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Original Article

Number of negative lymph nodes should be considered for incorporation into staging for breast cancer

San-Gang Wu[1*], Yan Wang[2*], Juan Zhou[3*], Jia-Yuan Sun[2], Feng-Yan Li[2], Huan-Xin Lin[2], Zhen-Yu He[2]

[1]Xiamen City Cancer Center, Department of Radiation Oncology, The First Affiliated Hospital of Xiamen University, Xiamen, People's Republic of China; [2]Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Department of Radiation Oncology, Collaborative Innovation Center of Cancer Medicine, Guangzhou, People's Republic of China; [3]Xiamen City Cancer Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Xiamen University, Xiamen, People's Republic of China. [*]Equal contributors.

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Abstract: This study aimed to investigate the prognostic value of the number of involved lymph nodes (pN), number of removed lymph nodes (RLNs), lymph node ratio (LNR), number of negative lymph nodes (NLNs), and log odds of positive lymph nodes (LODDS) in breast cancer patients. The records of 2,515 breast cancer patients who received a mastectomy or breast-conserving surgery were retrospectively reviewed. The log-rank test was used to compare survival curves, and Cox regression analysis was performed to identify prognostic factors. The median follow-up time was 64.2 months, and the 8-year disease-free survival (DFS) and overall survival (OS) were 74.6% and 82.3%, respectively. Univariate analysis showed that pN stage, LNR, number of RLNs, and number of NLNs were significant prognostic factors for DFS and OS (all, $P < 0.05$). LODDS was a significant prognostic factor for OS ($P = 0.021$). Multivariate analysis indicated that pN stage and the number of NLNs were independent prognostic factors for DFS and OS. A higher number of NLNs was associated with higher DFS and OS, and a higher number of involved lymph nodes were associated with poorer DFS and OS. Patients with a NLNs count > 9 had better survival ($P < 0.001$). Subgroup analysis showed that the NLNs count had a prognostic value in patients with different pT stages and different lymph node status (log-rank $P < 0.05$). For breast cancer, pN stage and NLNs count have a better prognostic value compared to the RLNs count, LNR, and LODDS. Number of negative lymph nodes should be considered for incorporation into staging for breast cancer.

Keywords: Breast cancer, lymph node staging, prognosis, lymph node ratio, negative lymph nodes

Introduction

The axillary lymph node status is an important prognostic factor, and provides guides for therapy in breast cancer patients. Axillary lymph node dissection (ALND) yields critical information on the status of axillary lymph nodes. Although the survival rate is comparable between patients who receive a sentinel lymph node biopsy and those who receive an axillary lymph node dissection [1, 2], the pathologic nodal (pN) stage is still based on the number of involved lymph nodes in the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) (7th edition, 2009) staging system for breast cancer.

In recent years, studies have revealed that other parameters related to the status of lymph nodes, such as number of removed lymph nodes (RLNs), number of negative of lymph nodes (NLNs), ratio of involved to removed nodes (lymph node ratio, LNR), and log odds of positive lymph nodes (LODDS) significantly impact the prognosis of breast cancer patients [3-17]. These factors are related to the degree of axillary lymph node dissection. Theoretically, more lymph nodes collected during axillary lymph node dissection should improve survival. However, the importance of the number of RLNs in the prognosis of breast cancer is still controversial [3-5]. The LNR may serve as a supplement to the previously used pN stage in the evaluation of prognosis, and its value may be superior to that of pN stage [6-10]. In patients with gastric cancer, pancreatic cancer, and colorectal cancer studies have shown that LODDS has a clear prognostic superiority over

both pN and LNR [11-13], and may provide an approach to the modeling of nodal involvement in breast cancer. However, studies have failed to demonstrate any significant advantage over LNR [14, 15]. The number of NLNs is the difference in number between dissected lymph nodes and positive lymph nodes. More negative lymph nodes may reduce the possibility of occult lesions, and studies have shown that the number of NLNs may be used to determine prognosis [16, 17]. However, there is still controversy with respect to the prognostic value of the different lymph node staging methods in breast cancer, and to date there have been no studies comparing them. The purpose of this study was to compare the prognostic value of pN stage, LNR, NLNs, RLNs, and LODDS in patients with breast cancer.

