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ORIGINAL ARTICLE

Axillary lymph node status, adjusted for pathologic complete response in breast and axilla after neoadjuvant chemotherapy, predicts differential disease-free survival in breast cancer

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ABSTRACT

Background

Our retrospective study in breast cancer patients evaluated whether integrating subtype and pathologic complete response (pCR) information into axillary lymph node restaging after neoadjuvant chemotherapy (NAC) adds significance to its prognostic values.

Methods

Patients included in the analysis had stage II or III disease, with post-NAC axillary lymph node dissection (ALND), without sentinel lymph node biopsy before completion of NAC, with definitive subtyping data and subtype-oriented adjuvant treatments. The ypN grading system was used to restage axillary lymph node status, and ypN0 was adjusted by pCR in both breast and axilla into ypN0(pCR) and ypN0(non-pCR). Univariate and multivariate survival analyses were performed.

Results

Among the 301 patients analyzed, 145 had tumours that were hormone receptor–positive (HR+) and negative for the human epidermal growth factor receptor (HER2–), 101 had tumours that were positive for HER2 (HER2+), and 55 had tumours that were triple-negative. The rate of pCR in both breast and axilla was 11.7%, 43.6%, and 25.5% respectively for the 3 subtypes. Compared with the non-pCR patients, the pCR patients had better disease-free survival (DFS) and overall survival (OS): $p = 0.002$ for DFS and $p = 0.011$ for OS. In non-pCR patients, DFS and OS were similar in the ypN0(non-pCR) and ypN1 subgroups, and in the ypN2 and ypN3 subgroups. We therefore grouped the ypN grading results into ypN0(pCR) ($n = 75$), ypN0–1(non-pCR) ($n = 175$), and ypN2–3 ($n = 51$). In those groups, the 3-year DFS was 98%, 91%, and 56%, and the 3-year OS was 100%, 91%, and 82% respectively. The differences in DFS and OS between those three subgroups were significant (all $p < 0.05$ in paired comparisons). Multivariate Cox regression showed that subtype and ypN staging adjusted by pCR were the only two independent factors predicting DFS.

Conclusions

Axillary lymph node status after NAC, adjusted for pCR in breast and axilla, predicts differential DFS in patients without prior sentinel lymph node biopsy.

KEY WORDS

Breast cancer, neoadjuvant chemotherapy, axillary restaging, pathologic complete response, prognosis

1. INTRODUCTION

In breast cancer management, surgical nodal staging reflects initial axillary status in patients having surgery as initial treatment, and it is always an important prognostic factor[1,2]. However, in neoadjuvant settings, nodal metastasis can readily be eradicated by preoperative treatment, which changes the initial axillary status substantially[3–5]. Therefore, whether axillary restaging is still able to predict prognosis after neoadjuvant chemotherapy (NAC) is of interest. On the other hand, because of receptor status–based subtype stratification and subtype-oriented therapeutic strategies, breast cancer is no longer viewed as a single disease[6–8]. Evaluations of any prognostic factor should therefore take subtypes and treatments into account.

Moreover, pathologic complete response (pCR) after NAC has been established as an independent prognostic factor, at least in the human epidermal growth factor receptor 2–positive (HER2+) and triple-negative breast cancer (TNBC) subtypes[9–11]. Nonetheless, the reported pCR rate of 16% overall from an update analysis of the National Surgical Adjuvant Breast and Bowel Project B18 and B27 trials suggests

that most patients will eventually miss pCR[12]. For this large group of non-pCR patients, the search for surrogates to further stratify subgroups with different long-term outcomes is clinically significant in adjuvant decision-making.

Currently, the ypN classification, an axillary restaging after NAC, is used in the American Joint Committee on Cancer staging system for breast malignancies[13]. However, considering the critical roles of pCR and subtype, it is reasonable to assume that integrating the ypN system with pCR and subtype might possibly improve the power of the system to predict prognosis.

To test that hypothesis, we retrospectively analyzed a cohort of Chinese breast cancer patients who received NAC and standard subtype-oriented local or systemic adjuvant treatments. We evaluated the feasibility and clinical significance of axillary restaging refined with pCR and subtype.

2. METHODS

2.1 Patients

We reviewed medical records at our institution from June 2006 to June 2011. Inclusion criteria were stage II or III non-inflammatory breast cancer in patients who had been treated with at least 1 cycle of NAC, who received complete axillary lymph node dissection (ALND), for whom complete estrogen receptor (ER), progesterone receptor (PR), and HER2 status were available, and who had received subtype-oriented and standard local or systemic adjuvant treatments.

To ensure that the study population had undergone all standard treatments currently available and to minimize possible confounding factors, we set up exclusion criteria. To be specific, HER2+ patients who had not received adjuvant trastuzumab treatment were excluded regardless of whether neoadjuvant trastuzumab had been used. Patients who were ER positive (ER+) and who had not received adjuvant endocrine therapy were also excluded. Patients who had not received radiotherapy per clinical guidelines were not eligible. Patients undergoing sentinel lymph node biopsy (SLNB) before neoadjuvant treatment were excluded because the SLNB would affect the results of axillary restaging after NAC.

Clinicopathologic characteristics, evaluations of clinical and pathologic response, and follow-up data were retrieved for analysis.

2.2 Subtype Classification

At our institution, ER status, PR status, HER2 status, and Ki67 labelling index are routinely examined using standard immunohistochemical methods as reported elsewhere[14]. Positivity for ER or PR was defined using a cut-off of 10% or more tumour cells stained in the nucleus. Positivity for HER2 was defined as an immunohistochemical 3+ or a positive gene amplification result with fluorescence *in situ* hybridization. The Ki67 index was reported as the percentage of positive tumour nuclei. Patients were then classified into a hormone receptor–positive [HR+ (ER or PR positive, or both)] subtype, a HER2-negative (HER2–) subtype, a HER2+ subtype (HER2+ regardless of HR status), and a TNBC subtype (negative for ER, PR, and HER2).

Although the Ki67 index was adopted to refine breast cancer subtyping in the 2011 St. Gallen Consensus[8], we did not use it to further stratify our HR+ HER2– patients because of the lack of a standardized examination method. However, we included it in the multivariate Cox regression analysis to test its association with prognosis.

2.3 Neoadjuvant Regimens and Response

No specific neoadjuvant regimens or number of cycles were required in our study. Regimens were classified as anthracycline-based, taxane-based, anthracycline with taxane, platinum with taxane, and trastuzumab with taxane.

Clinical response was evaluated according to already-established criteria described elsewhere. By comparing the pre-surgery imaging results with the baseline imaging results from both breast and axilla, patients were graded as having complete response, partial response, stable disease, or progressive disease[10]. The pathologic response was evaluated on the final specimens from breast-conserving surgery or mastectomy with ALND. A pCR was defined as no evidence of residual invasive cancer cells in both breast and axilla[15].

