SLN metastasis in SLN micrometastasis and macrometastasis are 5-25% and 40-60%, respectively (Abdessalam et al., 2001; Galimberti et al., 2013).

Numerous studies have been designed to identify variables that are predictive of non-SLN metastasis to avoid a complete ALND. Such studies have shown that

different pathological characteristics of the primary tumor along with SLN metastasis are linked with higher chances in finding additional positive non-SLN (Van Zee et al., 2003; Ozmen et al., 2006; Mittendorf et al., 2012; Koca et al., 2014; Chen et al., 2015).

Inflammatory response plays an important role in the development and progression of various cancers, including breast cancer (Mantovani et al., 2008; Colotta et al., 2009; Mantovani et al., 2009). The cancer related inflammatory response helps proliferation and survival of malignant cells coupled with the angiogenesis and metastasis of breast cancer (Hanahan and Weinberg, 2011).

Peripheral blood tests before treatment could reflect inflammatory conditions within the tumor. Pre-treatment neutrophil-lymphocyte ratio (NLR) is an inflammation-related marker that has been shown to be associated with outcomes in cancer patients. It can also be administered more easily, conveniently and at a lower cost (Azab et al., 2012).

In unselected patients with breast cancer elevated pre-treatment NLR was associated with larger tumors

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and stage of disease. It appears that the NLR is a more consistently independently prognostic in patients with a group of solid organ malignancies that tend to present at the later stage with more advanced features (Azab et al., 2012; Kohet al., 2015).

The aim of this study was to determine whether the neutrophil-lymphocyte ratio (NLR) can be a predictive factor of non-sentinel lymph node (SLN) metastasis in early breast cancer patients.

Materials and Methods

The study design was approved by: The Songklanagarind Hospital Ethics Committee.

Patients

The records of 448 SLNs, from 214 consecutive patients with cT1-T3N0 invasive breast cancer, who had undergone intraoperative SLN evaluation by One-Step Nucleic Acid Amplification (OSNA) assay at Songklanagarind Hospital between the periods of the 1st March 2011 and the 30th of May 2016 were examined.

The records of patients who had undergone systemic neoadjuvant chemotherapy, breast cancer with metastasis at the time of diagnosis, patients with active infection, active bleeding, hematological disorder, acute-chronic inflammation, post-splenectomy or steroid usage were excluded. The data collected from the medical records included; age, tumor size, histologic subtype, grade, estrogen receptor (ER) and progesterone receptor (PR) status, HER2 status, presence of lymphovascular invasion (LVI), total number of SLNs, number of positive and negative SLNs and the number of positive/negative non-SLNs.

SLN evaluation by OSNA assay

The OSNA assay (Sysmex Corporation, Kobe, Japan) was developed as a molecular analysis procedure through the detection and amplification of cytokeratin 19 (CK19) mRNA to evaluate lymph node metastasis. The results were assessed by the cut off level of calculated CK19 mRNA copies per μL : macrometastasis was defined as $> 5,000$ copies/ μL of CK19 mRNA, micrometastasis as 250-5,000 copies/ μL , and non-metastasis as <250 copies/ μL (Tsujiimoto et al., 2007). Several studies have found that the OSNA assay could accurately detect SLN metastasis at the rates comparable with a conventional pathological examination (Tsujiimoto et al., 2007; Visser et al., 2008; Schem et al., 2009; Tamaki et al., 2009). Axillary lymph node dissection at level I and II was performed in cases of SLN metastasis.

NLR evaluation

Retrospective data on preoperative blood cell counts were collected. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count.

Statistical analysis

Statistical analysis was carried out in SPSS for

Windows version 17.0 (SPSS Inc., Chicago, IL, United States). Categorical data were analyzed by Pearson's Chi-square test. Non-parametric data were analyzed by Mann-Whitney U test. Univariate and multivariate logistic regression models were used to evaluate the risk factors, so as to determine whether there was a difference between the non-SLN positive and negative groups, after a positive SLN. The power of the NLR was quantified with the receiver operating characteristics (ROC) curve as measured by the areas under receiver operating characteristic curves (AUC). A p-value of <0.05 indicated statistical significance. All tests were two-tailed with a 95% confidence interval.