Materials and methods

Patients

The records of patients with breast cancer who were treated at the Sun Yat-Sen University Cancer Center between January 1998 and December 2007 were retrospectively analyzed. Criteria for inclusion in the analysis were: 1) Females with pathologically confirmed unilateral invasive breast cancer; (2) Received mastectomy or breast-conserving surgery including level I-II axillary lymph node dissection; (3) Complete resection of the tumor and the surgical margin was negative; (4) Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2) status determined immunohistochemistry; and (5) Neoadjuvant chemotherapy was not performed before surgery, and corresponding therapies (chemotherapy, radiotherapy, and endocrine therapy) were given after surgery according to cancer stage and hormone receptor status. The study was approved by the ethics committee of Sun Yat-Sen University Cancer Center. All patients provided written consent for storage of their information in the hospital database, and for the research use of the information.

Clinicopathological factors and lymph node status

Clinicopathological factors and different lymph node staging methods were used to assess the risk of disease recurrence and death. Factors examined included age, menstrual status, pT stage, pN stage, and molecular subtypes (a categorical variables), and number of RLNs, number of NLNs, LNR, and LODDS (as continuous variables). ER and PR positive was defined as > 1% positive cells on immunohistochemical staining, and Her2 positive was defined as a 3+ or 2+ score on immunohistochemical evaluation, and was confirmed using a fluorescence *in-situ* hybridization (FISH) assay for Her2. The molecular subtypes were not determined according to the criteria developed in the St. Gallen International Breast Cancer Conference because some patients did not have immunohistochemistry testing for Ki-67 [18]. The categorization of breast cancer subtype was based on ER, PR, and Her2 status as follows: 1) Luminal A (ER+ or PR+, and Her2-); 2) Luminal B (ER+ or PR+, and Her2+); 3) Her2 positive (ER-, PR-, and Her2+); and 4) triple negative (ER-, PR-, and Her2-).

The pT/pN stages were determined according to the 7th edition of AJCC/UICC staging system. Stage pN1 stage was defined as metastases in 1-3 lymph nodes, stage pN2 as metastases in 4-9 lymph nodes, and stage pN3 as metastases in 10 or more lymph nodes. The number of NLNs was obtained by subtracting the number of positive lymph nodes from the total number of removed axillary lymph nodes. The LNR was defined as the ratio of the number of positive axillary lymph nodes to the number of removed axillary lymph nodes. The total number of RLNs was the total number of dissected axillary lymph nodes. The LODDS was defined as $\log_e$ ([pnod + 0.5]/[tnod + 0.5]), where pnod is the number of positive lymph nodes, tnod is the total number of lymph nodes harvested, and 0.5 is added to both numerator and denomination to avoid singularity [14].

Follow-up and survival endpoints

Follow up was performed every 3-6 months by hospital visit, telephone, or mail after surgery. The endpoints of the study were disease-free survival (DFS) and overall survival (OS). For patients with disease recurrence, survival time was determined from the date of surgery to the date of locoregional recurrence and/or distant metastasis. OS was calculated as the period from the date of diagnosis to the date of death from any cause or the date of last follow-up.

Statistical analysis

The χ^2 and Fisher's exact tests were used to analyze the differences between qualitative data. The variance inflation factor (VIF) was

Table 1. Clinical and histopathological characteristics of the 2515 patients

Characteristic	Number
Age (years)	
< 35	265
≥ 35	2250
Menopause	
Premenopause	1648
Postmenopause	867
pT stage	
T1	879
T2	1415
T3	153
T4	68
pN stage	
N0	1319
N1	802
N2	205
N3	189
ER	
Negative	1079
Positive	1439
PR	
Negative	926
Positive	1589
Her2	
Negative	1711
Positive	804
Breast cancer subtype	
Luminal A	1301
Luminal B	450
Her2 positive	354
Triple negative	410

ER, estrogen receptor; PR, progesterone receptor; Her-2, human epidermal growth factor receptor-2.

used to identify multicollinearity of pN, RLNs, NLNs, LNR, and LODDS; a VIF of ≥ 10 indicates multicollinearity. Univariate and multivariate Cox regression analyses were performed. Cut-off point analyses were then performed to determine whether there was a cut-off that was related to the greatest difference in DFS and OS. In this analysis, we sequentially dichotomized patients using thresholds of 9-16 NLNs (the interquartile range of NLN count for all patients) with an interval of 1 node (i.e., 9 or fewer nodes vs. more than 9; 10 or fewer nodes vs. more than 10; etc.), and then performed a series of log-rank tests, with the highest χ^2 statistic representing the greatest group difference. Calculation of survival rates were plotted by the Kaplan-Meier method, and compared using the log-rank test. All data were analyzed the SPSS statistical software package, version 16.0 (IBM Corporation, Armonk, NY, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Clinicopathological factors, lymph node resection, and survival

