RESEARCH ARTICLE

Metastatic Axillary Lymph Node Ratio (LNR) is Prognostically Superior to pN Staging in Patients with Breast Cancer -- Results for 804 Chinese Patients from a Single Institution

Xiang-Sheng Xiao[1,2], Hai-Lin Tang[1,2], Xin-Hua Xie[1,2], Lai-Sheng Li[3], Ya-Nan Kong[1,2], Min-Qing Wu[1,2], Lu Yang[1,2], Jie Gao[1,2], Wei-Dong Wei[1,2]*, Xiaoming Xie[1,2]*

Abstract

The number of axillary lymph nodes involved and retrieved are important prognostic factors in breast cancer. The purpose of our study was to investigate whether the lymph node ratio (LNR) is a better prognostic factor in predicting disease-free survival (DFS) for breast cancer patients as compared with pN staging. The analysis was based on 804 breast cancer patients who had underwent axillary lymph node dissection between 1999 and 2008 in Sun Yat-Sen University Cancer Center. Optimal cutoff points of LNR were calculated using X-tile software and validated by bootstrapping. Patients were then divided into three groups (low-, intermediate-, and high-risk) according to the cutoff points. Predicting risk factors for relapse were performed according to Cox proportional hazards analysis. DFS was estimated using the Kaplan-Meier method and compared by the log-rank test. The 5-year DFS rate decreased significantly with increasing LNRs and pN. Univariate analysis found that the pT , pN, LNR, molecule type, HER2, pTNM stage and radiotherapy well classified patients with significantly different prognosis. By multivariate analysis, only LNR classification was retained as an independent prognostic factor. Furthermore, there was a significant prognostic difference among different LNR categories for pN2 category, but no apparent prognostic difference was seen between different pN categories in any LNR category. Therefore, LNR rather than pN staging is preferable in predicting DFS in node positive breast cancer patients, and routine clinical decision-making should take the LNR into consideration.

Keywords: Breast cancer - LNR - prognostic factors - DFS

Asian Pac J Cancer Prev, **14 (9)**, 5219-5223

Introduction

Breast cancer is the most commonly diagnosed cancer among women in China and numerous other parts of the world. Axillary lymph nodes (ALN) status is one of the most important prognostic factors in breast cancer and the number of involved nodes has been incorporated into routine clinical decision making (Fitzgibbons et al., 2000). According to guidelines of the 7th edition American Joint Committee on Cancer (AJCC) staging system, patients with different positive ALNS have been classified as various pN disease (pN0: zero positive node, pN1: 1–3 positive nodes, pN2: 4-9 positive nodes and pN3: ≥ 10 positive nodes) (Singletary et al., 2002; Vinh-Hung et al., 2003). Recently, however, a few studies have suggested that, not only the number of postive nodes but also the total number of lymph nodes removed should be taken into consideration. Therefore, a new prognostic factor of lymph node ratio (LNR), which was defined as the ratio of the number of positive to total removed lymph nodes, was proposed (Vinh-Hung et al., 2004; Yildirim and Berberoglu, 2007; Tausch et al., 2012; Zhu and Wu, 2012). Some studies have revealed that the LNR can improve prognostic information and reduce the stage migration as compared with the pN staging (Ahn et al., 2011; Chagpar et al., 2011; Peparini and Chirletti, 2011).

In many of the above studies, however, additional factors affecting DFS, such as tumor receptor status, HER2 expression, and use of neoadjuvant or adjuvant treatment regimens, were not considered. The cutoff values were often various in different institutions as well. What's more, up to now, the recommendation of using LNR as an alternative to the current pN staging has no fomal guideline to follow. Hence, in this article, we sought to determine the prognostic impact of LNR in comparison to pN staging and other established prognostic factors in Chinese breast cancer patients for the first time. Based on the caculated optimal cutoff values, we then further evaluated the independent prognostic value of LNR compared to pN staging in predicting DFS of breast cancer.