2.4 Axillary Restaging After NAC

Patients were classified using the American Joint Committee on Cancer staging system into ypN0 (zero positive nodes), ypN1 (1–3 positive nodes), ypN2 (4–9 positive nodes) and ypN3 (≥10 positive nodes)[13]. Patients classified ypN0 were further classified into ypN0(pCR) and ypN0(non-pCR) depending on whether pCR was achieved in both breast and axilla.

2.5 Statistics

Disease-free survival (DFS) was defined as the time interval between the date of definitive surgery and the date of a first recurrence event (locoregional or distant) or the date of last follow-up if no recurrence event was recorded. Overall survival (OS) was defined as the time interval between the date of definitive surgery and the date of death or last follow-up.

Kaplan–Meier survival analysis was performed to determine DFS and OS. Log-rank tests were used to compare differences between subgroups. Multivariate Cox regression was performed to test the independency of various factors.

All analyses were performed using the SPSS software application (version 19.0: SPSS, Chicago, IL, U.S.A.), and significance was set at $p = 0.05$.

2.6 Substituting ypN with Lymph Node Ratio

Considering that lymph node ratio (LNR) staging has also been suggested as an alternative axillary grading system for breast cancer as reported by others[16], we used the suggested criteria to categorize LNR in our patients. To be specific, LNR was first calculated as the number of positive nodes divided by the number of harvested nodes. Then, based on the LNR range, patients were classified as LNR0 (LNR = 0), LNR1 ($0 < \text{LNR} \leq 0.2$), LNR2 ($0.2 < \text{LNR} \leq 0.65$), and LNR3 ($0.65 < \text{LNR} \leq 1.0$). Similarly, we further used PCR to stratify patients who were LNR0 into LNR0(PCR) and LNR0(non-PCR). All analyses performed for the ypN grading were also performed for the LNR grading (see Appendix A).

3. RESULTS

3.1 Patient Characteristics

In total, 301 patients were eligible for inclusion in the present study (Table I). Median age was 46 years, with 267 patients (88.7%) being older than 35 years and 34 (11.3%) being younger than 35 years. In 284 patients (94.4%), the diagnosis was invasive ductal carcinoma; only 17 patients (5.6%) had other histologic types. The disease was classified as grade 3 in 58 patients (19.3%) and grade 1 or 2 in 243 (80.7%). In 233 patients (77.4%), the disease was classified as stage II, and in 68 (22.6%), as stage III. In this cohort, 48 patients (15.9%) underwent breast-conserving surgery and 253 (84.1%), mastectomy. All patients underwent complete ALND without pre-chemotherapy SLNB. Written informed consent was provided by all patients before treatment.

3.2 Subtype Distribution

The cohort included 145 HR+ HER2– patients (48.2%), 101 HER2+ patients (33.6%), and 55 TNBC patients (18.3%). The HR+ HER2– patients were not further stratified into luminal A or luminal B subtypes by Ki67 index, and HER2+ patients were not further stratified into the HER2+ or luminal B subtype (HER2+) according to HR status.

3.3 NAC Regimens and Cycles

Among the 301 patients, 92.7% received taxane-containing regimens; only 7.3% received anthracycline-based regimens. In HER2+ patients, most received trastuzumab-containing regimens (96 of 101, 95.0%). The NAC cycles ranged from 2 to 10, with a mean cycle number of 4.1.

3.4 Response Evaluation

The overall response rate (clinical complete and partial responses) was 80.0% in HR+ HER2– patients, 92.1% in HER2+ patients, and 80.0% in TNBC patients. The PCR rate was just 11.7% (17 of 145) in HR+ HER2– patients, 25.5% (14 of 55) in TNBC patients, and as high as 43.6% (44 of 101) in HER2+ patients. The PCR rate was significantly lower in HR+ HER2– patients compared with either HER2+ ($\chi^2 = 32.36$, $p < 0.001$) or TNBC patients ($\chi^2 = 5.74$, $p = 0.017$).

3.5 Kaplan–Meier Survival

Median follow-up was 36.2 months. In total, 33 DFS events and 16 deaths were recorded. Table II shows the distribution of events in the subgroups. In the overall cohort, Kaplan–Meier survival analysis showed that, compared with non-PCR patients, PCR patients experienced better DFS and OS [$p = 0.002$ and 0.011 respectively, Figure 1(A,B)].

We further used PCR to adjust ypN classifications. Patients were stratified into ypN0(PCR), ypN0(non-PCR), ypN1, ypN2, and ypN3. Pairwise comparisons of DFS and OS showed that ypN0(PCR) patients had the best prognosis and that patients classified as ypN0(non-PCR) and ypN1 had overlapping survival curves. Similarly, patients classified as ypN2 and ypN3 also had overlapping survival curves. Figure 2(A,B) shows the Kaplan–Meier survival curves.

Based on those results, we then combined the subgroups with overlapping survival curves. Patients classified as ypN0(non-PCR) and ypN1 were merged as ypN0–1(non-PCR), and those classified as ypN2 and ypN3 were merged as ypN2–3. Kaplan–Meier survival analysis of all patients stratified by ypN0(PCR), ypN0–1(non-PCR), and ypN2–3 showed that DFS and OS were significantly different between these 3 classifications [Figure 3(A,B)]. The expected 3-year DFS was 98%, 91%, and 56% respectively. The expected 3-year OS was 100%, 91%, and 82% respectively.

Subtype-based analyses using ypN0(PCR), ypN0–1(non-PCR), and ypN2–3 were then performed to compare DFS and OS. As Figure 4 shows, this axillary restaging classification effectively discriminated the various DFS results in the 3 subtypes (all $p < 0.001$). On the other hand, in OS analysis, these classifications did not shown statistical significance for the HR+ HER2– subtype or the TNBC subtype, probably because only 16 OS events occurred, because the HR+ HER2– subgroup contained fewer PCR patients, and because the TNBC subtype contained relatively fewer patients overall. However, a notable trend remained in the HR+ HER2–and TNBC subgroups, with patients classified as ypN2–3 having the poorest OS [Figure 4(D,F)].

