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Research Article

# Significance of Neutrophil-Lymphocyte Ratio and Thrombocyte-Lymphocyte Ratio in Predicting Complete Pathological Response in Patients with Local Advanced Breast Cancer

**Gulcan Bulut,[1] Zehra Narli Ozdemir[2]**

[1]Department of Medical Oncology, Medicana International Izmir Hospital, Izmir, Turkey
[2]Department of Hematology, Izmir Bozyaka Training and Research Hospital, Izmir, Turkey

## Abstract

**Objectives:** Pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC) is com-monly accepted as the gold standard to assess outcome after NAC in patients with locally ad-vanced breast cancer (LABC). The immune system plays a key role in the response to NAC of patients with LABC. Neutrophil-to-lymphocyte ratio (NLR) and thrombocyte-to-lymphocyte ratio (TLR) are peripheral blood indicators of inflammatory response. Here, we investigate the relationship between pCR and NLR-TLR and other clinicopathological features before patients receive NAC.

**Methods:** Forty-two female patients with LABC, who received NAC between 2013 and 2021 were evaluated retrospectively. NLR and TLR were calculated with the values of neutrophils, lymphocytes, and thrombocytes in complete blood count at the time of diagnosis. The cut off values of NLR and TLR were determined using receiver operating characteristic (ROC) curve analysis.

**Results:** Median age was 54.5 years (31-82). pCR was achieved in 16 (31.8%) of patients. Pa-tients divided into 2 groups according to NLR cut-off and TLR cut-off values as NLR/TLR high and low groups. The cut-off values of NLR and TLR were 2.27 and 130.25, respectively. There was no significant difference in pCR between NLR (high/low) and TLR (high/low) groups (p=0.13/0.38 and 0.13/0.13, respectively). The pCR rate was higher in patients with ER negative and HER2 positive subgroups (p = 0.002, and 0.040, respectively).

**Conclusion:** Pre-NAC; NLR and TLR were not successful in predicting the pathological complete response in patients with LABC.

**Keywords:** BreastCancer, Neoadjuvant chemotherapy, Neutrophil-to-lymphocyte ratio, trombocyte-to-lymphocyte ratio.

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Breast cancer is the most common cancer and the second cause of cancer death in women. Neo-adjuvant chemotherapy (NAC) is the standard treatment for locally advanced breast cancer (LABC) and tumor downstage to achieve breast-conserving surgery.[1] Pathological complete response (pCR) after NAC is considered a good predictive marker for disease-free survival (DFS) and overall survival (OS), particularly in patients with more aggressive subtypes such as triple-negative or HER2-positive breast cancer.[2,3] Therefore, there are numerous studies ex-amin-

**Address for correspondence:** Gulcan Bulut, MD. Tibbi Onkoloji Anabilim Dali, Medicana International Izmir Hastanesi, Izmir, Turkey
**Phone:** +90 506 700 75 65 **E-mail:** gulcanbulut07@gmail.com

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ing clinicopathological features that can be used to predict pCR in patients receiving NAC.[4] Clinical tumor size (cT) and tumor grade are other clinicopathological features used to pre-dict pCR.[4,5] Apart from molecular subtypes, no other biomarker including Ki 67 value, and residual cancer burden has so far been validated as predictive markers for pCR after NAC.

Recently, several studies showed that inflammatory markers in peripheral blood and immune-related indicators predict survival and chemotherapy response in different tumor types.[6–8] High neutrophil-thrombocyte counts, and low lymphocyte counts in the pretreatment period were associated with poor survival.[9] In addition, neutrophil-lymphocyte ratio (NLR) and thrombocyte-lymphocyte ratio (TLR), which are easily calculable factors of systemic inflamma-tory response, have been defined as prognostic factors in breast cancer.[10-12] Previous stud-ies claimed that high pCR rates were related with low NLR than compared with high NLR in patients with LABC.[13,14] In contrast, there was no association between NLR and pCR in some manuscripts.[15,16] There was limited data on the relationship between TLR and re-sponse to NAC. Asano et al noted out that low TLR is an independent predictive factor of pCR.[17]

The primary aim of this study was to determine whether the clinicopathological features and systemic inflammatory indicators (NLR, TLR) are predictive for pCR after NAC in patients with LABC. In addition, it is aimed to define a threshold value for NLR and TLR.

