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ORIGINAL ARTICLE – BREAST ONCOLOGY

Lymph Node Ratio Analysis After Neoadjuvant Chemotherapy is Prognostic in Hormone Receptor-Positive and Triple-Negative Breast Cancer

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ABSTRACT

Background. Lymph node ratios (LNR), the proportion of positive lymph nodes over the number excised, both defined as ranges and single ratio values are prognostic of outcome. Little is known of the prognostic value of LNR after neoadjuvant chemotherapy (NAC) according to molecular subtype.

Methods. From 2003 to 2014, patients who underwent definitive surgery after NAC were identified. LNR was calculated for node-positive patients who received axillary dissection or had at least 6 nodes removed. DFS was calculated using the Kaplan-Meier log rank test for yp N0-3 status, LNR categories (LNRC) $\leq$0.20 (low), 0.21–0.65 (intermediate), >0.65 (high), and single LNR values.

Results. Of 428 NAC recipients, 263 were node negative and 165 (38.6 %) node positive: ypN1 = 97 (58.8 %), ypN2 = 43 (26.1 %), and ypN3 = 25 (15.2 %). Among node-positive cancers, the median number of LN removed was 14 (range, 6–51) and the median LNR was 0.22 (range, 0.03–1.0). Nodal stage was inversely associated with 5-year DFS: 91.5 % (ypN0), 74.5 % (ypN1), 49.8 % (ypN2), and 50.7 % (ypN3) ($p < 0.001$). LNRC was similarly inversely associated with DFS: 69.1 % (low), 71.4 % (intermediate), 49.3 % (high) ($p < 0.001$). Significant associations between LNRC and DFS were demonstrated in hormone receptor (HR)-positive and triple negative breast cancer (TNBC) subtypes, $p = 0.02$ and $p = 0.003$. A single-value LNR $\leq$ 0.15 in node-positive, HR-positive (94.1 vs 67.7 %; $p = 0.04$) and TNBC (94.1 vs 47.8 %; $p = 0.001$) groups was also significant.

Conclusions. Residual nodal disease after NAC, analyzed by LNRC or LNR = 0.15 cutoff value, is prognostic and can discriminate between favorable and unfavorable outcomes for HR-positive and TNBC cancers.

Nodal status or the degree of nodal involvement is a significant prognostic indicator of survival in breast cancer.[1,2] Neoadjuvant chemotherapy (NAC) use is rising steadily. It provides a mechanism for testing tumor chemosensitivity as well as downstaging of primary cancer to facilitate breast conservation or potentially avoid axillary node dissection.[3,4] TNM staging for NAC was incorporated into the American Joint Committee on Cancer (AJCC) staging classification of 2003, using the designation (yp).[5] The revision was prompted by observations that stage of disease after NAC is associated with prognosis, to the same degree as for those who receive primary upfront surgery.[6]

The extent of axillary node dissection and the number of nodes removed therein has been a topic of discussion for decades. Clinical studies have suggested improved survival with more extensive nodal dissections in both node-negative and node-positive patients.[7,8]

Lymph node ratio (LNR) is defined as the proportion of positive lymph nodes over the total number of nodes excised. This approach has been proposed as a practical alternative or complementary analysis to the AJCC staging.[8,9] LNR provides prognostic information with