Results

A total of 448 SLNs from 214 patients were examined. The mean age of the patients was 53 years of age. With the exception of 2 patients having a tumor size of 6 cm, all other patients had a tumor size of less than 5 cm. ($<T3$ lesion). Seventy-one of these patients (33%) had SLNs metastasis. Patient and disease characteristics were divided by negative and positive SLNs, which are reported in Table 1.

In the group of patients with SLN metastasis, 34 patients (47.8%) had an additional non-SLN metastasis. Patients with only SLN metastasis, 23 (62.2%) had a micrometastasis in the SLN, whilst 14 patients (37.8%) had a macrometastasis in the SLN. Of the patients, with only SLN metastasis, 81.1% had one positive SLN. In the group of patients with additional metastasis in the non-SLNs, 7 patients (20.6%) had a micrometastasis in the SLN while 27 patients (79.4%) had a macrometastasis in the SLN. A mean of 2.8 NLR was shown in non-SLNs metastasis group. Characteristics of patients with ALND are described in Table 2.

Univariate and multivariate analysis of non-SLN metastasis

The univariate analysis included; age, SLN identification technique, average number of SLNs, tumor size, histologic type, ER, PR, HER2 status, LVI status, type of SLN metastasis, number of positive SLNs, and

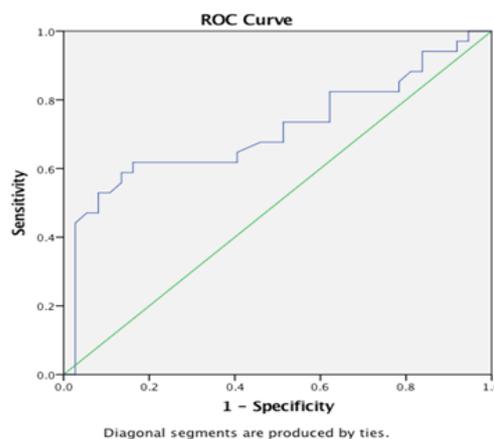


Figure 1. Area Under the Receiver Operating Characteristics (ROC) Curve of NLR = 0.7

Table 1. Patient and Tumor Characteristics

Characteristic	SLN		P-value
	Negative (N=143)	Positive (N=71)	
Mean Age (Years)	53.1	52.7	0.571*
< 45 years (%)	39 (27.3)	16 (22.5)	0.468 ^a
≥ 45 years (%)	104 (72.7)	55 (77.5)	
SLN identification			
Dye alone	38 (26.6)	23 (32.4)	0.373 ^a
Combined	105 (73.4)	48 (67.6)	
Average SLNs (node)	2.2	2	0.224*
Mean tumor size, mm	20.6	23.8	0.087*
Pathological T stage, no. (%)			
pT1a	5 (3.5)	4 (5.6)	0.631 ^a
pT1b	16 (11.2)	5 (7.0)	
pT1c	61 (42.7)	26 (36.6)	
pT2	60 (42.0)	35 (49.3)	
pT3	1 (0.7)	1 (1.4)	
Histologic type, no. (%)			
Invasive ductal carcinoma	127 (88.8)	69 (97.2)	0.188 ^a
Invasive lobular carcinoma	4 (2.8)	2 (2.8)	
Mucinous carcinoma	9 (6.3)	0 (0.0)	
Medullary carcinoma	1 (0.7)	0 (0.0)	
Invasive papillary carcinoma	2 (1.4)	0 (0)	
Histologic grade, no. (%)			
Grade I	40 (28.0)	11 (15.5)	0.124 ^a
Grade II	51 (35.7)	32 (45.1)	
Grade III	52 (36.4)	28 (39.4)	
Estrogen receptor			
Positive	106 (74.1)	55 (77.5)	0.590 ^a
Negative	37 (25.9)	16 (22.5)	
Progesterone receptor			
Positive	87 (60.8)	25 (35.2)	0.586 ^a
Negative	56 (39.2)	46 (64.8)	
HER2 status			
Positive	21 (14.7)	17 (23.9)	0.242 ^a
Negative	119 (83.2)	53 (74.6)	
Unknown	3 (2.1)	1 (1.4)	
Lymphovascular invasion			
Present	25 (17.5)	37 (52.1)	0.002 ^a
Absent	117 (81.8)	33 (46.5)	
Unknown	1 (0.7)	1 (1.4)	

*, P-value by Mann-Whitney U test; ^a, P-value by Chi-Square test

NLR, which were then analyzed. The NLR (p=0.002, OR 2.5, 95%CI 1.4-4.4), tumor size (p=0.011, OR 1.9, 95%CI 1.1-3.1), presence of LVI (p=0.001, OR 6.7, 95%CI 2.4-18.9) and macrometastasis in the SLN (p=0.001, OR 6.3, 95%CI 2.2-18.4) were statistically significant predictive factors of non-SLN metastasis.