*[1]Department of Breast Oncology, [2]State Key Laboratory of Oncology in South China, Sun Yat-Sen University Cancer Center, [3]Department of Laboratory Medicine, The First Affliated Hospital of Sun Yat-Sen University, Guangzhou, China *For correspondence: xiexm@sysucc.org.cn*

Materials and Methods

This study population consisted of 804 female patients who were confirmed to have the diagnosis of nonmetastatic primary invasive breast cancer and have underwent axillary lymph nodes dissection at Sun Yat-Sen University Cancer Center from 1999 to 2008. After institutional review board approval, we used medical records and tumor registry information to collect data on these patients. Patients were excluded from this study who (1) had distant metastases at diagnosis; (2) had incomplete pathological data entries; (3) underwent neoadjuvant treatment; (4) were lost during follw-up; and (5) had secondary malignancies except for non-melanoma skin cancer or in situ cervix cancer. Patients received treatment according to the standard care. Patient-related characteristics (age, body mass index, pT stage, pN stage, LNR, pTNM stage, estrogen/progesterone receptor status, and human epidermal growth factor receptor 2 status), and treatment-related factors (chemotherapy, radiotherapy and hormone therapy) were analyzed.

Postoperative follow-up included physical and laboratory examinations every 3 months for the first 3 years, every 6 months for the fourth and fifth years, and annually after 5 years. End point for survival analyses was disease-free survival (DFS), which was defined as time from operation to documentation of the first of the following events: any recurrence (local, regional, or distant) of breast cancer, a contralateral breast cancer, a second primary cancer, or death due to any cause. Patients who were alive without any of these events were censored at the date of their last disease evaluation. Follow-up was provided for the entire study population until July 2011.

According to the AJCC seventh edition staging system, all of our cases were restaged. The clinicopathologic characteristics associated with survival outcomes were analyzed. The optimal cutoff points of LNR were determined by the X-tile software (http://www.tissuearray.org/rimmlab/) and validated by bootstrap method (Wasserman and Bockenholt, 1989; Camp et al., 2004; Zuo et al., 2010). In our cohort, the caculated cutoff points from X-tile for LNR were 0, 0.30 and 0.81. For convenience use in everyday clinical practice, we adjusted the cutoff point of 0.81 to 0.80. Then four subgroups (LNR0, 0%; LNR1, 1%-30%; LNR2, 31%-80%; and LNR3, 81%-100%) were obtained for our study.

Statistical analysis

Comparisons were made by chi-square analysis for categorical variables and 2-sided t tests for continuous variables. A Cox proportional hazards model was used to compare parameters and to adjust for known prognostic clinical and pathological variables. DFS was calculated using the Kaplan–Meier method and the differences in survival time were estimated by the log-rank test. A multivariate Cox regression analysis (forward stepwise) was carried out to compare and identify independent prognostic factors. The 95% confidences interval (95% CI) was used to quantify the relationship between survival time and each independent factor. Differences were considered to be significant if the *P* values from a

Table 1. Characteristics of Patients and Tumors

Characteristics	No. of patients	5-year diseast free survival rate (%)	*P*
All	804		
Age (years)			0.263
≤ 35	113	94.3	
> 35	691	89.6	
BMI			0.185
≤ 25	576	90.7	
> 25	207	89.2	
T stage			<0.001
T1	134	93.7	
T2	568	91.1	
T3	102	80.8	
N stage (%)			<0.001
N0	443	97.1	
N1	196	92.5	
N2	101	70.2	
N3	64	65.9	
Nodes examined			0.294
≤15	467	91.3	
>15	337	88.8	
LNR (%)			<0.001
LNR0	443	97.1	
LNR1	223	92.4	
LNR2	98	68.9	
LNR3	40	52	
ER status (%)			0.539
Negative	327	90.1	
Positive	443	90.4	
Unknown	9	90	
PR status (%)			0.375
Negative	289	89.7	
Positive	481	90.6	
Unknown	14	92.6	
HR status (%)			0.411
Negative	234	90.1	
Positive	536	90.4	
Unknown	34	92.8	
HER2 status (%)			<0.05
Negative	492	88.3	
Positive	94	93	
Unknown	218	93.3	
Molecular type			<0.05
Luminal A	390	89.2	
Luminal B	18	94.4	
Basal-like	89	86.4	
ERBB2+	86	91.2	
Unknown	221	92.9	
pTNM stage			<0.001
I	171	96.9	
II	449	93.6	
III	184	75.3	
Radiotherapy			<0.001
No	626	92.2	
Yes	173	84.5	
Unknown	5	60	
Chemotherapy			0.84
No	66	95.1	
Yes	726	89.8	
Unknown	12	90.9	
Hormone-therapy			0.413
No	261	91.1	
Yes	474	89.7	
Unknown	69	92.2	