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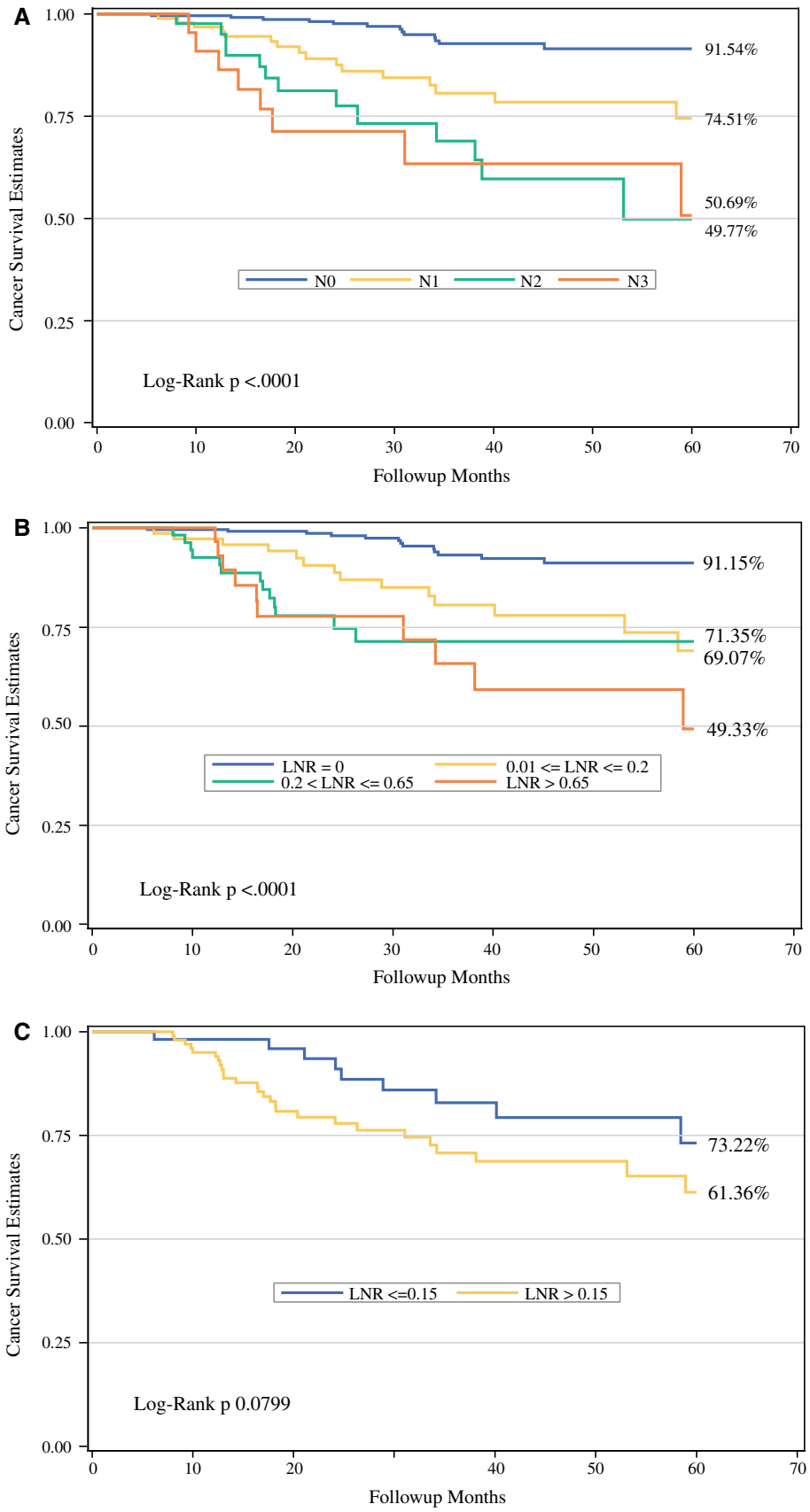

FIG. 1 Comparison of 5-year disease-free survival by Kaplan–Meier analysis among all node-positive patients by different ypN nodal stage and lymph node ratio (LNR). **a** ypN staging, **b** LNRC, **c** single LNR value 0.15