A total of 2,515 patients with a median age of 47 years (range, 14-92 years) were included in the study, and their characteristics are shown in **Table 1**. There were 2,417 patients (96.1%) who received a mastectomy, and 98 patients (3.9%) received breast-conserving surgery. Among all patients, 2,294 (91.2%) were T1/T2 stage, 1,439 (57.2%) were ER+, 1,301 (51.7%) were luminal A, and 410 (16.3%) were triple negative subtype.

The median number of RLNs was 14 (25th percentile 11, 75th percentile 18; range, 1-73). Overall, 1,319 (52.4%) patients had node-negative disease, and 1,196 (47.6%) had nodal metastases. The median number of NLNs was 12 (25th percentile 9, 75th percentile 16; range, 0-40). In patients with nodal metastases, the median number of involved lymph nodes was 2 (range, 1-67), the median number of NLNs was 11 (25th percentile 7, 75th percentile 15; range, 0-40), and the median LNR was 0.18 (range, 0.01-1.00).

A total of 2,310 patients received chemotherapy, and of these 235 received a regimen consisting of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) and 2,075 received regimens containing anthracycline and/or taxane. All patients with a positive hormone receptor status were treated with endocrine therapy using tamoxifen or an aromatase inhibitor after chemotherapy. Trastuzumab was used for 16 patients with Her2 overexpression. A total of 615 patients (24.5%) received adjuvant radiotherapy within 6 months after surgery.

The median follow-up time was 64.2 months (range, 6-144 months), and the 5-year and 8-year DFS were 80.5% and 74.6%, respective-

Table 2. Univariate analysis of prognostic factors influencing the survival of breast cancer patients

Characteristic	DFS			OS		
	Univariate			Univariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	0.636	0.497-0.814	< 0.001*	0.651	0.480-0.882	0.006*
Menopause status	1.022	0.849-1.230	0.919	1.098	0.875-1.379	0.420
pT stage	1.497	1.332-1.683	< 0.001*	1.589	1.377-1.834	< 0.001*
pN stage	1.730	1.595-1.877	< 0.001*	1.839	1.666-2.029	< 0.001*
Breast cancer subtypes	1.241	1.155-1.334	< 0.001*	1.376	1.260-1.503	< 0.001*
Number of RLNs	1.016	1.002-1.031	0.029*	1.028	1.010-1.046	0.002*
Number of NLNs	0.932	0.917-0.947	< 0.001*	0.922	0.904-0.941	< 0.001*
LODDS	1.109	0.995-1.236	0.063	1.171	1.024-1.340	0.021*
LNR	7.111	5.439-9.296	< 0.001*	8.536	6.212-11.729	< 0.001*

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; RLNs, removed lymph nodes; LODDS, log odds of positive lymph nodes; LNR, lymph node ratio; NLNs, negative lymph nodes. *$P < 0.05$, indicates a significant difference.

Table 3. Multivariate analysis of prognostic factors influencing the survival of breast cancer patients

