RESEARCH ARTICLE

Lymph Node Ratio is More Predictive than Traditional Lymph Node Stratification in Lymph Node Positive Invasive Breast Cancer

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Abstract

Objective: To evaluate the relationships between lymph node ratio (LNR, the ratio of positive lymph nodes in excised axillary lymph nodes) and disease-free survival (DFS) by comparing with traditional absolute positive lymph node number (pN classification) for prediction of breast cancer (BC) progrnosis. Methods and Patients: We retrospectively reviewed patients who received comprehensive therapy in Department of Breast Surgery, Hubei Cancer Hospital, China from Jan 2002 to Dec 2006 (Group A), and Department of Breast and Thyroid Surgery, Renmin Hospital of Wuhan University, China from Jun 2008 to May 2012 (Group B). Patients were allocated to low-risk (≤0.20), intermediate-risk (> 0.20 but ≤ 0.65), high-risk (>0.65) groups by LNR. The primary endpoint was 5-DFS. Results: A total of 294 patients were included in our study. LNR was verified as a negative prognostic factor for DFS (*P*= 0.002 in Group A, *P*< 0.0001 in Group B). Then we found the effects of pN and LNR delamination on disease-free survival (DFS) had statistical significance (*P*=0.012 for pN and *P*=0.031 for LNR stratification in Group A, both of them *P*<0.001 in Group B). Compared to pN staging, LNR staging displayed superior performance in prognosis, the adjusted hazard ratio of recurrence being 2.07 (95%CI, 1.07 to 4.0) for intermediate risk group (*P*=0.030) and 2.44 (95%CI, 1.21 to 4.92) for high risk group (*P*=0.013) in Group A. Conclusions: LNR stratification proved an adverse prognostic factor of DFS in lymph nodes positive invasive BC using cut-off values 0.20 and 0.65, and was more predictive than traditional pN classification for 5-DFS.

Keywords: Breast neoplasm - survival analysis - lymph node ratio - prognosis

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Introduction

Over the past three decades, the breast cancer (BC) incidence has been steadily increasing and becoming the most common malignancy in large cities, like Shanghai (Fan et al., 2009) in China. Accurate evaluation for each patient is fundamental for BC personalized care. TNM staging system is the essential classification for BC treatment decision and prognosis prediction over the past 60 years, which consists of the tumor size (T), lymph nodes metastasis (N) and distant metastasis (M) on the basis of anatomy (Singletary and Connolly, 2006; Goldhirsch et al., 2009; Veronesi et al., 2009). Among of them, lymph nodes status is the most important prognostic factor in early BC, making it one of the most important indicators for chemotherapy stretagies and radiotherapy seclection. Currently, the BC patients could be divided into four grades (N0-N3) by absolute positive numbers of lymph nodes metastasis (traditional lymph nodes classification, pN classification) according to the International Union Against Cancer (UICC)/American Joint Committee on Cancer (AJCC) guideline, resulting different treatment and clinical outcome for BC patients. This stratification was simple and intelligible, making it widely used in clinical practice. The numbers of lymph nodes involvement depend on the extensive axillary lymph node dissection (ALND), and accurate and careful lymph nodes identification. Generally, the more lymph nodes identified from the specimens, the more accurate judgment could be made for lymph nodes evaluation. However, in clinical practice, this process could be limited by many factors, such as surgical procedures, pathological identification, individual variances, and so on, resulting a wide range (1 to 57) of total number of lymph nodes identification among BC patients and institutions (Voordeckers et al., 2004; Truong et al., 2005; Danko et al., 2010).

Furthermore, the concerns of these variances on BC personalized care have been becoming increasingly evident in clinical practice. For example, the prognosis might be completely different in two types of BC patients with three positive lymph nodes, one patient with total 10 lymph nodes evaluation and the other with total 30

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Table 1. Clinicopathological Characteristics of 294 BC Patients with Positive Lymph Nodes