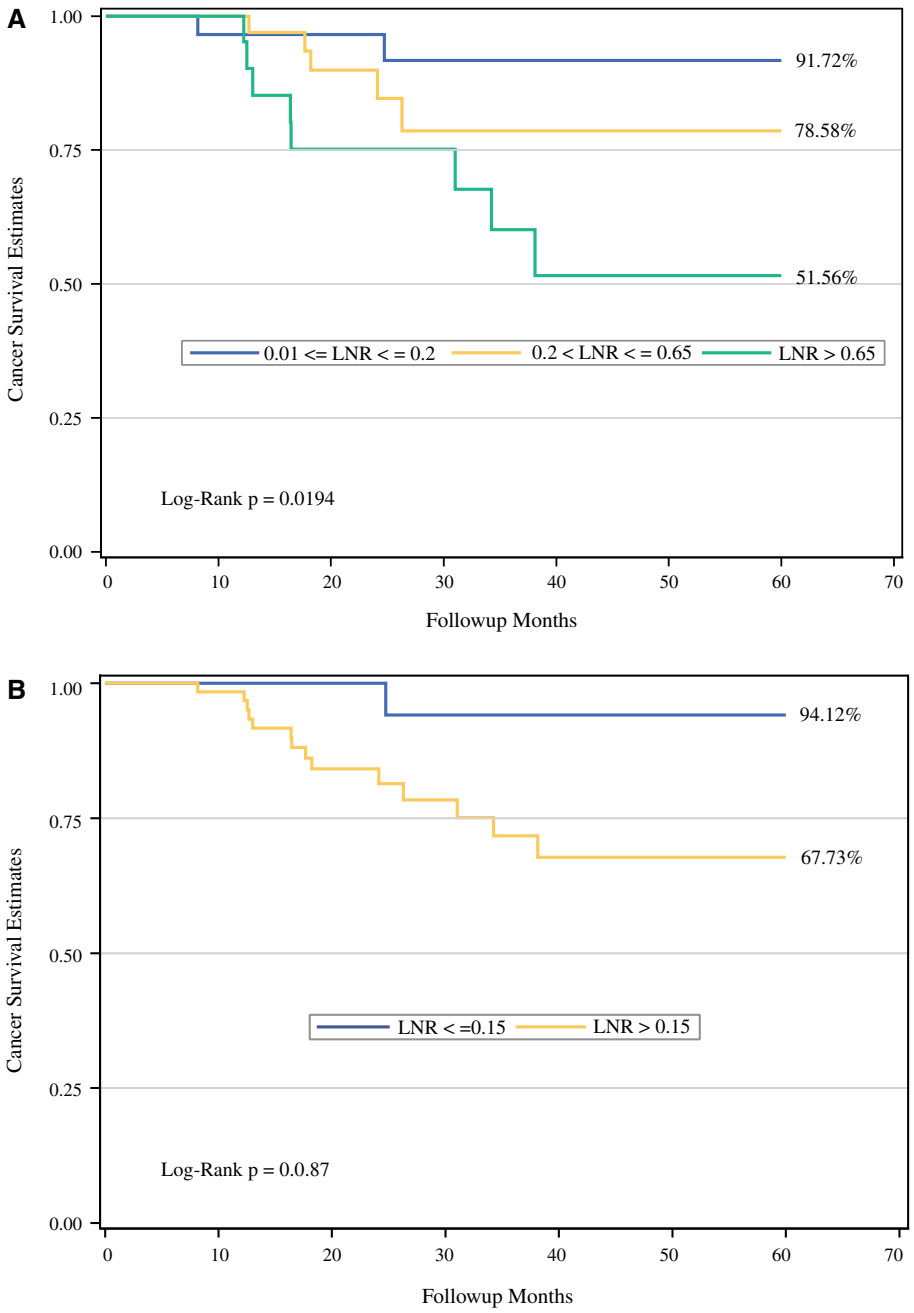

FIG. 2 Five-year disease-free survival by Kaplan–Meier analysis in node-positive, hormone receptor-positive patients by **a** lymph node ratio categories (LNRC), **b** single LNR value 0.15

respect to disease-free survival (DFS) and overall survival (OS), when analyzed either as a continuous variable or using specific cutoff ratios.[10–13] A number of studies have validated the use of three LNR categories (LNRC), ≤0.2 (low), 0.21–0.65 (intermediate), and >0.65 (high), as tools to discriminate between favorable and unfavorable breast cancer-specific or overall survival outcomes.[8,14,15] In a study of 1436 patients by Schiffman et al. LNR analysis could discriminate for better OS in N1 and N2 subgroups.[10] Similarly, LNRC provided additional prognostic survival information in a cohort of 309,216 patients with T1–T2 tumors and 1–2 involved nodes from the National Cancer Database.[14] More simply, even a single cutoff ratio value (0.25) has been found to be a valid discriminant.[13]