TABLE I Clinicopathologic characteristics of the study patients

Characteristic	*Patient group*			
	Overall	HR+ *HER2*–[a]	*HER2*+[b]	*TNBC*[c]
Patients [*n* (%)]	301	145 (48.2)	101 (33.6)	55 (18.3)
Age (years)				
Median	46	46	47	47
Range	19–75	26–75	19–69	29–71
Age group [*n* (%)]				
≤35 Years	34 (11.3)	12 (8.3)	15 (14.9)	7 (12.7)
>35 Years	267 (88.7)	133 (91.7)	86 (85.1)	48 (87.3)
Histologic type [*n* (%)]				
Invasive ductal	284 (94.4)	132 (91.0)	100 (99.0)	52 (94.5)
Others	17 (5.6)	13 (9.0)	1 (1.0)	3 (5.5)
Histologic grade [*n* (%)]				
Grade 1	17 (5.6)	17 (11.7)	0 (0)	0 (0)
Grade 2	226 (75.1)	112 (77.2)	78 (77.2)	36 (65.5)
Grade 3	58 (19.3)	16 (11.0)	23 (22.8)	19 (34.5)
Primary tumour staging [*n* (%)]				
T1	25 (8.3)	13 (9.0)	8 (7.9)	4 (7.3)
T2	214 (71.1)	109 (75.2)	66 (65.3)	39 (70.9)
T3	35 (11.6)	11 (7.6)	17 (16.8)	7 (12.7)
T4	27 (9.0)	12 (8.3)	10 (9.9)	5 (9.1)
Initial clinical lymph node status [*n* (%)]				
N0	132 (43.9)	61 (42.1)	42 (41.6)	29 (52.7)
N1	131 (43.5)	64 (44.1)	46 (45.5)	21 (38.2)
N2	26 (8.6)	14 (9.7)	9 (8.9)	3 (5.5)
N3	12 (4.0)	6 (4.1)	4 (4.0)	2 (3.6)
Initial clinical staging [*n* (%)]				
Stage II	233 (77.4)	114 (78.6)	75 (74.3)	44 (80.0)
Stage III	68 (22.6)	31 (21.4)	26 (25.7)	11 (20.0)
Neoadjuvant regimens [*n* (%)]				
Anthracycline-based	22 (7.3)	13 (9.0)	3 (3.0)	6 (10.9)
Taxane-based	154 (51.2)	116 (80.0)	1 (1.0)	37 (67.3)
Anthracycline with taxane	29 (9.6)	16 (11.0)	1 (1.0)	12 (21.8)
Trastuzumab with taxane	96 (31.9)	0 (0)	96 (95.0)	0 (0)
Number of cycles [*n* (%)]				
≤4	269 (89.4)	127 (87.6)	94 (93.1)	48 (87.3)
>4	32 (10.6)	18 (12.4)	7 (6.9)	7 (12.7)
Mean (*n*)	4.1	4.2	4.0	4.1
Range (*n*)	2–10	2–10	2–6	2–7
Clinical response [*n* (%)]				
Complete	107 (35.5)	32 (22.1)	54 (53.5)	21 (38.2)
Partial	146 (48.5)	84 (57.9)	39 (38.6)	23 (41.8)
Stable disease	47 (15.6)	28 (19.3)	8 (7.9)	11 (20.0)
Progressed	1 (0.3)	1 (0.7)	0 (0)	0 (0)
Overall response rate (%)	84.10	80.00	92.10	80.00
Surgery type [*n* (%)]				
Breast-conserving	48 (15.9)	23 (15.9)	16 (15.8)	7 (12.7)
Mastectomy	253 (84.1)	122 (84.1)	85 (84.2)	48 (87.3)

TABLE I Continued

Characteristic	*Patient group*			
	Overall	HR+ *HER2*–[a]	*HER2*+[b]	*TNBC*[c]
Pathologic response [*n* (%)]				
Complete (pCR)	75 (25.2)	17 (11.7)	44 (43.6)	14 (25.5)
Non-pCR	226 (74.8)	128 (88.3)	57 (56.4)	41 (74.5)
Breast pCR	97 (32.2)	29 (20.0)	50 (49.5)	18 (32.7)
Mean positive	2.0	2.5	1.4	1.6
ypN staging [*n* (%)][d]				
ypN0	178 (59.1)	67 (46.2)	73 (72.3)	38 (69.1)
pCR	75 (42.1)	17 (25.4)	44 (60.3)	14 (36.8)
Non-pCR	103 (57.9)	50 (74.6)	29 (39.7)	24 (63.2)
ypN1	72 (23.9)	46 (31.7)	16 (15.8)	10 (18.2)
ypN2	35 (11.6)	25 (17.2)	7 (6.9)	3 (5.5)
ypN3	16 (5.3)	7 (4.8)	5 (5.0)	4 (7.3)

[a] Hormone receptor–positive (estrogen or progesterone or both), and negative for the human epidermal growth factor receptor 2.
[b] Positive for the human epidermal growth factor receptor 2 (any hormone receptor status).
[c] Negative for the human epidermal growth factor receptor 2 and for both hormone receptors.
[d] Restaged axillary lymph node status after neoadjuvant therapy.
HR = hormone receptor; HER2 = human epidermal growth factor receptor 2; TNBC = triple-negative breast cancer; ypN0 = no positive nodes among all axillary nodes harvested; ypN1 = 1–3 positive nodes among all axillary nodes harvested; ypN2 = 4–9 positive nodes among all axillary nodes harvested; ypN3 = ≥10 positive nodes among all axillary nodes harvested.

3.6 Multivariate Cox Regression Analysis

In multivariate Cox regression analysis, ypN was adjusted for pCR, age, initial clinical stage, initial Ki67 index; intrinsic subtype and initial tumour grade were included as calibrators. Status as ypN1 was used as the reference for other ypN statuses. Similarly, the HR+ HER2– subtype was used as the reference for other subtypes. In DFS analysis, ypN adjusted by pCR and intrinsic subtype were two independent predicting factors ($p < 0.001$ and 0.002 respectively). However, in OS analysis, ypN classification was nonsignificant ($p = 0.100$), and intrinsic subtype had only borderline significance ($p = 0.052$).

3.7 Results by LNR

We performed the same set of analyses substituting LNR for ypN. The results were comparable to those using ypN (see Appendix A).

4. DISCUSSION

Compared with pre-chemotherapy staging by imaging evaluation and physical examination, post-chemotherapy pathologic restaging is superior in some respects. First, it is based on pathology results, which are definitive and accurate. Second, it measures tumour and axillary response, and thus reflects the efficacy of neoadjuvant treatments[17]. In this sense, axillary pathologic restaging is more objective and potentially feasible for clinical practice.

In recent years, some reports have shown that axillary restaging after NAC is a significant prognostic factor[18,19]. Nonetheless, those studies did not integrate subtype or adjust the restaging system with pCR, which could be problematic considering the increasing evidence showing better prognosis in pCR than in non-pCR patients[10–12,15,20]. The probable result of simply putting those patients into the single ypN0 group is an underestimation of survival in pCR patients and an overestimation in non-pCR patients, thus impairing the accuracy of the axillary restaging system.