Characteristic	DFS			OS		
	Multivariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Number of RLNs (Model 1)						
Age	0.687	0.537-0.880	0.003*	0.71	0.524-0.963	0.027*
Menopause status						
pT stage	1.250	1.102-1.418	0.001*	1.296	1.112-1.511	0.001*
pN stage	1.758	1.601-1.930	< 0.001*	1.766	1.603-1.966	< 0.001*
Breast cancer subtypes	1.288	1.197-1.385	< 0.001*	1.42	1.299-1.553	< 0.001*
Number of RLNs	0.982	0.967-0.997	0.022*	0.987	0.968-1.006	0.185
Number of NLNs (Model 2)						
Age	0.680	0.531-0.871	0.002*	0.708	0.522-0.960	0.026*
Menopause status						
pT stage	1.241	1.095-1.407	0.001*	1.29	1.107-1.503	0.001*
pN stage	1.552	1.411-1.708	< 0.001*	1.632	1.451-1.836	< 0.001*
Breast cancer subtypes	1.278	1.188-1.375	< 0.001*	1.414	1.293-1.545	<0.001*
Number of NLNs	0.970	0.954-0.987	0.001*	0.97	0.948-0.991	0.006*
LODDS (Model 3)						
Age	—	—	—	0.71	0.524-0.963	0.027*
Menopause status	—	—	—	—	—	—
pT stage	—	—	—	1.293	1.112-1.511	0.001*
pN stage	—	—	—	1.776	1.603-1.966	< 0.001*
Breast cancer subtypes	—	—	—	1.42	1.299-1.553	< 0.001*
LODDS	—	—	—	1.106	0.943-1.296	0.216
LNR (Model 4)						
Age	0.691	0.539-0.885	0.003*	0.724	0.534-0.983	0.037*
Menopause status						
pT stage	1.242	1.096-1.407	0.001*	1.292	1.109-1.504	0.001*
pN stage	1.300	1.086-1.557	0.004*	1.346	1.082-1.674	0.008*
Breast cancer subtypes	1.270	1.180-1.366	< 0.001*	1.401	1.281-1.532	< 0.001*
LNR	2.758	1.490-5.110	0.001*	2.909	1.398-6.056	0.004*
Different lymph node staging methods (Model 5)						
Age	0.681	0.532-0.872	0.002*	0.709	0.523-0.961	0.027*
Menopause status						
pT stage	1.244	1.098-1.411	0.001*	1.293	1.110-1.507	0.001*
pN stage	1.545	1.403-1.702	< 0.001*	1.625	1.443-1.830	< 0.001*

Breast cancer subtype	1.278	1.188-1.374	< 0.001*	1.413	1.293-1.545	< 0.001*
Number of RLNs	1.001	0.975-1.028	0.919	1.008	0.979-1.039	0.582
Number of NLNs	0.969	0.952-0.986	<0.001*	0.969	0.947-0.991	0.005*
LODDS	—	—	—	1.038	0.882-1.222	0.651
LNR	1.588	0.630-4.002	0.327	1.571	0.513-4.813	0.429

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; RLNs, removed lymph nodes; LODDS, log odds of positive lymph nodes; LNR, lymph node ratio; NLNs, negative lymph nodes. *$P < .05$, indicates a significant difference.

Table 4. Cutoff point analyses of the relationship between negative lymph node count and survival

Dichotomized number of NLNs	Number	DFS			OS		
		Number of Events	χ^2	*P*	Number of Events	χ^2	*P*
> 9 vs. ≤ 9	733 vs. 1782	220 vs. 276	66.245	< 0.001*	152 vs. 171	47.835	< 0.001*
> 10 vs. ≤ 10	897 vs. 1618	253 vs. 243	57.150	< 0.001*	170 vs. 153	34.861	< 0.001*
> 11 vs. ≤ 11	1102 vs. 1413	282 vs. 214	36.374	< 0.001*	186 vs. 137	19.649	< 0.001*
> 12 vs. ≤ 12	1325 vs. 1190	326 vs. 170	33.484	< 0.001*	213 vs. 110	16.110	< 0.001*
> 13 vs. ≤ 13	1500 vs. 1015	353 vs. 143	24.525	< 0.001*	232 vs. 91	12.346	< 0.001*
> 14 vs. ≤ 14	1702 vs. 813	381 vs. 115	17.045	< 0.001*	248 vs. 75	7.084	0.008
> 15 vs. ≤ 15	1848 vs. 667	415 vs. 81	25.508	< 0.001*	1848 vs. 667	15.666	< 0.001*
> 16 vs. ≤ 16	1989 vs. 526	429 vs.67	14.702	< 0.001*	284 vs. 39	10.420	0.001

DFS, disease-free survival; OS, overall survival; NLNs, negative lymph nodes. *$P < .05$, indicates a significant difference.