Characteristics	Group A N (%)	Group B N (%)	*P* value
Total number	116	178	
Age at diagnosis (years)			
≤50	70 (60.3)	104(58.4)	0.744
<50	46 (39.7)	74(41.6)	
Tumor size (cm)			
T1(T≤2)	16(13.8)	53(29.8)	<0.0001
T2 (2<T≤5)	71(61.2)	105(59.0)	
T3 (T>5)	29(25.0)	20(11.2)	
Histological grade			
Grade 1	9 (7.8)	6(3.4)	0.095
Grade 2	68 (58.6)	123(69.1)	
Grade 3	39 (33.6)	49(27.5)	
Hormone receptor†			
Positive	87 (75.0)	134(75.3)	0.957
Negative	29 (25.0)	44(24.7)	
HER2 status‡			
Positive	32 (27.6)	48(27.0)	0.907
Negative	84 (72.4)	130(73.0)	
Traditional pN classifiction			
1 to 3	54(46.6)	88(49.4)	0.248
4 to 9	27(23.3)	51(28.7)	
>9	35(30.2)	39(21.9)	
LNR classification			
≤0.20	44(37.9)	79(44.4)	0.118
0.20<LNR ≤0.65	34(29.3)	60(33.7)	
>0.65	38(32.8)	39(21.9)	

†Hormone receptor included estrogen receptor (ER) and progesterone receptor (PR); ER (+) and/or PR(+) was viewed as hormone receptor positive; ‡HER2 (positive) means HER2 (+++) affirmed by immunohistochemistry (IHC) or gene amplication

lymph nodes evaluation, though the two patients are with the same morphology, TNM stage and treatment strategy. These indicate that the total lymph nodes should be also considered as well as the absolute positive lymph node number for lymph node stratification in the era of personalized medicine. Recent years, a new stratification by lymph node ratio (LNR, the ratio of positive lymph nodes with total lymph nodes evaluation) had showed better prognosis prediction than pN classification (Voordeckers et al., 2004; Vinh-Hung et al., 2009; Danko et al., 2010; Han et al., 2011). However, this new stratification by LNR should be further evaluated in different regions. This study retrospectively evaluated the value of LNR in two groups of BC patients with 5-year follow up in China.

Materials and Methods

Patients and study design

Two groups of BC patients who received operation, chemotherapy, endocrine therapy in Department of Breast surgery, Hubei Cancer Hospital, China from Jan 2002 to Dec 2006(Chen et al., 2010) (defined as Group A), and Department of Breast & Thyroid Surgery, Renmin Hospital of Wuhan University, China from Jun 2008 to May 2012 (defined as Group B) were enrolled. Estrogen receptor (ER), progesterone receptor (PR), and HER2 status determined by conventional immunohistochemstry (IHC) methods and HER2 IHC 2++ were further confirmed by gene amplification. The major pathological parameters and treatment information including types of surgery and adjuvant treatments (chemotherapy, radiotherapy, and endocrine therapy) were obtained from the medical records of each patient. The study protocol was approved by the Institutional Ethics Committee.

Patients were excluded as the follow criteria: (a) obtained neoadjuvant chemotherapy before operation, (b) the total excision lymph nodes less than 10 (Singletary et al., 2002; Vinh-Hung et al., 2009), and bilateral BC (Vinh-Hung et al., 2010). Two hundred and ninety four BC patients with lymph nodes positive were enrolled with 116 cases from Group A, and 178 cases from Group B. The traditional classification of positive lymph nodes (pN classification) were obtained, including 1 to 3 positive lymph nodes defined as N1, 4 to 9 positive lymph nodes defined as N2 and more than 9 positive lymph nodes defined as N3. The positive lymph node ratio (LNR) defined as the ratio of positive lymph nodes to the total lymph nodes detection. And the cut offs of new classification by LNR was as the follow (Vinh-Hung et al., 2009): LNR ≤0.20, 0.20, LNR≤0.65, and 0.65, LNR. All the patients were on regular follow-up schedule. Recurrence included locoregional recurrence and distant metastasis. The primary endpoint was disease-free survival, which was defined as the time interval from the date of BC surgery to the first evidence of recurrence (local, regional, or distant). If recurrence was not evident, patients were censored on the last follow-up. Patient characteristics were summarized in Table 1.

Statistical analysis

Pearson Chi-square test was used to compare clinicopathological parameters of patients among groups. The 5-year disease-free survival (5-DFS) was the primary endpoint, which was analyzed by Kaplan-Meier analysis and log-rank test. Multivariate Cox proportional hazards regression model was performed to analyze the independent prognostic factors. Hazard ratios with 95% confidence intervals (95% CI) were also obtained. Statistical analyses were performed using SPSS 16.0 software (SPSS Inc. Chicago, IL) and two-tailed $P<0.05$ was considered as statistically significant.