The present study adds to the importance and accuracy of the axillary restaging system by integrating pCR information. First, our analysis shows that, compared with ypN0(non-pCR) patients, ypN0 patients achieving pCR experienced significantly better OS. Second, differences in DFS between those two subgroups almost approached significance, with a borderline $p = 0.078$, suggesting a trend in favor of pCR. Those results are consistent with a recent report that has established the role of pCR as a significant prognostic factor[21]. In sum, we strongly recommend that pCR should be used for adjusting ypN0 for the best accuracy when considering axillary restaging.

There are also reports that LNR is a potential prognosis surrogate[16,22]. However, the original LNR classification was created in the non-neoadjuvant setting, with the rationale that nodal staging would be inaccurate in cases with fewer than 10 harvested axillary lymph nodes. Considering the substantially modified axillary status after NAC—and the mean of

TABLE II Multivariate Cox regression analysis of pathologic complete response (pCR) and axillary lymph nodes restaged after neoadjuvant therapy (ypN)

Factor	*Pts (n)*	*Disease-free survival*					*Overall survival*				
		Events (n)	*HR*	*95% CL*		p *Value*	*Events (n)*	*HR*	*95% CL*		p *Value*
				Lower	*Upper*				*Lower*	*Upper*	
ypN adjusted with pCR	301	33				<0.001[a]	16				0.100
ypN0 (pCR)	75	1	0.07	0.01	0.58	0.014[a]	0	—	—	—	0.949
ypN0 (non-pCR)	103	7	0.53	0.17	1.71	0.290	5	0.78	0.17	3.45	0.741
ypN1	72	6	1		Reference		3	1		Reference	
ypN2	35	14	5.51	1.95	15.56	0.001[a]	6	5.23	1.13	24.23	0.034[a]
ypN3	16	5	3.8	1	14.45	0.050	2	3.93	0.5	30.94	0.194
Age (years)											
≥35	267	29	1		Reference		15	1		Reference	
<35	34	4	2.05	0.66	6.38	0.218	1	0.8	0.1	6.65	0.840
Clinical staging											
Stage II	233	18	1		Reference		11	1		Reference	
Stage III	68	15	1.44	0.62	3.3	0.395	5	0.94	0.25	3.56	0.924
Ki67 (continuous)			2.45	0.42	14.43	0.320		11.56	0.89	149.78	0.061
Subtype						0.002[a]				0.053	
HR+ HER2–[b]	145	10	1		Reference		4	1		Reference	
HER2+[c]	101	14	4.39	1.86	10.33	0.001[a]	7	4.64	1.28	16.76	0.019[a]
TNBC[d]	55	9	3.65	1.36	9.79	0.010[a]	5	4.17	0.99	17.58	0.052
Tumour grade											
Grade 1/2	243	27	1		Reference		14	1		Reference	
Grade 3	58	6	1.13	0.37	3.46	0.835	2	2.56	0.46	14.26	0.282

[a] Statistically significant.
[b] Hormone receptor–positive (estrogen or progesterone or both), and negative for the human epidermal growth factor receptor 2.
[c] Positive for the human epidermal growth factor receptor 2 (any hormone receptor status).
[d] Negative for the human epidermal growth factor receptor 2 and for both hormone receptors.

Pts = patients; HR = hazard ratio; CL = confidence limits; ypN0 = no positive nodes among all axillary nodes harvested; ypN1 = 1–3 positive nodes among all axillary nodes harvested; ypN2 = 4–9 positive nodes among all axillary nodes harvested; ypN3 = ≥10 positive nodes among all axillary nodes harvested; HR = hormone receptor; HER2 = human epidermal growth factor receptor 2; TNBC = triple-negative breast cancer.

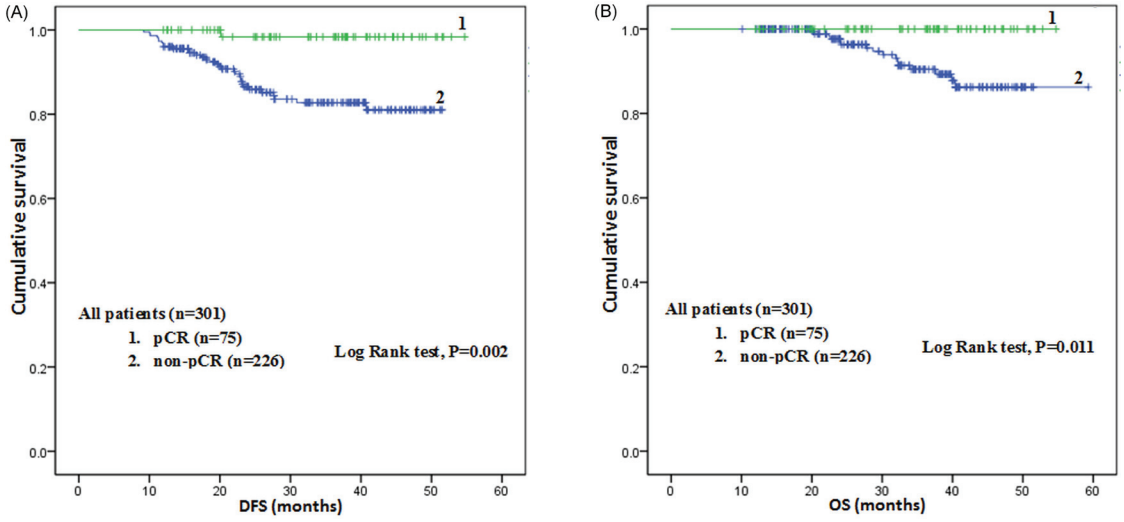

FIGURE 1 Pathologic complete response (pCR) predicts better survival. Compared with patients not having a pCR, those with a pCR had (A) significantly better disease-free survival (DFS) and (B) significantly better overall survival (OS).