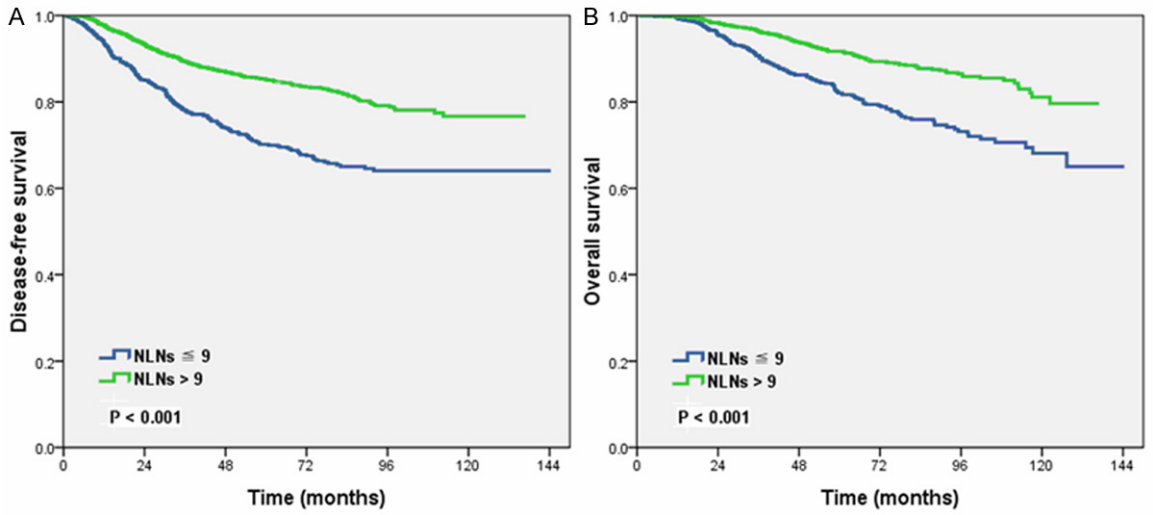

Figure 1. Impact of the number of negative lymph nodes on disease-free survival (A) and overall survival (B).

ly. The 5-year and 8-year OS were 88.8% and 82.3%, respectively.

Prognosis

The VIF values for pN stage, RLNs, LNR, NLNs, and LODDS were 5.306, 6.074, 8.452, 7.998, and 1.176, respectively. Thus, no multicollinearity was identified.

Univariate Cox survival analysis showed that pN stage, LNR, RLN count, and NLN count were significant prognostic factors for DFS and OS (all, $P < 0.05$). In addition, LODDS was a significant prognostic factor for OS ($P = 0.021$), but not for DFS ($P = 0.063$). Other significant prognostic factors included age, pT stage, and molecular subtype (all, $P < 0.05$) (**Table 2**).

Multivariate Cox analysis adjusted for significant factors from the univariate analysis was used to assess the association of survival with RLNs (Model 1), NLNs (Model 2), LOODS (Model 3), and LNR (Model 4), separately, and combined together (Model 5). The analysis showed that pN, RLNs, NLNs, and LNR were significant