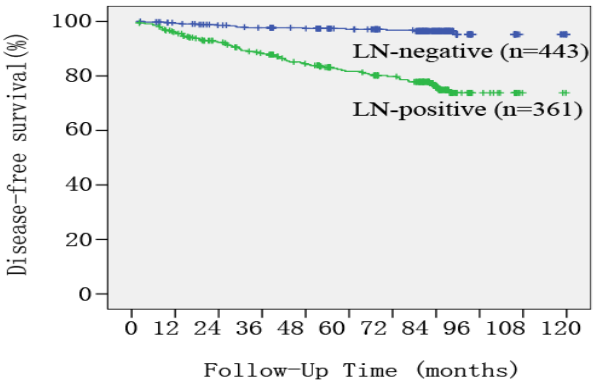

Figure 1. Kaplan-Meier DFS for Patients with Breast Cancer According to Lymph Node Status

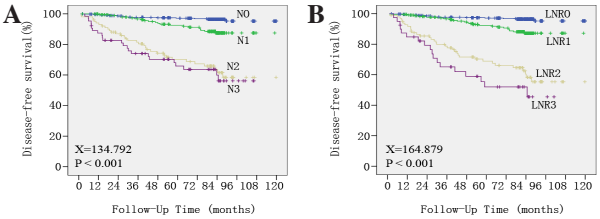

Figure 2. Kaplan-Meier Survival Estimates According to Risk Groups: (A) risk groups defined by pN; (B) risk groups defined by lymph node ratio (LNR)

two-tailed test was less than 0.05. All statistical analyses were conducted with SPSS software version 18.0 (SPSS, Chicago, IL).

Results

There were 804 cases with breast cancer identified for analysis and the corresponding clinicopathological characteristics were shown in Table 1. The 5-year disease-free survival rate for patients with pN positive or negative were 96.6% and 80.0%, respectively, and 713 patients were alive when our follow-up was completed (Figure 1).

Univariate and multivariate survival analyses

Seven factors were found to have statistically significant associations with the disease-free survival on univariate analysis, which were pathological T (pT), pN, LNR, HER2 status, Molecular type, pTNM stage and Radiotherapy (Table 1). All the aforementioned 7 variables were included in a multivariate Cox proportional hazards model to adjust for the effects of covariates. Based on the number of pN stage, 196 patients are classified as pN1, 101 as pN2 and 64 as pN3. Using the LNR stage, 223 patients are classified as LNR1, 98 as LNR2 and 40 as LNR3. The 5-year survival rates according to each pN and LNR stage are shown in Figure 2. With both the pN and the LNR stage, the Kaplan–Meier plots showed a good discriminatory ability (Figure 2A and 2B). The 5-year disease-free survival(DFS) rates of LNR0, LNR1, LNR2, and LNR3 patients were 97.1%, 92.4%, 68.9%, and 52.0%, respectively (log-rank $x^2 = 164.879$). In addition, pN classification showed an imbalance in prognostic separation, with the pN2 and pN3 survival curves closed to each other, while the LNR curves remained separated exceeding 5 years. For patients in the pN2 category, the