While LNR has been reported for patients who undergo surgery first, few analyses have been undertaken in the NAC setting.[16–18] In this study, we evaluate LNR in early-stage breast cancer patients receiving NAC followed by definitive surgery to determine the prognostic value across molecular subtypes.

TABLE 1 Clinical and pathologic characteristics ($n = 428$)

	Node−	Node+	*P* value
N	263 (61.4 %)	165 (38.6 %)	
Nodes removed (median)	4	14	
Nodes positive (median)	0	7	
Pathology (%)			
IDC	242 (92 %)	145 (87.8 %)	0.15
ILC	12 (4.6 %)	15 (9.1 %)	0.06
Other	9 (3.4 %)	5 (3.0 %)	0.82
Mastectomy (%)	146 (55.5 %)	115 (69.7 %)	0.003
Receptor subtypes (%)			
HR+	70 (26.6 %)	92 (59.2 %)	<0.0001
HER2+	91 (34.6 %)	33 (18.7 %)	0.0004
TNBC	102 (38.8 %)	40 (21.5 %)	0.0002

IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *HR+* hormone receptor (estrogen receptor and/or progesterone receptor positive and HER2 negative), *HER2 +* HER2 overexpressed or amplified with any ER/PR status, *TNBC* triple negative breast cancer (ER negative, PR negative, HER2 negative)

METHODS

The institutional cancer registry was queried for patients who underwent NAC followed by definitive surgery during the years 2003–2014. Pretreatment nodal staging was based on clinical and/or radiologic examinations, with or without accompanying cytopathological evaluation. Patients who had distant metastases, incomplete tumor estrogen receptor (ER), progesterone receptor (PR) and HER2 data, or fewer than six lymph nodes removed when node positive, were excluded. Node-negative patients were included regardless of the number of nodes resected.

Nodal involvement was not qualified as micrometastasis or macrometastasis. LNR was calculated for node-positive patients, by dividing the number of positive lymph nodes by the total number of lymph nodes examined. These values were grouped into LNRC defined as: low ≤ 0.20, intermediate 0.21–0.65, and high > 0.65.[10] DFS was analyzed using the Kaplan-Meier method according to the LNRC and AJCC ypN staging classification methods described previously.[5] Additionally, we set out to determine whether a single LNR value would more simply discriminate between favorable and unfavorable outcomes. Subgroup analyses were then performed by breast cancer subtype defined by immunohistochemistry for ER and PR and immunohistochemistry or FISH for HER2. Hormone receptor-positive tumors were defined as ER- and/or PR-positive and HER2-negative. HER2-positive tumors included all patients with HER2 receptor positivity regardless of hormone receptor status. Triple-negative tumors included those that were ER-negative, PR-negative, and HER2 not overexpressed nor amplified.

RESULTS

After approval from our institutional review board, a total of 428 patients who received NAC followed by definitive surgery from 2003 to 2014 were identified from our institutional registry. The mean follow-up time was 36.9 months (range, 5.6–132 months). Of these patients, 263 (61.4 %) were pathologically staged as node negative, and 165 (38.6 %) as node positive (Table 1). Specifically, 97 (58.8 %) were classified as ypN1, 43 (26.1 %) as ypN2, and 25 (15.2 %) as ypN3. The median number of lymph nodes removed was 14 (range, 6–51) in the node-positive cohort versus 4 (1–37) in the node-negative cohort ($p < 0.001$). LNRs in the node-positive subgroup ranged from 0.03 to 1.0 with a mean of 0.38 and median of 0.22.