Results

Clinicopathological characteristics of lymph nodes positive BC patients

Two hundred and ninety four BC patients with lymph nodes positive were selected and 93 patients were developed recurrences with 29 (16.3%) patients of Group B at the media follow up 28 months (6 to 60 months) and 64 (55.2%) of Group A at the media follow up 41 months (2 to 60 months) . The clinicopathological characteristics of the patients were showed in Table1, in which more patients from Group A have significant larger tumor size ($P<0.05$) and lymph nodes metastasis (but with no significant statistical differences, $P>0.05$). The distribution of positive lymph nodes in two groups

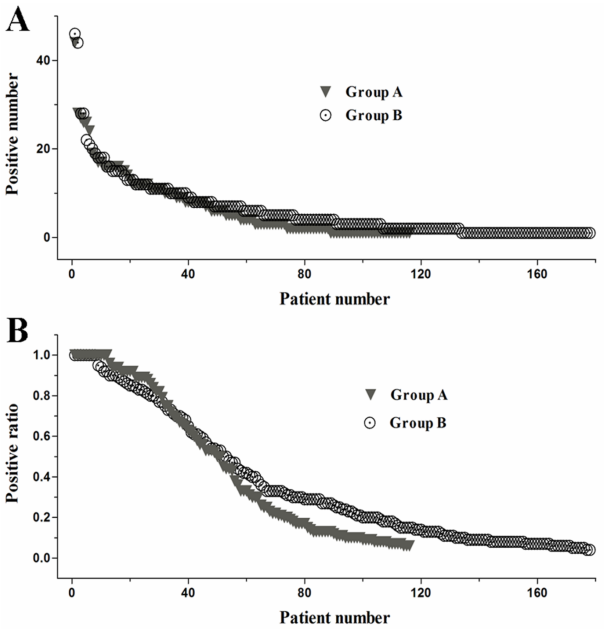

Figure 1. The Distribution of Lymph Nodes Involvement of Two Groups. A, the distribution of absolute positive lymph nodes, B, the distribution of LNR

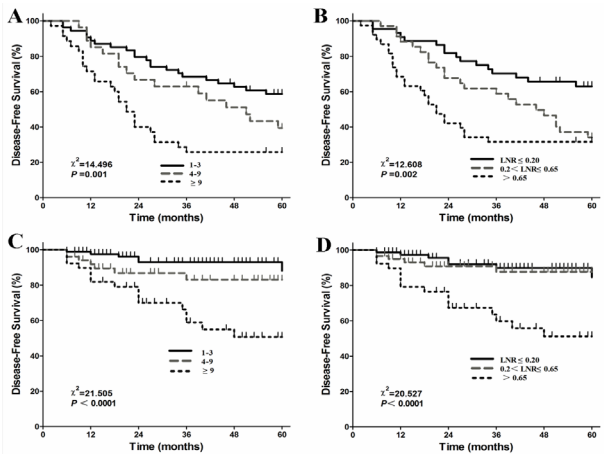

Figure 2. Comparison of 5-year Disease-free Survival (DFS) with Different Lymph Nodes Classification. The differences of 5-DFS of three groups by traditional lymph nodes staging in Group A (A) and Group B (C). The differences of 5-DFS of three groups by LNR classification in Group A (B) and Group B (D)

were illustrated in Figure 1A, with media number of 5 (1 to 44) for Group A and 4 (1-46) for Group B. In contrast, the distribution of LNR were illustrated in Figure 1B, with media LNR 0.33 (0.06 to 1.00) for Group A and 0.27 (0.04 to 1.00) for Group B.

Univariate analysis by different lymph nodes classification

Three subgroups with different lymph nodes status were defined as low risk group (1-3 lymph nodes positive or LNR ≤0.20), intermediate risk group (4-9 lymph nodes positive or 0.20 < LNR ≤0.65) and high risk group (9<lymph nodes positive or 0.65 < LNR), respectively. The 5-DFS in three risk subgroups achieved significantly statistical differences (P<0.01) by either traditional pN classification (Figure 2A and C) or LNR (Figure 2B and D) in two groups. For further analysis in Group A, there were statistical significant differences in 5-DFS only between the low risk group and high risk group (P<0.01) but no differences between the other groups (low vs intermediate: P=0.137; high vs intermediate: P=0.063) by traditional pN classification (Figure 2A). In contrast, there were statistical significant differences in 5-DFS between the low risk group and the other two groups (low vs high: P=0.001; low vs intermediate: P=0.022) but no differences between the high and intermediate groups (P=0.189) by LNR (Figure 2B).