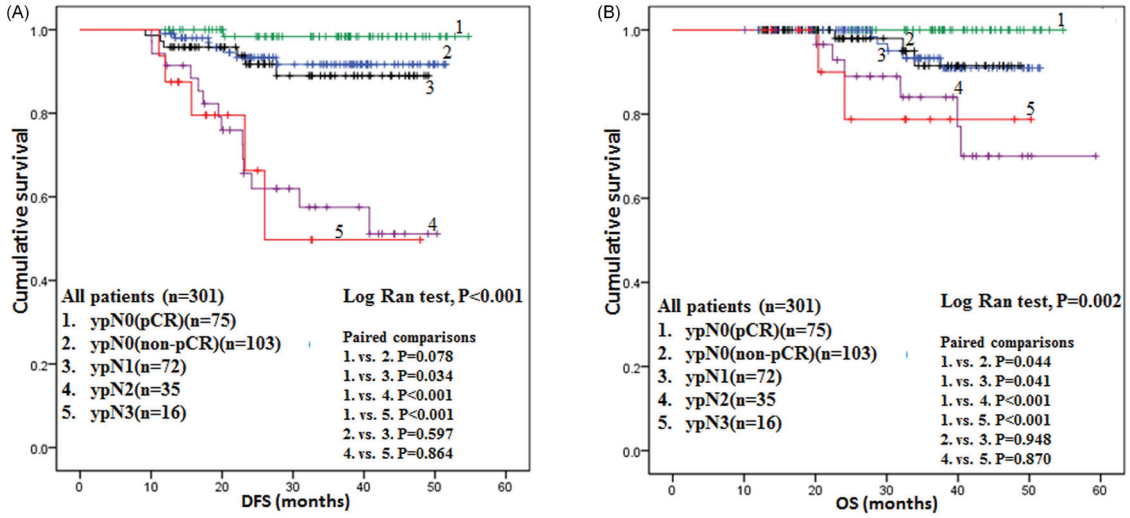

FIGURE 2 *Survival analysis of all patients, stratified by ypN status and adjusted by pathologic complete response (pCR). (A) The difference in disease-free survival (DFS) between ypN0(pCR), ypN0(non-pCR), ypN1, ypN2, and ypN3 patients was significant. (B) The difference in overall survival (OS) between ypN0(pCR), ypN0(non-pCR), ypN1, ypN2, and ypN3 patients was significant.*

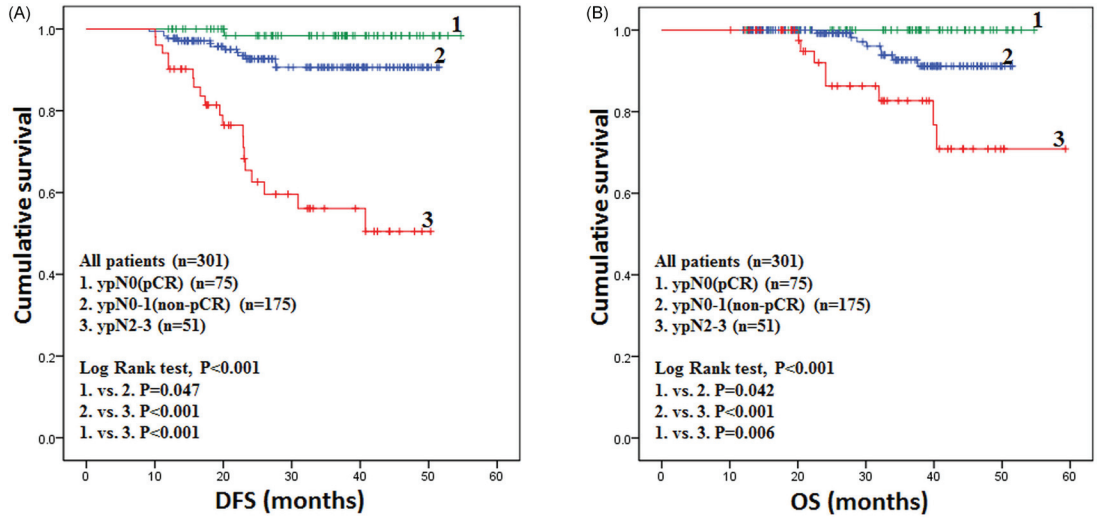

FIGURE 3 *Survival analysis for all patients, stratified by pathologic complete response (pCR). (A) Disease-free survival (DFS) was better for ypN0(pCR) patients than for ypN0–1(non-pCR) and ypN2–3 patients, and for ypN0–1(non-pCR) patients than for ypN2–3 patients. (B) Overall survival (OS) was better for ypN0(pCR) patients than for ypN0–1(non-pCR) and ypN2–3 patients, and for ypN0–1(non-pCR) patients than for ypN2–3 patients.*

18.6 harvested axillary lymph nodes, with only 3% of patients (9 of 301) having fewer than 10 harvested nodes (Table I)—ypN staging and LNR classification should be comparable, as was expected and observed in the present study (see Appendix A). In addition, our results also support the conclusion drawn by another study that NAC does not affect the number of harvested axillary lymph nodes[23]. The ypN classification should therefore be preferred over LNR given that it is already a universally accepted grading system.

Subtype stratification was also performed in the present study with the purpose of refining the findings. Rates of pCR were noted to be significantly different between the subtypes. The pCR rate was particularly low in the HR+ HER2– patients than in the HER2+ or TNBC patients. But the HR+ HER2– patients had the best prognosis regardless of response to NAC, after that subtype was adjusted for other clinicopathologic variables (Table II). In addition, patients with the HR+ HER2– subtype achieving pCR did not experience better survival than ypN0(non-pCR) and ypN1 patients [Figure 4(A,D)]. On the contrary, our results showed that pCR, ypN0–1(non-pCR), and ypN2–3 patients of the HER2+ subtype all had significantly different prognoses [Figure 4(B,E)].

These results suggest that that axillary restaging adjusted with pCR may have the power to predict prognosis for the disease subtypes. But, given the