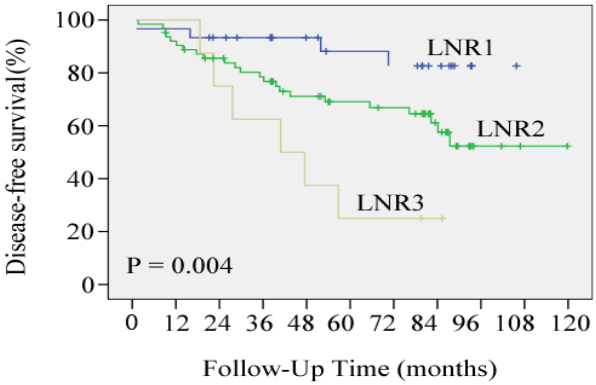

Figure 3. Comparison of Survival Curves for Patients with pN2 Stage Stratified by LNR

Table 2. DFS Rates on the Basis of pN Category According to the LNR Category

	LNR1		LNR2		LNR3		P^a
	No.of patients	5-year DFS rate (%)	No.of patients	5-year DFS rate (%)	No.of patients	5-year DFS rate (%)	
pN1	192	93	4	66.7			0.182
pN2	30	88.1	63	69.1	8	25	0.004
pN3	1	100	31	68.9	32	61.5	0.682
P^b	0.641		0.928		0.186		

Table 3. Effect of LNR and pN Staging on Breast Cancer DFS among Patients with Lymph Node Positive Breast Cancer

Variable	Hazard Ratio	95% CI	*P*
LNR			<0.0001
LNR1, ≤0.30	1	Reference	
LNR2, >0.30 and ≤0.80	4.211	2.486 to 7.130	
LNR3, >0.80	6.324	3.407 to 11.741	
pN			<0.0001
pN1	1	Reference	
pN2	3.921	2.248 to 6.839	
pN3	4.61	2.530 to 8.402	

five year DFS rates for LNR1-3 were 88.1%, 69.1%, and 25.0%, respectively ($P<0.05$; Table 2 and Figure 3). However, when we compared the survival rates among the pN categories in different LNR categories, there was no significant prognostic difference between patients in different pN categories for any any LNR categories. By comparison, the 5 years DFS rates for patients with pN0, pN1, pN2, and pN3 stage were 97.1%, 92.5%, 70.2%, and 65.9%, respectively (log-rank $x^2 = 134.792$). Compared with patients with pN1 stage, the adjusted hazard ratio of DFS was 4.211 for patients with pN2 and 6.324 for patients with pN3, while 3.921 for patients with LNR2 and 6.324 for patients with LNR3 compared with patients with LNR1 stage.

In multivariate survival analyses, we demonstrated that only LNR remained independent prognostic factors (data not shown).

Discussion

Although the current TNM classification system is still the mainstay of breast cancer staging, the role of the total number of lymph nodes retrieved is still unknown

(Li et al., 2012; Wang et al., 2012). To ensure optimal treatment for individual breast cancer patients in this era of personalized medicine, it is necessary to escalate the use of AJCC staging system. Recently, the concept of LNR has aroused deep concern around the world and the role of pN has been challenged (Kim et al., 2011). The positive nodes harvested in the axilla are dependent on the surgeon (technique and philosophy), the pathologist (specimen evaluation technique and philosophy), and the patient (tumor site, T stage, immune response, and age) (Chen et al., 2011). So, any of the above factors could affect the pN staging, and then patients may be inaccurately staged.

However, the LNR-namely, the ratio of the number of positive to total removed lymph nodes-attempts to standardize against the inconsistency and variability of nodal assessment. Previous studies have demonstrated that the effects of LNR staging is superior to that of pN staging in predicting prognosis of patients for many cancer sites: colon (Chen et al., 2011), gastric (Wang et al., 2011; Yu et al., 2011), pancreas (Pawlik et al., 2007; Keck et al., 2012), esophagus (Greenstein et al., 2008), corpus uteri (Chan et al., 2007) and bladder (Herr, 2003).