For the patients in Group B, there were statistical significant differences in 5-DFS between the high risk group and the other two groups (high vs low: P< 0.0001; high vs intermediate: P=0.014) but no differences between the low and intermediate groups (P=0.126) by traditional pN classification (Figure 2C). Similarly, the differences of 5-DFS between the high risk group and the other two groups achieved statistical significances (high vs low: P< 0.0001; high vs intermediate: P=0.001) but no significances between the low and intermediate groups (P=0.131) by LNR (Figure 2D).

Multivariate analysis by different lymph node classification

To further explore the differences of 5-DFS among three subgroups, a multivariate analysis was performed, and the low risk group of lymph node classification was used as reference adjusted with other variables including age, tumor size, tumor grade, hormone receptor and HER2 status (Table 2 and 3). The results demonstrated that, among others, the significance of lymph node status by different classification both were predictive for 5-DFS (P<0.05). Further analysis showed that all the differences

Table 2. Multivariate Analysis by Traditional pN and LNR Classification Among BC Patients in Group A

Variable	Hazard Ratio	95% CI	*P* value
pN Classification			**0.012**
Low (1-3)	Reference*	-	-
Intermediate (4-9)	1.524	0.778-2.983	0.219
High (>9)	2.608	1.384-4.916	0.003
LNR Classification			**0.031**
Low (0.01-0.20)	Reference*	-	-
Intermediate (0.20<LNR ≤0.65)	2.071	1.073-3.998	**0.03**
High (>0.65)	2.442	1.211-4.921	0.013

*Low risk group used as reference state in multivariable analysis; *CI, confidence interval; LNR, lymph node ratio; Hazard ratios were adjusted for age, tumor size, tumor grade, hormone receptor and HER2 status

Table 3. Multivariate Analysis by Traditional pN and LNR Classification Among BC Patients in Group B

Variable	Hazard Ratio	95% CI	*P* value
pN Classification			**<0.001**
Low (1-3)	Reference*	-	
Intermediate (4-9)	2.292	0.752-6.991	0.145
High (>9)	6.158	2.389-15.870	<0.001
LNR Classification			**<0.001**
Low (0.01-0.20)	Reference*	-	
Intermediate (0.20<LNR ≤0.65)	1.295	0.431-3.893	0.645
High (>0.65)	5.013	2.022-12.430	0.001

*Low risk group used as reference state in multivariable analysis; *CI, confidence interval; LNR, lymph node ratio; Hazard ratios were adjusted for age, tumor size, tumor grade, hormone receptor and HER2 status

Table 4. Summary of Studies to Evaluate the Value of LNR in BC Prediction

Author (year)	Number	Cut-off value	Median follow up
Schmoor et al. (2001)	141	1.0(<1.0 vs 1.0)	8.0 years[a]
van der Wal et al. (2002)	453	0.2	6.1 years
Voordeckers et al. (2004)	741	0.10/0.50	6.2 years
Truong et al. (2005)	542	0.25	7.5 years
Vinh-Hung et al. (2009)	1829	0.20/0.65	25.0 years[b]
Hatoum et al. (2009)	669	0.25/0.50/0.75	3.4 years
Mersin et al. (2009)	185	0.25 and 0.30[c]	3.0 years
Vinh-Hung et al. (2010)	17685	0.20/0.65	12.8 years
Danko et al. (2010)	1788	0.20/0.65	8.2 years
Karihtala et al. (2010)	269	0.20/0.65	6.2 years
Ibrahim et al. (2010)	217	0.20/0.65	3.3 years
Schiffman et al. (2011)	1436	0.20/0.65	5.4 years
Duraker et al. (2011)	924	0.15	9.0 years
Han et al. (2011)	130	0.15	4.9 years
Kim et al. (2011)	330	0.25/0.55	7.5 years
Tausch et al. (2012)	2718	0.10/0.20	8.2 years

[a]The median follow-up time was approximately 8 years when event-free survival was observed as endpoint; [b]Longest observation; [c]0.25 for locoregional recurrence, 0.30 for distant recurrence

of 5-DFS between low and high risk groups achieve statistical significances. Notably, the significance between low and intermediate risk groups were predictive by LNR classification in Group A (Table 2, $P = 0.030$).