In the multivariate analysis, the NLR (p=0.033, OR 2.3, 95%CI 1.1-4.8), presence of LVI (p=0.002, OR 8.4, 95%CI 2.3-31.3) and macrometastasis in the SLN (p=0.005, OR 6.6, 95%CI 1.8-24.7) were statistically

significant predictive factors of non-SLN metastasis. (Table 3)

The area under the receiver operating characteristics (ROC) curve of NLR was 0.7 (95%CI 0.6-0.8) and is shown in Figure 1. The optimal cut-off for NLR was 2.6, giving a sensitivity of 62%, a specificity of 83.8%, a positive predictive value of 77.3% and a negative predictive value of 70.5%.

Table 2. Characteristics of Patients after SLN Metastasis

Characteristic	Non-SLN in ALND		P-value
	Negative (N=37)	Positive (N=34)	
Mean age (Years)	54.8	50.5	0.097*
< 45 years (%)	5 (13.5)	11 (32.4)	0.062 ^a
≥ 45 years (%)	32 (86.5)	23 (67.6)	
Average SLNs (node)	2.12	1.74	0.022*
Mean tumor size, mm	20.1	27.8	0.028*
Pathological T stage, no. (%)			
pT1a	4 (10.8)	0 (0.0)	0.284 ^a
pT1b	3 (8.1)	2 (5.9)	
pT1c	13 (35.1)	13 (38.2)	
pT2	17 (46.0)	18 (52.9)	
pT3	0 (0.0)	1 (2.9)	
Histologic type, no. (%)			
Invasive ductal carcinoma	37 (100.0)	32 (94.1)	0.143 ^a
Invasive lobular carcinoma	0 (0.0)	2 (5.9)	
Histologic grade, no. (%)			
Grade I	7 (19.0)	4 (11.8)	0.411 ^a
Grade II	18 (48.6)	14 (41.2)	
Grade III	12 (32.4)	16 (47.0)	
Estrogen receptor			
Positive	29 (78.4)	26 (76.5)	0.859 ^a
Negative	8 (21.6)	8 (23.5)	
Progesterone receptor			
Positive	24 (64.9)	22 (64.7)	0.982 ^a
Negative	13 (35.1)	12 (35.3)	
HER2 status			
Positive	9 (24.3)	8 (23.5)	0.620 ^a
Negative	27 (73.0)	26 (76.5)	
Unknown	1 (2.7)	0 (0.0)	
Lymphovascular invasion			
Present	12 (32.4)	25 (73.6)	0.001 ^a
Absent	25 (67.6)	8 (23.5)	
Unknown	0 (0)	1 (2.9)	
Type of SLN metastasis			
Micrometastasis	23 (62.2)	7 (20.6)	<0.001*
Macrometastasis	14 (37.8)	27 (79.4)	
Number of Positive SLN			
1	30 (81.1)	26 (76.5)	0.763 ^a
≥ 2	7 (18.9)	8 (23.5)	
Mean NLR	2	2.8	0.002*

*, P-value by Mann-Whitney U test; ^a, P-value by Chi-Square test

Discussion

The hypothesis that the systemic inflammatory response, in particular the NLR, could be predict survival in breast cancer patients is one that has attracted a lot of interest in the last decade. Elevated NLR (>3.3) was associated with larger tumors and more advanced stage (Azab et al., 2012). Lymph node metastasis and NLR (>2.5) were significantly associated with disease free

survival as well as breast cancer specific survival (Nakano et al., 2014). Patients with higher NLR (NLR ≥ 2.5) showed significantly lower disease-specific survival rates than those with lower NLR (Noh et al., 2013). A recent study, which stratified the NLR into quintiles, reported that higher NLR quintiles were significantly associated with poorer survival and elevated NLR (>4) had larger tumors, higher tumor grade, lymph node and distant metastasis (Koh et al., 2015).