Approximately 61 % of patients underwent mastectomy after NAC: 55.5 % in the node-negative cohort and 69.7 % in node-positive cohort. The use of mastectomy increased with increasing LNRC, with 81.8 % receiving mastectomy ($n = 33$) in the LNR high group, which likely correlates with an overall greater burden of disease.

In all patients, nodal stage was inversely correlated with 5-year DFS: 91.5 % (ypN0), 74.5 % (ypN1), 49.8 % (ypN2), and 50.7 % (ypN3), log-rank $p < 0.001$ (Fig. 1a). These differences were only significant when comparing ypN0 to the ypN+ categories. The differences between ypN1 and ypN2/N3 were not statistically significant, $p > 0.05$. The median number of positive LNs for the low, intermediate, and high LNRCs was 1, 4, and 9, respectively. LNRC was similarly inversely correlated with 5-year DFS: 69.1 % (low), 71.4 % (intermediate), and 49.3 % (high), log rank $p < 0.001$ (Fig. 1b).

TABLE 2 Five-year disease-free survival by lymph node ratio categories and receptor subtypes

	Total (*N*)	≤0.2 (*n* = 73)		>0.21 to ≤0.65 (*n* = 59)		>0.65 (*n* = 33)		*P* value*
		N	DFS (%)	*N*	DFS (%)	*N*	DFS (%)	
HR+	92	30	91.7	38	78.6	24	51.6	0.019
HER2+	33	19	45.5	9	87.5	5	100	0.315
TNBC	40	25	64.2	11	30.3	4	0	0.003

HR + hormone receptor (estrogen receptor and/or progesterone receptor positive and HER2 negative), *HER2* + HER2 overexpressed or amplified with any ER/PR status, *TNBC* triple negative breast cancer (ER negative, PR negative, HER2 negative)

*Log-rank *p* value

The distribution of all cancers by molecular subtypes were 37.9 % HR-positive, 28.9 % HER2-positive, and 33.2 % for TNBC. Among node-positive cancers after NAC, the largest proportion of cases were HR-positive (59.2 %), whereas among the node-negative group, TNBC and HER2 subtypes predominated (Table 1). Analyzing outcomes by molecular subgroups revealed that the proportion of HR-positive patients increased with higher LNRC: low 40.5 % ($n = 30$), intermediate 64.4 % ($n = 38$), and high 72.7 % ($n = 24$) compared with 26.6 % of the node-negative ($n = 70$) cohort. In the subgroup of node-positive, HR-positive cancers, 5-year DFS was significantly associated with increasing LNRC, 91.7 % (low), 78.6 % (intermediate), 51.6 % (high), $p = 0.02$ (Fig. 2a; Table 2). The 5-year DFS was also significantly associated with increasing LNRC was found in the node-positive TNBC group: 64.2 % DFS (low), 30.3 % (intermediate), 0 % (high), log-rank $p = 0.003$ (Table 2).

Pretreatment clinical nodal staging was stated in 96 % ($n = 410$) of the patients in this registry (Supplemental Table 1). A total of 122 (29.8 %) of the 410 evaluable patients were downstaged from clinically node positive to pathologically node negative. In contrast, 15 (28 %) of the HR-positive, 3 (9 %) of HER2-positive, and 2 (3 %) of TNBC cancers were upstaged. The latter cases were associated with a median LNR 0.29 (0.07–1.0) in HR-positive, 0.10 (0.07–0.13) in TNBC, and 0.67 (0.17–0.86) in HER2-positive subgroups.

Several new LNR cutoff values were explored for all patients with node-positive cancers, and no single LNR value was found to significantly discriminate between favorable and unfavorable DFS. Specifically, DFS analyzed by a LNR value ≤0.15 versus a LNR value of >0.15 was 73.2 versus 61.4 %, log-rank $p = 0.08$ (Fig. 1c). However, when analyzing cases by molecular subtypes, LNR ≤ 0.15 was significantly associated with DFS in HR-positive cases, 94.1 versus 67.7 %; $p = 0.04$ (Fig. 2b) and in TNBC cases, 94.1 versus 47.8 %; $p = 0.001$. Neither this cutoff, nor other higher values, were significant for HER2-positive tumors.