## Methods

### Patients

Medical records of the patients who received NAC after diagnosis of LABC in the Medical On-cology Division of Defne Hospital between 2013 and 2020, were retrospectively evaluated. The inclusion criteria to study were female gender, clinical status of II to III according to the 8th Edition tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer Staging,[18] complete blood count performed prior NAC, availability of postoperative patholo-gy reports after surgical procedure. As part of the NAC regimen, the patients were administered taxane -based regimens (paclitaxel 80 mg/m2 for 12 weeks or 4 cycles of docetaxel 75 mg/m$^2$, every 3 weeks) combined and anthracycline-based regimens (4 cycles of doxorubicin 60 mg/m$^2$ and cyclophosphamide 600 mg/m$^2$, or cyclophosphamide 600 mg/m$^2$ and epirubicin 50 mg/m$^2$, every 3 weeks). HER-2-positive patients treated with trastuzumab (At the time of the study, there was no access to pertuzumab in neoadjuvant therapy in our country). The exclusion crite-ria is co-morbidities that may alter complete blood count (presence of rheumatological diseases, existing chronic liver or chronic renal diseases) and drug use (antibiotic use within the last week before NAC, corticosteroid use), incomplete NAC, and absence of surgical treatment.

### Subtypes of Breast Cancer

Tumor size and lymph node involvement level were evaluated in all patients included in the study. Needle biopsy specimens performed before NAC and tissues removed by surgical pro-cedure were subjected to histopathological and immunohistochemical (IHC) examinations.

Estrogen receptor (ER), progesterone receptor (PR), and HER-2 status were determined by the IHC method; the specimens of patients with a staining level of ≥ 1% in the tumor cells were considered as having positive ER and PR status; further, HER-2 status was regarded positive if it was 3+ and negative if it was ≤1+. Then, HER-2 status was confirmed by fluorescence in situ hybridization (FISH) for patients with 2+ HER-2 status on IHC testing. Breast cancer was clas-sified into four subtypes: HR+, Her2+; HR+, Her2-; HR-, Her2+; and HR-, Her2-.

### pCR

In the postoperative pathological evaluation, the absence of invasive tumors in the breast tissue or lymph node (regardless of the presence of an in-situ component) was defined as pCR (ypT0/ypN0).

### NLR and TLR

Peripheral complete blood count was performed before administering NAC. Neutrophil, lym-phocyte, thrombocyte counts, and all laboratory indexes were evaluated prior to starting NAC. NLR was calculated as the rate of absolute neutrophil count to absolute lymphocyte count, TLR, as the rate of absolute thrombocyte count to absolute lymphocyte count.

NLR was divided into two groups according to the cutoff points ≥2.27 or <2.27 as NLR high and low (area under the curve: 0.577, specificity: 0.56, sensitivity: 0.58). TLR was divided into two groups based on the cut-off points (≥ 130.25 or <130.25) as TLR high and low (area under the curve: 0.606, specificity: 0.63, sensitivity: 0.62). The cut off values of NLR and TLR were determined using ROC curve analysis.

All the procedures were conducted according to the Declaration of Helsinki, and the study pro-tocol was approved by the Ethics Committee of Mustafa Kemal University, School of Medicine (Hatay, Turkey).

### Statistical Analysis

Chi-square, Fisher's exact and Mann-Whitney U tests were

used for comparison of age, radio-logical tumor size, lymph node involvement, hormone receptor status and HER2 status among groups as appropriate. Univariate and multivariate analysis were performed using logistic re-gression model. SPSS 22.0 (SPSS Inc., Chicago,IL, USA) software was used in all statistical analyses. A p value of <0.05 was considered as significant.

## Results

Forty-two female patients were included in the study between December 2013-December 2020. Median age was 54.5 years (31-82). Demographic features of the patients including age, radio-logical tumor size, lymph node involvement, hormone receptor status and HER2 status were given in table 1.

Univariate analysis of the patient and tumor characteristics to pCR were given in table 2. In terms of pCR; HR negative group was found statistically significant compared with HR positive group (p=0.02). And HER-2 positive group was found statistically significant compared with HER-2 negative group (p=0.04). There was no difference in terms of pCR between groups in NLR-low/NLR-high and TLR-low/TLR-high.

However, these results determined by univariate analysis; could not be confirmed by multivar-iate analyses in table 3.

## Discussion

The prognostic significance of the NLR and TLR have been investigated in many tumors.[19,20] Previous studies claimed that increased systemic inflammatory markers such as NLR and TLR are associated with poor prognosis in metastatic breast cancer.[11] In our study, we analyzed the association between pre-NAC NLR and TLR levels and pathological response in patients with locally advance breast cancer. Here, we reported that ER status, and HER2 status were independent predictive factors for pCR in patients with LABC but NLR and TLR were not associated with pCR.

Previous studies used similar NLR cut-offs, such as 2 or 5, to stratify patients into low- and high groups.[21,22] However, we defined a more accurate threshold level using the ROC curve analysis and used the 2.27 value to classify the patients into NLR low group (<2.27) and NLR high group (≥2.27). In our cohort, patients with low NLR did not have better pCR ( (31%, 45%; p= 0.38, p= 0.13, respectively).