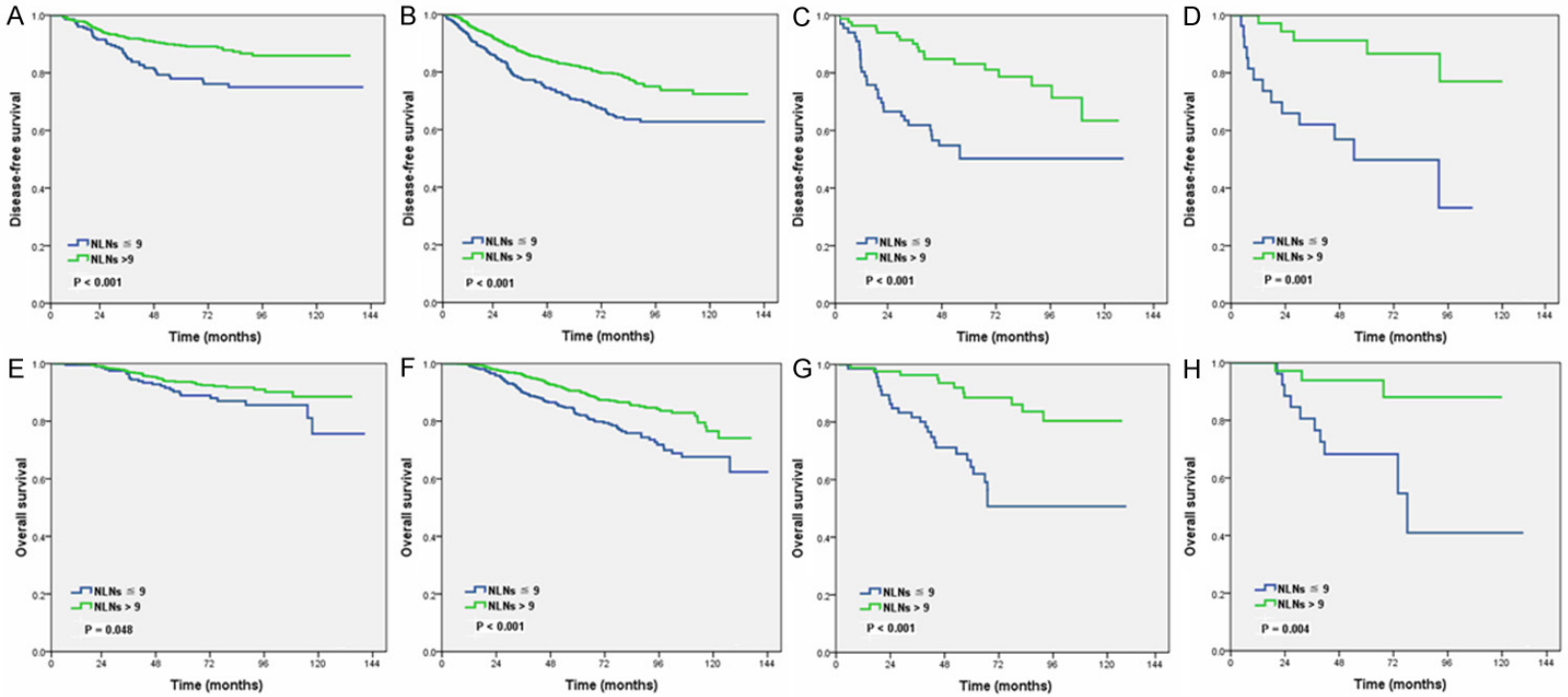

Figure 2. Impact of the number of negative lymph nodes on the disease-free survival of pT1 (A), pT2 (B), pT3 (C), and pT4 (D) stage patients, and overall survival of pT1 (E), pT2 (F), pT3 (G), and pT4 (H) stage patients.

