Int J Clin Oncol (2015) 20:70–81
DOI 10.1007/s10147-014-0672-8

ORIGINAL ARTICLE

Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer?

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Received: 27 August 2013 / Accepted: 25 January 2014 / Published online: 18 February 2014
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Abstract
Background The neutrophil-to-lymphocyte ratio (NLR) is a strong predictor of mortality in patients with cancer. Similarly, a study in a large series has shown that the newly defined derived NLR (dNLR; neutrophil/leukocyte–lymphocyte ratio) also has prognostic value. The present study retrospectively evaluates the prognostic significance of NLR and dNLR in breast cancer.
Methods Hematological parameters and clinicopathological data during diagnosis were retrospectively recorded for 1,527 patients diagnosed with breast cancer at Izmir Katip Celebi University Ataturk Research and Training Hospital from January 2006 to December 2011. The cut-off values were determined by calculating the NLR and dNLR of the patients.
Results The cut-off values were determined as 4 and 2 for NLR and dNLR, respectively. The association between NLR and dNLR assessed by Spearman's rank correlation analysis was 0.935 ($P < 0.001$). There was a significant difference regarding disease free survival (DFS) and overall survival (OS) in patients with NLR <4 and NLR ≥4 (respectively, $P < 0.00$, $P < 0.001$). Similarly, there was a significant difference regarding DFS and OS in patients with dNLR <2 and dNLR ≥2 (respectively, $P < 0.001$, $P < 0.001$). Furthermore, NLR and dNLR demonstrated a significant association with the American Joint Committee on Cancer (AJCC) staging ($P < 0.001$). Assessment using the Cox proportional multivariate model showed that high NLR, pN, pT, luminal A-like, luminal B-like (HER2 positive), basal-like, and AJCC staging are independent prognostic factors.
Discussion NLR was shown to be better than dNLR in terms of predicting prognosis in patients with breast cancer. However, large prospective studies are required to further demonstrate the prognostic significance of these two values.

Keywords Breast cancer · Neutrophil · Lymphocyte · Prognosis

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Introduction

Breast cancer is the most common type of cancer among women. It is the main cause of cancer-related death in the age group of 40–59 years [1]. The prognostic factors of breast cancer include lymph node status, tumor size, histological type, histological grade, lymphatic–vascular invasion [2–6]. HER2 (human epidermal growth factor receptor 2) over-expression has also been shown to be a significant predictive and prognostic factor in the presence of estrogen (ER) and progesterone (PR) expression [7–11]. Cancer-related inflammation is considered to be effective in cancer prognosis and there are increasing studies on this subject. The host leukocytes, particularly tumor-associated

macrophages (TAM) in the microenvironment of the neoplastic tissue, play a key role. The transcription factors (such as nuclear factor-kB [NF-kB], signal transducer and activator of transcription-3 [Stat3]), cytokines (such as TNF, IL-1, IL-6), and chemokines (such as CCL2 and CXCL8) released from tumor cells and the leukocytes in this microenvironment lead to tumor angiogenesis, tumor cell survival, and proliferation [12].

The inflammation markers with prognostic significance in patients with cancer include C-reactive protein [13, 14] and abnormal inflammation-based score (Glasgow Prognostic Score, GPS) [15, 16]. The significance of neutrophil-to-lymphocyte ratio (NLR) as an inflammation marker has been increasing.

The production and release of neutrophils increases in the bone marrow in response to inflammation [17, 18]. The chemokines playing a significant role in cancer-related inflammation induce neutrophil release from the bone marrow and accumulation of neutrophils in peripheral tissue [19]. NF-kB activates human neutrophils and prolongs neutrophil survival by inhibiting neutrophil apoptosis [20]. In-vivo studies have shown that IL-1 induces neutrophil sequestration by regulating chemokine expression [21]. In contrast to such an effect on neutrophils, inflammation shows an inverse effect on lymphocyte count due to cytokines, particularly TNFα and IL-1β [22, 23]. Similarly, the CXXL2 and stem cell factors induced by the inflammation lead to granulopoiesis and inhibit lymphoid development [14].