DISCUSSION

We applied LNR analyses to a cohort of patients who received NAC before definitive surgery in order to characterize the significance of residual cancer burden in their axillary nodes. Previously validated LNR categories and the identification in our dataset of a single discriminant LNR value (LNR ≤ 0.15) were both found to significantly distinguish between favorable and unfavorable outcomes in HR-positive and TNBC cancers. Neither LRNC or LNR ≤ 0.15 was significantly correlated with DFS in HER2-positive tumors in this dataset.

Residual disease after NAC in breast or lymph nodes at the time of definitive surgery portends poor prognosis. Calculation of the residual cancer burden (RCB), as put forth by Symmans et al., is an additional method for qualifying the burden of residual disease beyond the scope of the AJCC yp staging. Importantly, patients with minimal residual disease, or RCB 1, have prognoses that are as favorable as those in patients who achieve a complete pathological response (pCR). However, calculation of RCB is currently not widely implemented in clinical practice.

The adequacy of lymph node dissection is important for proper staging of the axilla.[19] Currently, different organizations accept 6–10 lymph nodes as adequate for axillary node dissections.[20–22] Differences in intrinsic anatomy and pathology techniques may explain some of the variability encountered with respect to the number of lymph nodes retrieved. Anatomic surgical technique is a determinant of the number of axillary nodes removed during an axillary dissection. In a multicenter study, Fisher et al. reported in 1970 that the number of nodes resected had no impact on survival or local recurrence in both node-negative or node-positive axillae.[23] Nodal involvement is less common in TNBC cancers compared with the HER2-positive or HR-positive subtypes. However, TNBCs and HER2-positive tumors are much more likely to achieve a pCR to NAC than HR-positive cancers.[24–26] These differences in tumor subtype response to NAC are reflected in our study,

wherein HR-positive patients had the highest proportion of node-positive cancers following NAC (Table 1).

Previous studies reported an association between LNRC and survival in the adjuvant and neoadjuvant setting.[10,14,16,27] In a retrospective analysis of endocrine-responsive cancers derived from multiple trials of the Austrian Breast and Colorectal Cancer Study Group, increasing LNR correlated with worsening DFS and OS in the subset of patients who underwent mastectomy and had 1–3 positive lymph nodes.[28] In contrast, Liao et al. have shown LNR only to be valuable and predictive of survival in luminal tumors.[29] In the neoadjuvant setting, LNR has been demonstrated to have some prognostic value.[17,30] In our neoadjuvant series, increasing LNRC correlated with worse DFS. Additionally, LNRC were significant predictors of outcomes in both HR-positive and TNBC subgroups, but not in the HER2-positive cohort, which included only 33 cases.

Single cutoff values have been proposed to simplify the LNR analysis. Keam et al. found LNR > 0.25 to be associated with poor survival among 205 stage II/III patients who received NAC.[16] In our study, LNRC and LNR ≤ 0.15 were found to discriminate between favorable and unfavorable outcomes among HR-positive and TNBC cancers.

Limitations of this study include data from a registry and the limited number of cases in the HER2-postive and TNBC subtypes, which may weaken the strength of our conclusions. Additionally, the type and duration of chemotherapy prescribed was not examined. Endocrine responsive tumors are heterogeneous and the prognostic significance of residual disease after NAC is complex and difficult to interpret, especially in the presence of nodal involvement. In terms of evaluating residual cancer burden, LNR is a "no cost" simple calculation, which provides additional prognostic information beyond that obtained from pathologic staging or molecular biomarkers. Our study suggests that LNR is useful in discerning favorable subpopulations from those whose risk for distant disease is higher.

In conclusion, after NAC, calculation of LNR in node-positive cases can provide additional insights on prognosis, especially in hormone receptor-positive and triple-negative cases. Our identification of a single value of 0.15 as a discriminant LNR can help distinguish a subpopulation of HR-positive patients with a very favorable 5-year DFS.

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