Table 3. Univariate and Multivariate Analysis Predicting of Non-SLNS Metastasis

	Univariate		Multivariate	
	Odds ratio (95%CI)	P-value	Odds ratio (95%CI)	P-value
Age, ≥ 45 yr vs. < 45 yr	0.4 (0.1; 1.1)	0.069		
Average SLNs	0.7 (0.4; 1.1)	0.084		
Tumor size	1.9 (1.1; 3.1)	0.011	1.1 (0.6; 2.1)	0.773
Histologic grade, III vs. I/II	2.3 (0.6; 9.8)	0.42		
Estrogen receptor, +ve vs. -ve	0.9 (0.3; 2.7)	0.175		
Progesterone receptor +ve vs. -ve	0.9 (0.4; 2.6)	0.983		
HER2 status +ve vs. -ve	0.9 (0.3; 2.8)	0.877		
Presence of lymphovascular invasion	6.7 (2.4; 18.9)	0.001	8.4 (2.3; 31.3)	0.002
SLN macro vs. micrometastasis	6.3 (2.2; 18.4)	0.001	6.6 (1.8; 24.7)	0.005
Number of Positive SLN	1.2 (0.6; 2.6)	0.692		
Neutrophil-Lymphocyte ratio	2.5 (1.4; 4.4)	0.002	2.3 (1.1; 4.8)	0.033

The correlation between the high NLR and high stage of disease, it might be explained by lymphocytes can reduce malignant progression. Tumor infiltrative lymphocytes (TILs) have been shown to improve the survival rates of cancer patients (Ohashi et al., 2006). So, a low level of lymphocytes caused a high NLR which may indicate a relationship with high stage including axillary lymph node metastasis.

Our study shows patients with SLN metastasis, 47.8% had an additional non-SLN metastasis. Therefore, more than 50% of these patients received no benefit from ALND (Abdessalam et al., 2001; Galimberti et al., 2013). This is just one of the reasons to identify predictive factors of non-SLN metastasis.

Several studies have tried to identify the variables that are predictive of non-SLN metastasis, in selected SLN metastasis patients who meet ACOSOG Z0011 eligibility criteria, but may not however completely meet all criterias. LVI, the number of positive and negative SLNs, along with the size of SLN metastasis were identified as predictors of non-SLNs metastasis (Chen et al., 2015). As Koca (2014) defined; tumor size, extranodal extension of SLN, presence of LVI, multifocality, the number of negative SLNs, and large size of metastatic SLN were found to be independent predictive factors for non-SLN metastasis. As Ozmen (2006) defined; tumor size > 2 cm, macrometastasis in SLNs, extranodal extension of SLN were more likely to have non-SLN metastases in both univariate and multivariate analyses. Six predictors, tumor size, number of SLN identified, SLN metastasis size, number of positive SLN, extranodal extension, and histology were identified in MD Anderson nomogram for predicting the likelihood of having additional axillary metastasis (Mittendorf et al., 2012). A MSKCC nomogram was created using; pathological tumor size, tumor type, nuclear grade, LVI, multifocality, ER status, method of detection of SLN metastases, and number of positive and negative SLNs as predictors of additional non-SLNs metastasis (Van Zee et al., 2003)

In patients with additional non-SLN metastasis, almost 80% of SLN are macrometastasis while patients without non-SLN metastasis, 62% of SLN are micrometastasis. From univariate and multivariate analysis it is shown

that macrometastasis is one of the predictors of non-SLN metastasis statistical significance as reported from Ozmen (2006).

The presence of LVI is one of the predictors of non-SLN metastasis within our study with statistical significance as reported from Van zee (2003), Koca (2014) and Chen (2015).

To our knowledge in the literature this is the first study evaluating the preoperative NLR as a predictor of non-SLN metastasis in early breast cancer patients. Our study demonstrated the preoperative NLR, presence of LVI and macrometastasis in the SLN were predictive factors of non-SLN metastasis with statistically significant differences, and the AUC curve of NLR was 0.7. The optimal cut-off for NLR was 2.6 almost similar with Japan and Korea populations (Nakano et al., 2014; Koh et al., 2015).

This study had one important limitation: It was a retrospective study. Hence, future prospective studies, with a larger number of patients, are warranted.

In conclusion, the predictive factors of non-SLN metastasis in early breast cancer with SLN metastasis are a presence of LVI, macrometastasis as well as a high NLR. The NLR is information, which is commonly available and can help surgeons to make timely decisions concerning ALND for their patients.

Disclosure

No authors report any conflict of interest.

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