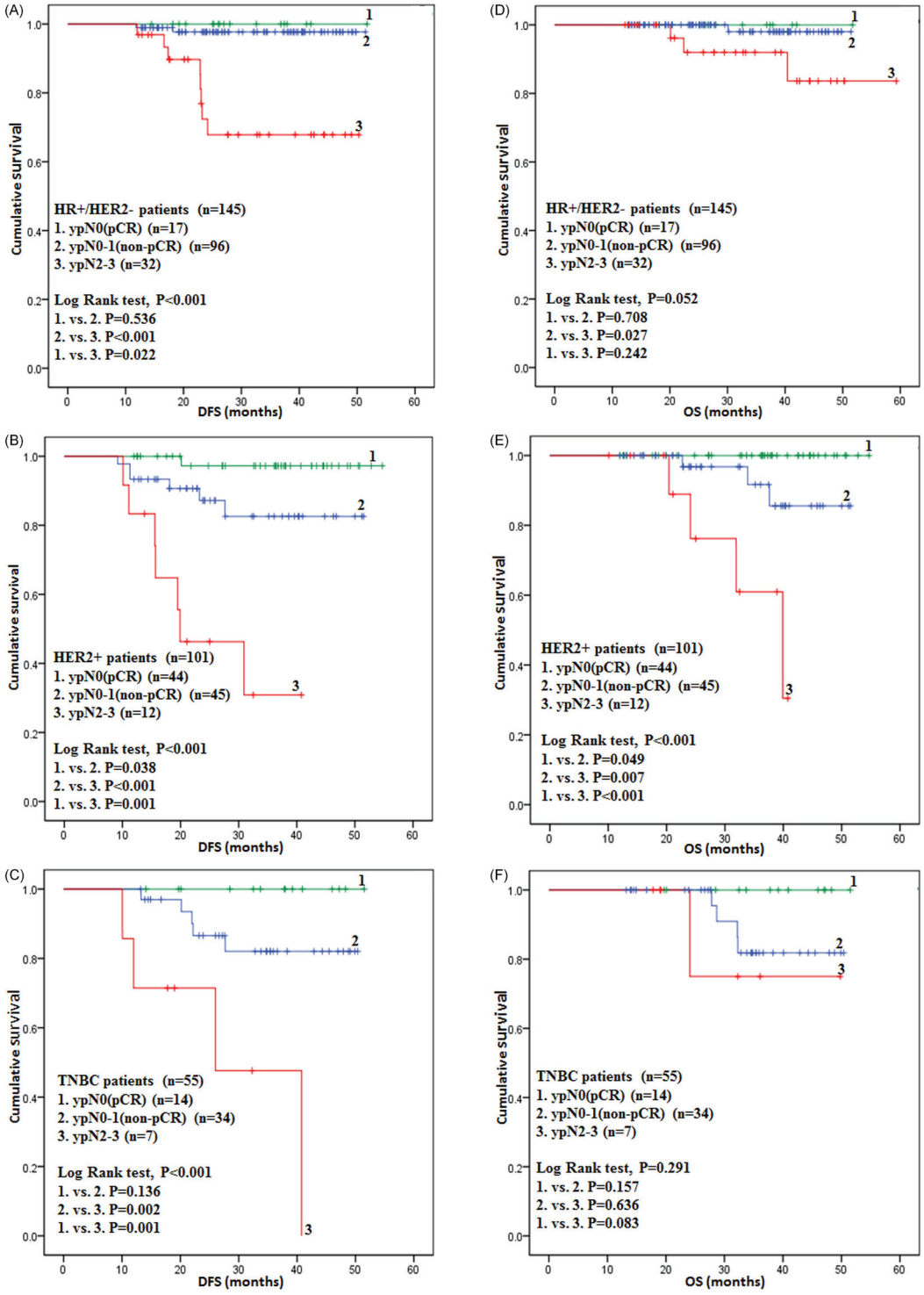

FIGURE 4 *Subtype-based survival analysis, stratified by ypN status and adjusted by pathologic complete response (pCR). (A) In the hormone receptor–positive (HR+), human epidermal growth factor receptor–negative (HER2–) subtype, the differences in disease-free survival (DFS) between ypN0(pCR), ypN0–1(non-pCR), and ypN2–3 patients were statistically significant. (B) In the HER2–positive (HER2+) subtype, DFS was better for ypN0(pCR) patients than for ypN0–1(non-pCR) and ypN2–3 patients, and for ypN0–1(non-pCR) patients than for ypN2–3 patients. (C) In the triple-negative breast cancer (TNBC) subtype, differences in DFS between ypN0(pCR), ypN0–1(non-pCR), and ypN2–3 patients were statistically significant. (D) In the HR+ HER2– subtype, differences in overall survival (OS) between ypN0(pCR), ypN0–1(non-pCR), and ypN2–3 patients were not statistically significant. (E) In the HER2+ subtype, OS was better for ypN0(pCR) patients than for ypN0–1(non-pCR) and ypN2–3 patients, and OS was better for ypN0–1(non-pCR) patients than for ypN2–3 patients. (F) In TNBC patients, differences in OS between ypN0(pCR), ypN0–1(non-pCR), and ypN2–3 patients were not statistically significant.*

significantly lower pCR rate in HR+ HER2– patients, pCR may not be as important in that particular subtype as in the others. Still, these results should not undermine the significance of axillary restaging, because ypN2–3 patients still had the poorest prognosis [Figures 4(A,D)] across all subtypes in our study. Moreover, in the present study, patients classified ypN2–3 had the worst long-term outcomes regardless of disease subtype despite all patients in that subpopulation having undergone standard systemic and local adjuvant treatment after NAC and curative surgery. That finding raises a question of general interest: Might more-aggressive adjuvant treatment in ypN2–3 patients improve the poor outcomes? In other words, might it be helpful to extend adjuvant trastuzumab to a 2-year duration or to add lapatinib, thus dually targeting HER2 in the HER2+ subtype? Or to apply ovarian ablation in premenopausal HR+ HER2– patients? Or to use an adjuvant chemotherapy regimen different from that used in the neoadjuvant setting for the TNBC subtype?

Such questions are beyond the scope of the present study, but they deserve to be resolved in future well-designed prospective clinical trials, because a prognostic factor will gain much more clinical significance when it can direct therapeutic decision-making and eventually lead to outcome improvements.

Several limitations of our study should be addressed. First, because of the patient selection criteria, our results are applicable only to patients not undergoing SLNB before completion of NAC. Whether our findings are applicable in the post-SLNB setting has to be determined in further studies. Second, the retrospective nature of the study means that the conclusions drawn should be treated with caution because of the possibility of hidden bias. Third, the relatively short follow-up period means that the subtype analysis cannot be considered definitive, especially for the TNBC subtype. Further follow-up is therefore warranted.

5. CONCLUSIONS

We demonstrated that, in patients without SLNB before completion of NAC, axillary restaging adjusted by pCR after completion of neoadjuvant treatment is an independent prognostic factor for breast cancer patients. The combination of ypN and pCR status has the power to predict differences in survival between intrinsic disease subtypes. The combined classification is convenient and provides valuable prognostic information. This work deserves further validation for its potential application in future clinical trials.

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7. CONFLICT OF INTEREST DISCLOSURES

The authors have no financial conflicts of interest to declare.

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APPENDIX A: SUPPLEMENTARY DATA

TABLE AI Multivariate Cox regression analysis of pathologic complete response (pCR) and lymph node ratio (LNR)[a] status