As expected, in our study, we demostrated that the power of LNR was much better than pN in predicting the breast cancer DFS. Then, for the first time in Chinese breast cancer patients, we proposed a categorization of the LNR which was validated by bootstrap resampling among patients. Patients in our study were then classified into four groups (LNR0, 0%; LNR1, 1%-30%; LNR2, 31%-80%; and LNR3, 81%-100%).

Our cutoff points that would be required for a staging classification are different from previous studies. Tsuchiya A et al. retrospectively reviewed the data for 37 breast cancer patients treated between 1987 and 1995. The corresponding cutoff points of LNR were 0.10 and 0.50 (Tsuchiya et al., 1997). Walker MJ et al. retrospectively reviewed the records of 141 patients who had breast cancer with > 10 positive nodes and they defined the cutoff points with 0.50 and 0.75 (Walker et al., 1995) Voordeckers M et al. also demostrated that the LNR was the most important factor in 810 breast cancer patients who are lymph node-positive, the LNR were categorized into three groups by 0.10 and 0.50 (Voordeckers et al., 2004). Vinh-Hung et al. analyzed 1,827 breast cancer patients with all T and N stages and demostrated prognostic importance of LNR, and then categorized the LNR into different risk groups with 0.20 and 0.65 (Vinh-Hung et al., 2009). To identify cutoff points that should be stable for specific population, we chose the X-tile software method to calculate the cutoff points and validated them with bootstrap method. In our cohort, the caculated cutoff points from X-tile for LNR were compatible with our cancer center population. Therefore, in distrinct areas, the cutoffs should be modified when the LNR was the significant factor predicting prognosis for breast cancer patients.

Moreover, the LNR in our research could discriminate subsets of patients with similar prognosis in pN staging: the five years DFS of LNR 1-3 were 88.1%, 69.1%, and 25.0% for patients in the pN2 category, respectively (P<0.05). These findings showed the ability of LNR classification to neutralize the risk of stage migration.

However, we acknowledge several limitations in our study. Firstly, it was retrospective and single center, then it might lead to bias in patients seletion. Moreover, the overall survival time would be obtained in the future. Therefore, the independent validation of LNR as a prognostic factor in breast cancer awaits further prospective clinical trials.

In conclusions, the LNR staging identified three cutoff points which could define breast cancer prognosis more accurately than the pN staging. As the LNR staging is an available and inexpensive prognostic factor for patients with breast cancer, therefore, it should be incorporated into the current AJCC staging system in future.

Acknowledgements

This research is supported by the Key Program of National Natural Science Foundation of China (31030061); National Natural Science Foundation of China (81272514).

References

Ahn SH, Kim HJ, Lee JW, et al (2011). Lymph node ratio and pN staging in patients with node-positive breast cancer: a report from the Korean breast cancer society. *Breast Cancer Res Treat*, **130**, 507-15.

Camp RL, Dolled-Filhart M, Rimm DL (2004). X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res*, **10**, 7252-9.

Chagpar AB, Camp RL, Rimm DL (2011). Lymph node ratio should be considered for incorporation into staging for breast cancer. *Ann Surg Oncol*, **18**, 3143-8.

Chan JK, Kapp DS, Cheung MK, et al (2007). The impact of the absolute number and ratio of positive lymph nodes on survival of endometrioid uterine cancer patients. *Br J Cancer,* **97**, 605-11.

Chen SL, Steele SR, Eberhardt J, et al (2011). Lymph node ratio as a quality and prognostic indicator in stage III colon cancer. *Ann Surg*, **253**, 82-7.

Fitzgibbons PL, Page DL, Weaver D, et al (2000). Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med*, **124**, 966-78.

Greenstein AJ, Litle VR, Swanson SJ, et al (2008). Prognostic significance of the number of lymph node metastases in esophageal cancer. *J Am Coll Surg*, **206**, 239-46.

Herr HW (2003). Superiority of ratio based lymph node staging for bladder cancer. *J Urol*, **169**, 943-5.

Keck T, Kusters S, Wellner UF, Hopt UT, Karcz KW (2012). Total laparoscopic partial pancreatoduodenectomy and reconstruction via laparoscopic pancreatogastrostomy. *Langenbecks Arch Surg,* **397**, 1009-12.