Due to all these effects of inflammation, the neutrophil count increases while lymphocyte count decreases in peripheral blood. Consequently, these changes increase the NLR. Since increased NLR may be a marker of cancer-related inflammation, it may also be associated with prognosis. Several studies have shown that high NLR may be associated with poor prognosis in various types of cancer [24–27]. However, there are limited studies in the literature demonstrating the prognostic significance of NLR in breast cancer. Furthermore, a prognostic significance similar to that of NLR has been shown for derived NLR (dNLR) defined as the neutrophil count to white cell count–neutrophil count by Proctor et al. [27]. Defining this new ratio was intended to be a way to record the lymphocyte count while recording routine treatment data of cancer patients who are not part of a study.

The aim of the present is to determine the prognostic significance of NLR and dNLR in 1,527 patients with breast cancer.

Patients and methods

The data were retrospectively evaluated for 1,648 patients diagnosed with breast cancer at Izmir Katip Celebi

Table 1 Patient characteristics

	n (%)
Age (years)	
≤35	92 (6.0)
36–50	619 (40.6)
>50	816 (53.4)
Menopausal status	
Premenopausal	665 (43.6)
Postmenopausal	849 (55.6)
Unknown	13 (0.8)
Histology	
Invasive ductal carcinoma (IDC)	1075 (70.4)
Invasive lobular carcinoma (ILC)	91 (5.9)
Mixed (ductal and lobular)	120 (7.9)
Other	241 (15.8)
Pathology (pN)	
pN0	539 (37.6)
pN2	423 (29.5)
pN2	286 (19.9)
pN3	155 (10.8)
Unknown	31 (2.2)
Primary tumor (pT)	
pT0	10 (0.7)
pT1	440 (30.6)
pT2	718 (50.1)
pT3	104 (7.3)
pT4	118 (8.2)
Unknown	44 (3.1)
AJCC stage	
0	8 (0.5)
I	237 (15.4)
II	667 (43.5)
III	522 (34.0)
IV	101 (6.6)
Tumor grade	
1/2	1218 (79.4)
3	315 (20.5)
Unknown	4 (0.1)
Estrogen receptor status	
Negative	483 (31.5)
Positive	1019 (66.4)
Unknown	33 (2.1)
Progesterone receptor status	
Negative	497 (32.4)
Positive	994 (64.7)
Unknown	44 (2.9)
HER-2/*neu* staining	
Negative	883 (58.2)
1+	152 (9.9)
2+	181 (11.8)

Table 1 continued

	n (%)
3+	249 (16.2)
Unknown	60 (3.9)
FISH	
Positive	53 (3.5)
Negative	75 (4.9)

Table 2 Treatments administered to the patients

Type of surgery	
Breast preservation	726 (47.3)
Mastectomy	809 (52.7)
Adjuvant chemotherapy	
Yes	1278 (83.3)
No	257 (16.7)
Neo-adjuvant chemotherapy	
Yes	148 (9.6)
No	1379 (90.4)
Adjuvant radiotherapy	
Yes	1221 (79.5)
No	294 (19.2)
Unknown	20 (1.3)
Adjuvant hormone therapy	
Tamoxifen (Tmx)	410 (26.7)
Aromatase inhibitors (Ai)	518 (33.7)
Tmx → Ai*	410 (9.9)

* The group switched from tamoxifen to aromatase inhibitors

University Ataturk Research and Training Hospital from January 2006 to December 2011. Informed written consent for this retrospective study was obtained from the participants currently receiving treatment. The study began following approval by the Academic Committee of the Oncology Clinic. Preoperative complete blood counts (leukocytes, neutrophils, lymphocytes) of the patients with histologically verified breast cancer were analyzed in this study. Patients with active infection (high fever, classical symptoms and signs of infection in systems such as the upper and lower respiratory system, urinary system etc., identification of microorganisms in cultures of serous effusions, and radiological signs of the infection), active bleeding, blood transfusion within the last 3 months, chronic inflammatory or autoimmune disease, or steroid treatment were excluded from the study. 1,527 patients were enrolled in the study after employing the exclusion criteria.