Factor	*Pts* (n)	*Disease-free survival*					*Overall survival*				
		Events (n)	*HR*	*95.0% CL*		p *Value*	*Events* (n)	*HR*	*95.0% CL*		p *Value*
				Lower	*Upper*				*Lower*	*Upper*	
LNR adjusted with pCR	301	33				**<0.001**	16				0.133
LNR0 (pCR)	75	1	0.07	0.01	0.59	**0.015**	0	—	—	—	0.946
LNR0 (non-pCR)	103	7	0.53	0.16	1.71	0.288	5	0.76	0.17	3.37	0.716
LNR1	72	6	1		Reference		3	1		Reference	
LNR2	35	12	4.8	1.65	13.93	**0.004**	4	3.91	0.77	19.77	0.099
LNR3	16	7	4.97	1.43	17.31	**0.012**	4	5.37	0.91	31.73	0.064
Patient age											
≥35 Years	267	29	1		Reference		15	1		Reference	
<35 Years	34	4	2.09	0.66	6.59	0.207	1	0.83	0.1	6.89	0.86
Clinical staging											
Stage II	233	18	1		Reference		11	1		Reference	
Stage III	68	15	1.39	0.6	3.2	0.438	5	0.88	0.22	3.42	0.848
Ki67 (continuous)			2.77	0.48	15.88	0.252		12.43	0.97	159.07	0.053
Subtype						**0.008**					0.083
HR+ HER2−	145	10	1		Reference		4	1		Reference	
HER2+	101	14	3.7	1.58	8.67	**0.003**	7	4	1.14	14.05	0.030
TNBC	55	9	3.17	1.17	8.56	**0.023**	5	3.56	0.85	15.01	0.083
Tumour grade											
Grades 1 and 2	243	27	1		Reference		14	1		Reference	
Grade 3	58	6	1.16	0.38	3.48	0.795	2	2.83	0.52	15.3	0.226

[a] Number of positive lymph nodes divided by the total number of axillary lymph nodes.
Pts = patients; HR = hazard ratio; CL = confidence limits; LNR0 = LNR of 0; LNR1 = 0 < LNR ≤ 0.2; LNR2 = 0.2 < LNR ≤ 0.65; LNR3 = 0.65 < LNR ≤ 1.0; HR = hormone receptor; HER2 = human epidermal growth factor receptor 2; TNBC = triple-negative breast cancer.

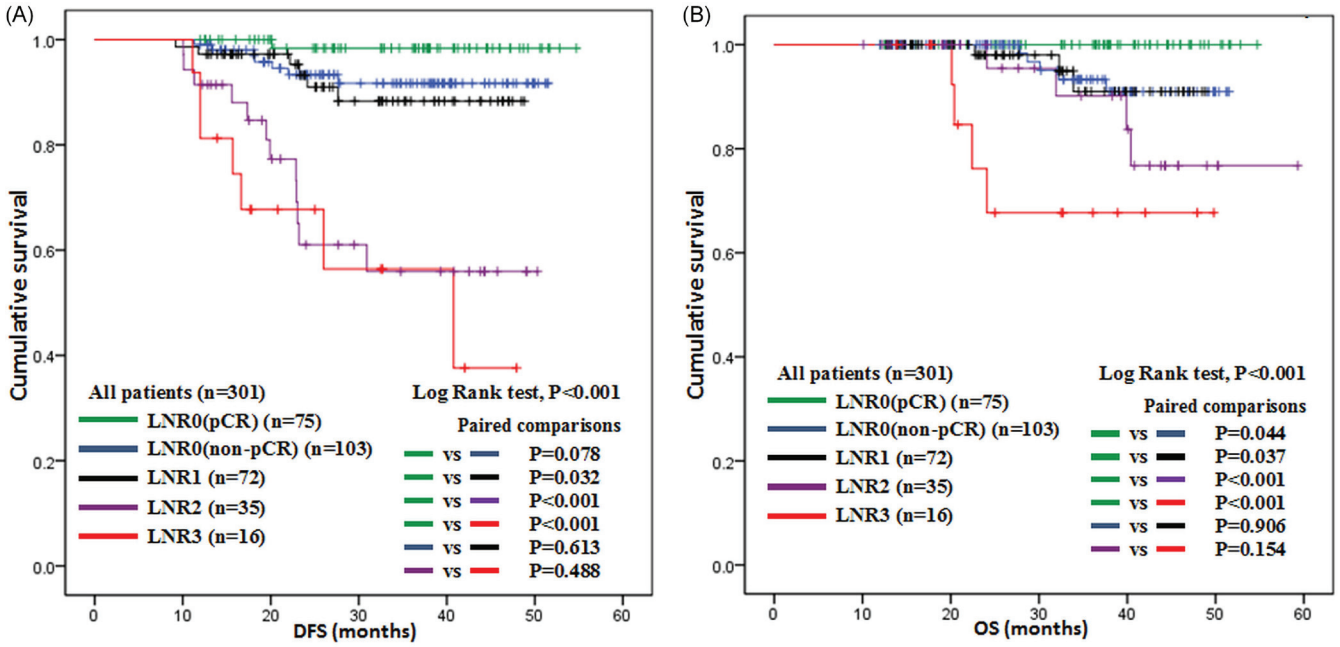

FIGURE A1 Survival analysis of all patients stratified by lymph node ratio (LNR), adjusted by pathologic complete response (pCR). (A) Disease-free survival (DFS) was significantly better for patients with LNR0(pCR) than for patients with LNR0(non-pCR), LNR1, LNR2, and LNR3. (B) Overall survival (OS) was significantly better for patients with LNR0(pCR) than for patients with LNR0(non-pCR), LNR1, LNR2, and LNR3.