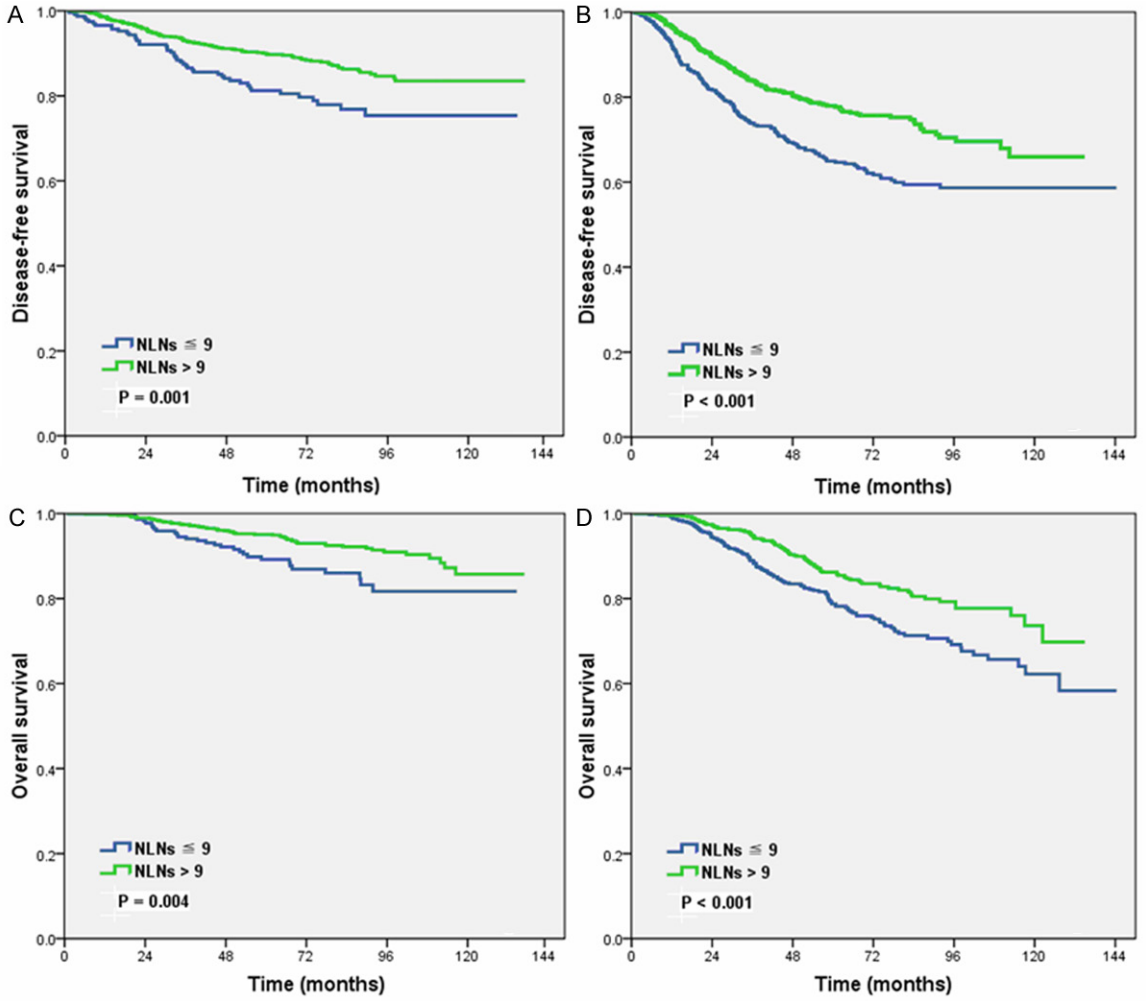

Figure 3. Impact of the number of negative lymph nodes on the disease-free survival of pN0 stage patients (A) and pN positive patients (B), overall survival of pN0 stage patients (C) and pN positive patients (D).

prognostic factors for DFS in Model 1, 2, and 4, but in Model 5, a higher number of NLNs was associated with better DFS (hazard ratio [HR] = 0.578, 95% confidence interval [CI]: 0.467-0.716, $P < 0.001$) and a higher number of involved lymph nodes was associated with poorer DFS, but RLN count and LNR exhibited no effect on DFS ($P > 0.05$) (**Table 3**).

In Model 1, 2, 3, and 4, pN stage, NLN count, and LNR were significant prognostic factors for OS, but RLN count and LODDS were not. When in Model 5, the pN stage and the number of NLNs remained an in dependent prognostic factor for OS, a higher number of NLNs was associated with better OS (HR = 0.578, 95% CI: 0.467-0.716, $P < 0.001$), and a higher number of involved lymph nodes was associated with poorer OS, but RLNs, LNR, and LODDS exhibited no effect on OS ($P > 0.05$) (**Table 3**).

Identification of optimal cut-off points

The optimal cut-off points for the number of NLNs were analyzed for all patients (**Table 4**). The results showed that 9 was the optimal cut-off point for the number of NLNs for all patients with respect to DFS ($\chi^2 = 66.245$, $P < 0.001$) and OS ($\chi^2 = 47.835$, $P < 0.001$) (**Figure 1**).

Effect of the number of NLNs on DFS and OS by pT stage

Whether the prognostic effect of the number of NLNs on DFS and OS was affected by pT stage was determined. Regardless of pT stage, a higher number of NLNs correlated with better

DFS and OS (all, $P < 0.05$ log-rank test) (**Figure 2**).

Effect of the number of NLNs on DFS and OS by lymph node stage

The prognostic effect of the number of NLNs was examined according to pN stage. pN0 and pN-positive patients, a higher number of NLNs correlated with better DFS and OS (all, $P < 0.05$ log-rank test) (**Figure 3**).

Discussion

In present study, we assessed the survival of Chinese patients with breast cancer to determine the prognostic value of different lymph node staging methods. The results showed that the number of involved lymph nodes and the number of NLNs were independent prognostic factors for DFS and OS, but RLN count, LNR, and LODDS did not influence prognosis.

The pN stage is an important factor used to evaluate the prognosis of breast cancer patients. However, the number of dissected lymph nodes may influence pN staging and subsequently affect the selection of auxiliary therapies and the prognosis of breast cancer patients. Thus, some investigators propose the concept of "stage migration", i.e., the dissection of more lymph nodes may provide more accurate information on lymph node status, which is helpful for pN staging and reduces the possibility of inaccurate staging. Schaapveld et al [19] found that patients with more dissected lymph nodes had better survival, supporting the notion of stage migration. Currently, studies show that the number of NLNs may also influence the prognosis of breast cancer patients [16, 17], and this finding is also observed in studies of esophageal cancer, cervical cancer, and colorectal cancer [16, 17, 20-22]. This may be explained because more NLNs are helpful to identify micrometastases and reduce the possibility of potential residual lesions. This also explains why patients with more NLNs had better survival, and also supports the hypothesis of stage migration.