Similarly, reports on the association of TLR with survival outcomes have generally used TLR cutoffs of 150 or 200, which divide patients into low- and high groups.[23] We used the value of 130.35 estimated by ROC curve analysis to classify patients as TLR low group (<130.35) and TLR high group (≥130.35). In our study, the pCR rate was higher in the low TLR group, but not reached statistical significance (50%, 27.3%; p=0.13, p=0.13, respectively).

**Table 1.** Demographic features of the patients according to NLR and TLR groups

| | All Patients N=42 (%) | NLR-low N=22 (52.4) | NLR-high N=20 (47.6) | p | TLR-low N=20 (47.6) | TLR-high N=22 (52.4) | p |
|---|---|---|---|---|---|---|---|
| Age (years) | | | | | | | |
| <50 | 14 (33.3) | 6 (27.3) | 8 (40) | .51 | 5 (25) | 9 (40.9) | .33 |
| >50 | 28 (66.7) | 16 (72.7) | 12 (60) | | 15 (75) | 13 (59.1) | |
| Stage | | | | | | | |
| <cT2 | 20 (45.2) | 12 (54.5) | 8 (40) | .37 | 11 (55) | 9 (40.9) | .53 |
| >cT2 | 22 (54.8) | 10 (45.5) | 12 (60) | | 9 (45) | 13 (59.1) | |
| Lymph node | | | | | | | |
| N1 | 11 (26.2) | 8 (36.4) | 3 (15) | .16 | 8 (40) | 3 (13.6) | .08 |
| N2+N3 | 31 (73.8) | 14 (63.6) | 17 (85) | | 12 (60) | 19 (86.4) | |
| Hormone receptor (HR) | | | | | | | |
| Negative | 19 (45.2) | 10 (45.5) | 9 (45) | .61 | 10 (50) | 9 (40.9) | .75 |
| Positive | 23 (54.8) | 12 (54.5) | 11 (55) | | 10 (50) | 13 (59.1) | |
| HER2 | | | | | | | |
| Negative | 27 (64.3) | 12 (54.5) | 15 (75) | .2 | 13 (65) | 14 (63.6) | .91 |
| Positive | 15 (35.7) | 10 (45.5) | 5 (25) | | 7 (35) | 8 (36.4) | |
| Molecular subtype | | | | | | | |
| HR+/Her2- | 18 (42.9) | 8 (36.4) | 10 (50) | | 8 (40) | 10 (45.5) | |
| HR-/Her2- | 9 (21.4) | 4 (18.2) | 5 (25) | | 5 (25) | 4 (18.2) | |
| HR-/Her2+ | 10 (23.8) | 6 (27.2) | 4 (20) | | 5 (25) | 5 (22.7) | |
| HR+/Her2+ | 5 (11.9) | 4 (18.2) | 1 (5) | | 2 (10) | 3 (13.6) | |

**Table 2.** Association of patient/tumor characteristics to pCR in univariate analysis.

| Variable | N=42 (%) | pCR (%) n=16 (38.1) | Odds ratio | %95 CI | p* |
|---|---|---|---|---|---|
| Age (years) | | | | | |
| <50 | 14 (33.3) | 7 (46.7) | 0.741 | 0.2-2.747 | .65 |
| >50 | 28 (66.7) | 9 (33.3) | 0.645 | | .19 |
| Stage | | | | | |
| <cT2 | 19 (45.2) | 9 (45) | 0.570 | 0.162-2.005 | .38 |
| >cT2 | 23 (54.8) | 7 (31.8) | 0.618 | | .13 |
| Lymph node | | | | | |
| N1 | 11 (26.2) | 6 (54.5) | 0.397 | 0.097-1.618 | .19 |
| N2+N3 | 31 (73.8) | 10 (32.3) | 0.756 | | .43 |
| Hormone receptor (HR) | | | | | |
| Negative | 19 (45.2) | 11 (57.9) | 0.202 | 0.53-0.776 | .02 |
| Positive | 23 (54.8) | 5 (21.7) | 1.375 | | .49 |
| HER2 | | | | | |
| Negative | 27 (64.3) | 8 (29.6) | 2.714 | 0.734-10.041 | .13 |
| Positive | 15 (35.7) | 8 (53.) | 0.421 | | .04 |
| NLR | | | | | |
| <2.27 | 22 (52.4) | 7 (31.8) | 0.57 | 0.162-2.005 | .38 |
| >2.27 | 20 (47.6) | 9 (45) | 0.618 | | .13 |
| TLR | | | | | |
| <130.35 | 20 (47.6) | 10 (50) | 0.375 | 0.104-1.354 | .13 |
| >130.25 | 22 (52.4) | 6 (27.3) | 0.612 | | .13 |