Kim JY, Ryu MR, Choi BO, et al (2011). The prognostic significance of the lymph node ratio in axillary lymph node positive breast cancer. *J Breast Cancer*, **14**, 204-12.

Li Y, Holmes E, Shah K, et al (2012). The prognostic value of lymph node cross-sectional cancer area in node-positive breast cancer: a comparison with N stage and lymph node ratio. *Patholog Res Int,* **2012**, 161964.

Pawlik TM, Gleisner AL, Cameron JL, et al (2007). Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. *Surgery*, **141**, 610-8.

Peparini N, Chirletti P (2011). Lymph node ratio, number of

excised nodes and sentinel-node concepts in breast cancer. *Breast Cancer Res Treat*, **126**, 829-33.
Singletary SE, Allred C, Ashley P, et al (2002). Revision of the American Joint Committee on Cancer staging system for breast cancer. *J Clin Oncol*, **20**, 3628-36.
Tausch C, Taucher S, Dubsky P, et al (2012). Prognostic value of number of removed lymph nodes, number of involved lymph nodes, and lymph node ratio in 7502 breast cancer patients enrolled onto trials of the Austrian Breast and Colorectal Cancer Study Group (ABCSG). *Ann Surg Oncol*, **19**, 1808-17.
Tsuchiya A, Kanno M, Abe R (1997). The impact of lymph node metastases on the survival of breast cancer patients with ten or more positive lymph nodes. *Surg Today*, **27**, 902-6.
Vinh-Hung V, Burzykowski T, Cserni G, et al (2003). Functional form of the effect of the numbers of axillary nodes on survival in early breast cancer. *Int J Oncol*, **22**, 697-704.
Vinh-Hung V, Verkooijen HM, Fioretta G, et al (2009). Lymph node ratio as an alternative to pN staging in node-positive breast cancer. *J Clin Oncol*, **27**, 1062-8.
Vinh-Hung V, Verschraegen C, Promish DI, et al (2004). Ratios of involved nodes in early breast cancer. *Breast Cancer Res*, **6**, R680-8.
Voordeckers M, Vinh-Hung V, Van de Steene J, Lamote J, Storme G (2004). The lymph node ratio as prognostic factor in node-positive breast cancer. *Radiother Oncol*, **70**, 225-30.
Walker MJ, Osborne MD, Young DC, et al (1995). The natural history of breast cancer with more than 10 positive nodes. *Am J Surg*, **169**, 575-9.
Wang F, He W, Qiu H, et al (2012). Lymph node ratio and pN staging show different superiority as prognostic predictors depending on the number of lymph nodes dissected in Chinese patients with luminal A breast cancer. *Clin Breast Cancer*, **12**, 404-11.
Wang W, Xu DZ, Li YF, et al (2011). Tumor-ratio-metastasis staging system as an alternative to the 7th edition UICC TNM system in gastric cancer after D2 resection--results of a single-institution study of 1343 Chinese patients. *Ann Oncol*, **22**, 2049-56.
Wasserman S, Bockenholt U (1989). Bootstrapping: applications to psychophysiology. *Psychophysiology*, **26**, 208-21.
Yildirim E, Berberoglu U (2007). Lymph node ratio is more valuable than level III involvement for prediction of outcome in node-positive breast carcinoma patients. *World J Surg*, **31**, 276-89.
Yu CJ, He WT, Shi W, Shu YQ, Liu P (2011). Prognostic value of metastatic lymph node ratio for patients with gastric cancer. *Zhonghua Wei Chang Wai Ke Za Zhi*, **14**, 516-9.
Zhu C, Wu XZ (2012). Proposal of new classification for stage III breast cancer on the number and ratio of metastatic lymph nodes. *J Surg Oncol*, **106**, 696-702.
Zuo G, Xu Z, Yu H, Hao B (2010). Jackknife and bootstrap tests of the composition vector trees. *Genomics Proteomics Bioinformatics*, **8**, 262-7.