Discussion

The increasing understanding of the BC biological behaviors has revolutionized BC care; and more and more patients could be diagnosis earlier and obtained personalized treatments, resulting improved survival and quality of life (Jemal et al., 2009; 2011). Accurate evaluation is fundamental for BC personalized treatment and lymph nodes status was the most important prognostic factor. The traditional BC classification by lymph node mainly on the base of number of positive lymph nodes involvement is insufficient to reveal the "real" condition of BC, and to meet the needs of individualized care. Over the past ten years, an increasing number of studies have been performed to evaluate the value of new BC classification by LNR for BC survival prediction (Schmoor et al., 2001; der Wal et al., 2002; Voordeckers et al., 2004; Overman et al., 2010). As showed in Table 4, the cut-off of LNR and follow up time were not uniform in initial small-scale studies. In 2009, Vinh-Hung et al (Vinh-Hung et al., 2009) evaluated the value of LNR in large-scale, long-term follow up BC patients, and obtained optimal cut-off values (0.20 and 0.65) for BC stratification using a reasonable mathematical method, which were more stable, reliable and favorable prognostic separation than traditional pN classification. Furthermore, the later large-scale studies also supported the rationalities of these cut-off values. Therefore, in this study, we also used these cut-off values for LNR stratification.

This study, we compared the predictive value of LNR with traditional pN classification in two groups BC patients with 5-year follow-up. And the results showed favorable stratification for 5-DFS prediction by LNR than by traditional pN classification in univariate and multivariable analysis in Group A patients. However, the predictive value of LNR in Group B patients is not superior to traditional pN classification in survival analysis. The reasons might be as follows. First, the patients were selected from different periods, the BC patients of Group A from 2002-2006 years periods and the patients of Group B from 2008-2012 years periods, which more patients in the latter group with low risk of lymph nodes involvements and small tumor size than that in the first group. Therefore, the 5-DFS of BC patients in Group A is worse than that in Group B. Second, the BC patients in this study should be longer follow up, especially in Group B. The variances in other studies might be also due to the involved lymph nodes and follow up time. One study (Voordeckers et al., 2004) only contained pN1 and pN2 patients, and the patients with pN1 or low risk LNR accounted more than half of the cases in another study (Vinh-Hung et al., 2009). Additionally, the predictive value was different from different follow up time in different studies(Schmoor et al., 2001; der Wal BC et al., 2002; Voordeckers et al., 2004; Overman et al., 2010) as showed in Table 4 and in same studies with different follow up interval periods (Vinh-Hung et al., 2009; 2010).

In this study, more than half BC patients diagnosed as intermediate or high risk (LNR>0.20) of lymph nodes involvement, especially in Group A. Nevertheless, with the development of new detection methods, especially the universal use of mammography in clinical practice, and improvement of awareness for high BC risk women, more and more patients could be diagnosed earlier with no or small number of lymph nodes involvement (Harper et al., 2009; Schootman et al., 2010; Pedraza et al., 2012), indicating a further evaluation of low risk of lymph nodes involvement (pN1) for BC patients. Recently, two studies tried to evaluate the role of LNR in pN1 BC. Han T et al (Han et al., 2011) evaluated the LNR in 1-3 lymph node-positive BC patients, and demonstrated that the patients with LNR>0.15 might derivate higher recurrence risk than that of LNR≤0.15. Duraker N and colleagues (2011) also studied the role of LNR in pN1 BC. The patients could be divided into two groups with significant different DFS by using survival analysis to obtain a reasonable cut-off value 0.20. They also demonstrated two different cut-off values of LNR, 0.15 and 0.30, for distant metastasis-free survival and locoregional recurrence-free survival prediction respectively. These studies indicated that the patients with pN1 have different outcomes and the new LNR for BC stratification could identify subtypes of patients with different DFS, though the optimal cut off of LNR should be further evaluated.

Moreover, the emerging molecular BC classifications over the past 20 years, especially that by multi-gene assays or the key molecules including ER, PR, HER2 and Ki67, which could divide BC at least four subtypes, including Luminal A, Luminal B, HER2 positive, and Triple negative (ductal), have become increasingly important in treatment selection, prognosis prediction and disease course monitoring (Cianfrocca and Gradishar, 2009; Sotiriou and Pusztai, 2009; Weigelt et al., 2010). Therefore, the value of LNR in different intrinsic subtypes

should be further evaluated, which also merits further exploration in our larger and longer time follow up studies.

In conclusion: In summary, we evaluated the value of LNR for BC stratification using the cut-off values 0.20 and 0.65 in two groups of lymph nodes positive patients, and demonstrated the more predictive role of LNR for BC 5-DFS than traditional pN classification. The role of LNR in early stage BC (pN1) and the association with molecular BC subtypes should be further investigated.

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