**Table 3.** Association of patient/tumor characteristics to pCR in multivariate analysis

| | p | OD ratio | 95% CI |
|---|---|---|---|
| HR status | .08 | 0.255 | 0.055-1.177 |
| HER2 status | .211 | 2.852 | 0.552-14.728 |
| NLR | .084 | 4.465 | 0.818-24.363 |
| TLR | .057 | 0.202 | 0.039-1.050 |

There were conflicting results on the relationship between NLR and pCR in patients receiving NAC for LABC.[16–19] Asano et al reported that among breast cancer patients receiving NAC, a higher rate of pCR was achieved in patients with a low NLR prior to therapy than in those with a high NLR.[17] Eren et al established that NLR is an independent predictive factor of pCR; while the pCR rate was 46.6% in patients with an NLR of <1.95, it was 11.6% in pa-tients with an NLR of ≥1.95 (OR: 3.438, 95%CI: 2.066–5.419, p< .001).[24] Suppan et al did not confirm the correlation between NLR and pCR.[15] Eryilmaz et al stated that NLR does not predict pCR.[16] In addition, we found that NLR is not an independent predictive factor of pCR; while the pCR rate was 31.8 % in patients with an NLR of <2.27, it was 45 % in patients with an NLR of ≥2.27 (OR: 4.46, 95%CI: 0.818–24.363, p=0.08). Similarly, TLR was not an independent predictive factor of pCR; while the pCR rate was 50 % in patients with an TLR of <130.35, it was 27.3% in patients with an NLR of ≥130.35 (OR: 0.202, 95%CI: 0.039–1.050, p=0.057)

The hormone receptor status is one of the important factors affecting pCR after NAC in breast cancer.[4] Battisti et al determined a significantly high pCR rate in ER-negative patients receiv-ing NAC as compared to in ER-positive patients.[25] Minckwitz et al. reported a pCR rate of 26% in ER-negative patients and of 7.6% in ER-positive patients (p<0.001).[4] Similar results were reported by Guarneri et al. 24% and 8% in ER-negative and ER-positive patients, respec-tively (p< .001).[26] Villa et al found significantly high pCR rates in ER-negative patients (OR: 0.87, 95%CI: 0.82–0.93; p<.001).[28] Eren et al. showed that the pCR rate was low in ER-positive patients compared to in ER-negative patients and that ER status was an independent predictive of pCR (OR: 0.250, 95%CI: 0.076–0.819; p=.022).[24] Our study showed that the higher pCR rate in ER-negative patients receiving NAC compared to ER-positive patients was consistent with the literature (OR: 0.20, 95% CI: 0.53-0.77; p < p=0.02). In addition, we re-ported a higher pCR rate (OR: 0.42, 95%CI: 0.73–10.04; p=0.04) in HER2-positive patients who received NAC compared to HER2-negative patients. However, these results determined by univariate analysis; could not be supported by multivariate analysis.

It is known that clinical and radiological tumor size at di-

agnosis is one of the factors predicting pCR after NAC in patients with LABC, and there is a significant decrease in pCR as cT in-creases.[5] Choi et al. found that cT is an independent predictive factor of pCR and pCR rates are higher in cT1 patients.[28] Univariate analysis by Villa et al found that cT was associated with pCR, while multivariate analysis showed that cT was not an independent predictor of pCR. [28] In our study, the pCR rate was similar in cT1-2 patients compared with cT≥ 3 patients (OR: 0.57, 95% CI: 0.162–2.00; p=0.38). Likewise, the pCR rate was similar in cN1 patients compared to cN2-3 patients (OR: 0.39, 95% CI: 0.097-1.618; p=0.19). There was no statisti-cal difference between the groups.

The major limitations of our study were its retrospective and small sample size single-arm tur-key-based cohort design. The molecular subgroups could not be included in the univariate anal-ysis since the number of patients in our study was small. Instead, hormone receptor status, which is the basis for molecular subgroups, namely HER-2 status was evaluated in the multi-variate analysis. Due to the small sample size, correlation analysis between NLR and molecular subgroups could not be performed separately.

The validity of the applied NLR and TLR cut-off should be investigated in a future prospective study with a larger sample size.

## Conclusion

In our study, we could not demonstrate successful prediction of the pathological complete re-sponse by NLR- TLR before treatment in patients with LABC treated with neoadjuvant chemo-therapy. We propose that these markers have limited prognostic value in NAC.

### Disclosures

**Ethics Committee Approval:** The approval of the Mustafa Kemal Üniversitesi Senate Ethics Committee was obtained before the study (Decision No: 8 Decision Date: 04.02.2021).

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship Contributions:** Concept – G.B.; Design – G.B., Z.N.O.; Supervision – G.B.; Materials – Z.N.O.; Data collection &/or processing – G.B.; Analysis and/or interpretation – Z.N.O.; Literature search – G.B.; Writing – G.B., Z.N.O.; Critical review – G.B.

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