Studies, including ours, have shown that the LNR is an important prognostic factor in patients with breast cancer [6-10]. In Model 4 of the present study, multivariate analysis showed that LNR was an independent determinant of breast cancer prognosis. However, when other methods used for lymph node staging were introduced into the multivariate analysis, LNR the prognostic value of NLN count was better than that of LNR. Kuru et al [16]. reported that the number of involved lymph nodes, RLN count, NLN count, and LNR were prognostic factors of breast cancer patients with positive lymph nodes, and patients with a NLN count > 15 had a better prognosis. Karlsson et al [17]. also reported that NLN count was an independent prognostic factor in breast cancer patients, and a NLN count of ≥ 10 was associated with a better prognosis as compared to a NLN count of < 10, but it had no influence on prognosis when axillary lymph nodes were negative [17]. In the current study, the number of NLNs had prognostic value not only in patients with positive lymph nodes, but also in those with negative lymph nodes. Previous studies have shown that the number of NLNs in breast cancer patients may represent a balance between host cells and cancer cells, and might influence the presence of circulating cancer cells [23]. In studies on colorectal cancer, the number of NLNs was related to the host immune response to cancer cells and the molecular biology of cancer cells [24, 25]. This may be another mechanism underlying the influence of NLN count on the prognosis of breast cancer. Breast cancer patients with more positive axillary lymph nodes are more susceptible to disease failure, and those with more negative lymph nodes may further avoid recurrence and metastasis. This may explain why patients with a higher pN stage had a poorer prognosis, and patients with more NLNs had a better prognosis in this study.

Studies have shown that axillary lymph node dissection may not affect the survival of breast cancer patients who receive breast-conserving therapy and have negative or 1-2 positive sentinel lymph nodes, on the basis of which investigators have proposed that axillary lymph node dissection should not be performed [1, 2]. Axillary lymph node dissection, however, remains controversial in breast cancer patients. In 2013, the 13th St. Gallen International Breast Cancer Consensus Conference recommended that sentinel lymph node biopsy in certain breast cancer patients, and axillary lymph node dissection should be avoided [18]. In our study, a majority of breast cancer patients received

complete mastectomy (96.1%), and axillary lymph node dissection was simultaneously performed, which was outside the Z0011 selection criteria (i.e., who still need ALND). Our results showed that in patients with different pT stages, and those with positive lymph nodes, a larger number of NLNs was associated with lower mortality. Furthermore, a more extensive axillary dissection may lead to unnecessary morbidity, and thus the balance between risks and benefits of more extensive axillary surgery must be considered on a patient-by-patient basis.

There are limitations of this study. This was a retrospective study conducted at a single center, and thus the findings may not be applicable to the general population. In addition, the optimal number of NLNs is not consistent with previous reports, which may be ascribed to differences in clinical characteristics and surgical patterns. More prospective, multicenter studies are required to confirm the exact value of the number of NLNs and the optimal cut-off point in breast cancer patients.

In conclusions, the results show that pN stage and NLN count have a better prognostic value compared to RLN count, LNR, and LODDS in patients with breast cancer. The number of NLNs is an important prognostic factors of breast cancer patients, and patients with more NLNs have a better prognosis. pN staging after addition of NLN count may be a better guide for the determination of prognosis of breast cancer patients.

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Disclosure of conflict of interest

No any actual or potential conflicts of interest exist.

Abbreviations

pN, pathologic nodal; UICC/AJCC, Union for International Cancer Control/American Joint Committee on Cancer; RLNs, removed lymph nodes; NLNs, negative of lymph nodes; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes; ER, estrogen receptor; PR, progesterone receptor; Her2, human epidermal growth factor receptor 2; FISH, fluorescence *in-situ* hybridization; DFS, disease-free survival; OS, overall survival; VIF, variance inflation factor; CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; HR, hazard ratio; CI, confidence interval; ALND, axillary lymph node dissection.

Address correspondence to: Zhen-Yu He, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Department of Radiation Oncology, Collaborative Innovation Center of Cancer Medicine, Guangzhou, People's Republic of China. Tel: +86 20 87343543; Fax: +86 20 87343392; E-mail: hezhy@sysucc.org.cn

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