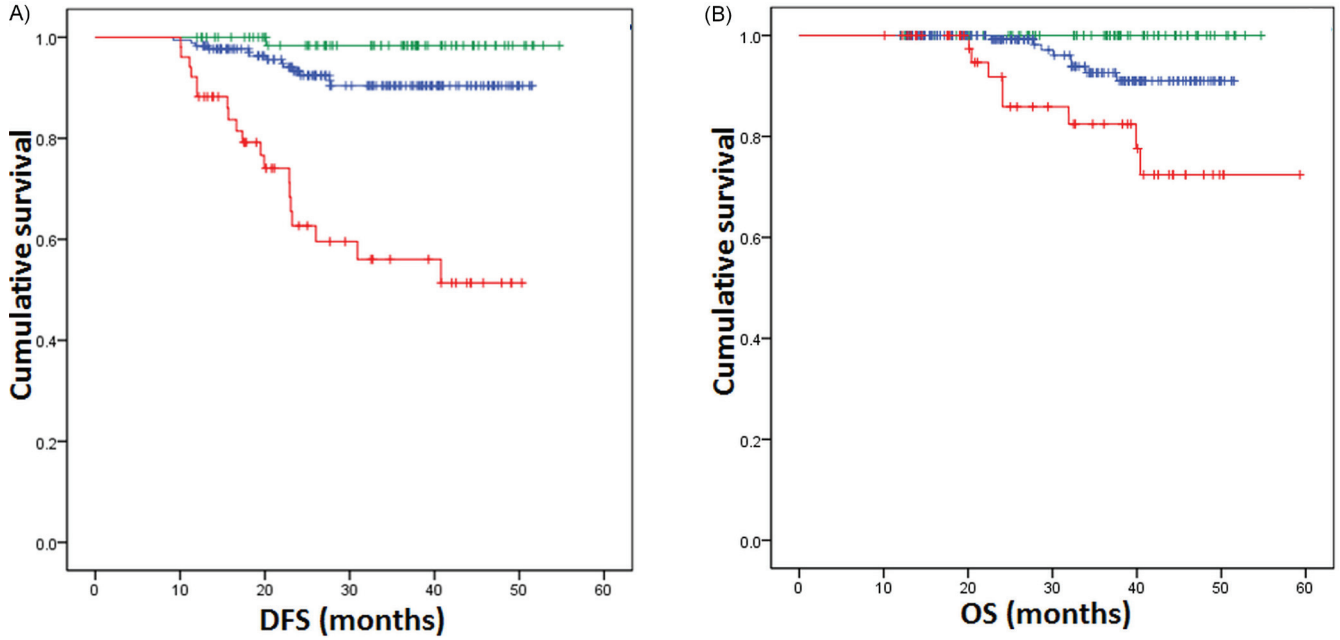

FIGURE A2 Survival analysis for all patients stratified by pathologic complete response (pCR), and comparing lymph node ratio (LNR) groups (LNR0–1 vs. LNR2–3). (A) Disease-free survival (DFS) was better for patients with LNR0(pCR) than for patients with LNR0–1(non-pCR) and LNR2–3; DFS was also better for patients with LNR0–1(non-pCR) than for patients with LNR2–3. (B) Overall survival (OS) was significantly better for patients with LNR0(pCR) than for patients with LNR0–1(non-pCR) and LNR2–3; OS was also significantly better for patients with LNR0–1(non-pCR) than for patients with LNR2–3.

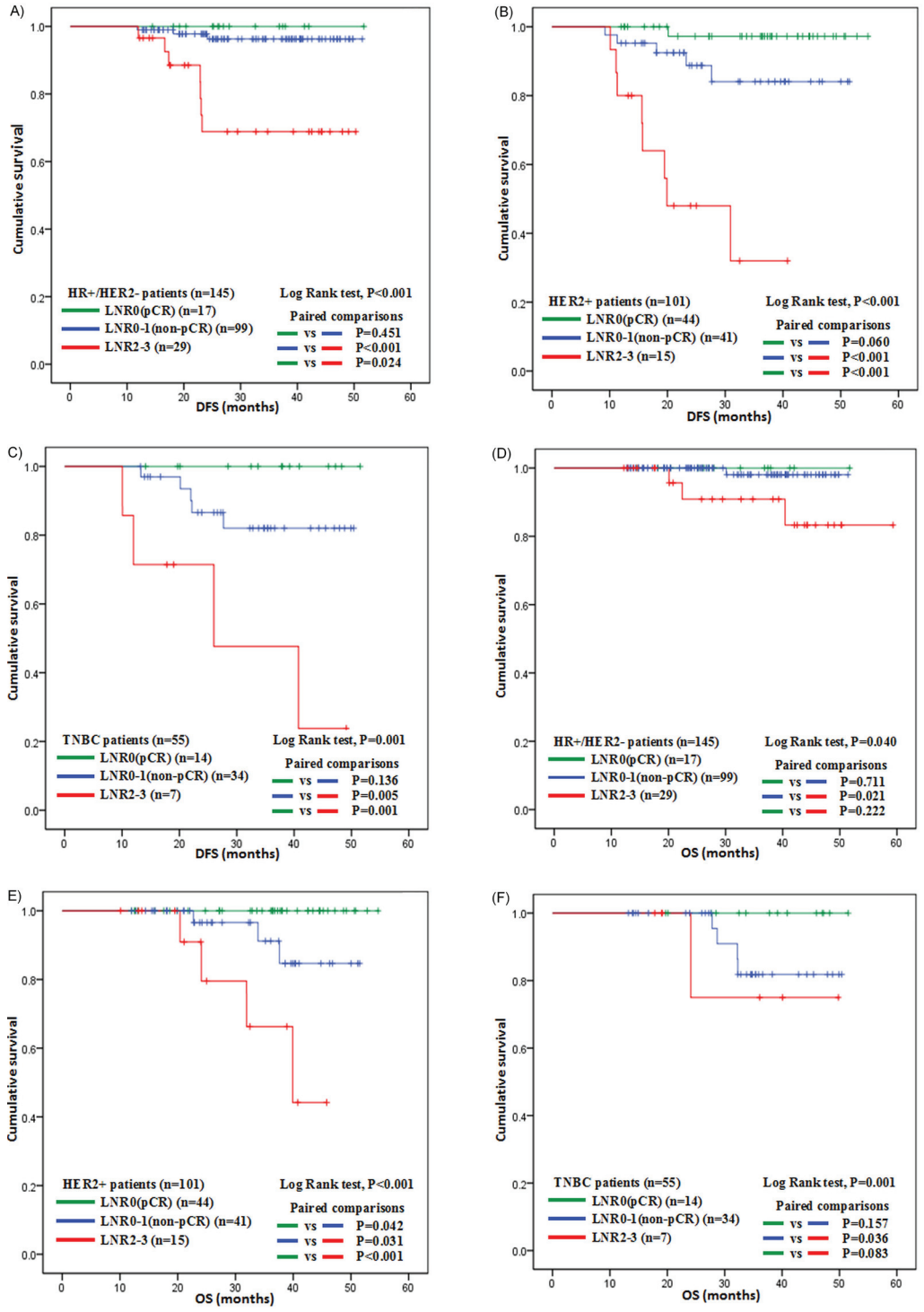

FIGURE 3 Subtype-based survival analysis stratified by lymph node ratio (LNR) status, adjusted by pathologic complete response (pCR). (A) In the subtype that is positive for either or both hormone receptors (HR+) and negative for the human epidermal growth factor receptor 2 (HER2–), statistically significant differences in disease-free survival (DFS) were observed between patients with LNR0(pCR), LNR0–1(non-pCR), and LNR2–3. (B) In the subtype positive for HER2 (HER2+), statistically significant differences in DFS were observed between patients with LNR0(pCR), LNR0–1(non-pCR), and LNR2–3. (C) In the triple-negative breast cancer (TNBC) subtype, statistically significant differences in DFS were observed between patients with LNR0(pCR), LNR0–1(non-pCR), and LNR2–3. (D) In the HR+, HER2– subtype, statistically significant differences in overall survival (OS) were observed between patients with LNR0(pCR), LNR0–1(non-pCR), and LNR2–3. (E) In the HER2+ subtype, OS was better for patients with LNR0(pCR) than for patients with LNR0–1(non-pCR) and ypN2–3 patients, and OS was better for patients with LNR0–1(non-pCR) than for patients with LNR2–3. (F) In the TNBC subtype, statistically significant differences in OS were observed between patients with LNR0(pCR), LNR0–1(non-pCR), and LNR2–3.