The hospital participating in the present study has been employing an automatization system since 2006 and complete blood count data were obtained for all patients.

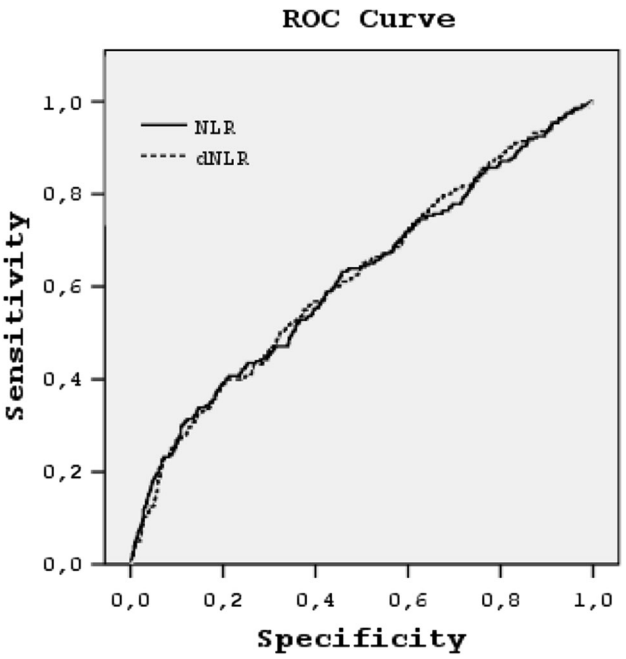


Fig. 1 Receiver-operating characteristics curve for cancer-specific survival

Furthermore, the age, pathological characteristics, staging, and all treatments received were recorded for these patients (Tables 1, 2). Disease staging was performed according to the Seventh Edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual. Complete blood counts were performed with an impedance-based analyzer (CELL-DYN 3700 ABBOTT, USA). Calibration of the analyzer was performed twice a day using control blood samples with high and low parameters. The NLR was calculated as follows: NLR = ratio of neutrophil count to lymphocyte count. The dNLR was calculated as follows: dNLR = ratio of neutrophil count to white cell count – neutrophil count.

Statistical analysis

The receiver-operating characteristic (ROC) curve was used to determine the sensitivity and specificity similarities between NLR and dNLR (Fig. 1). NLR values were compared using the Mann–Whitney *U* test. Fisher's exact probability test was used to determine the significance of differences between two groups. Survival probabilities were calculated using the product limit method of Kaplan and Meier considering overall deaths. Survival differences between groups were determined using the log-rank test. The influence of each significant predictor identified by univariate analysis was assessed by multivariate analysis using the Cox proportional hazards model.

Table 3 The distribution of all patients according to NLR and dNLR

Group	*n*	*Log-rank *P* value
NLR		
<1.0 vs 1.0 ≤ NLR < 2.0	60 vs 698	0.103
1.0 < NLR < 2.0 vs 2.0 ≤ NLR < 3.0	698 vs 458	0.395
2.0 ≤ NLR < 3.0 vs 3.0 ≤ NLR < 4.0	458 vs 170	0.592
3.0 ≤ NLR < 4.0 vs 4.0 ≤ NLR < 5.0	**170 vs 65**	**0.046**
4.0 ≤ NLR < 5.0 vs 5.0 ≤ NLR	65 vs 75	0.056
dNLR		
<1.0 vs 1.0 ≤ dNLR < 2.0	176 vs 953	0.232
1.0 < dNLR < 2.0 vs 2.0 ≤ dNLR < 3.0	**953 vs 277**	**0.047**
2.0 ≤ dNLR < 3.0 vs 3.0 ≤ dNLR < 4.0	277 vs 68	0.110
3.0 ≤ dNLR < 4.0 vs 4.0 ≤ dNLR < 5.0	68 vs 21	0.384
4.0 ≤ dNLR < 5.0 vs 5.0 ≤ dNLR	21 vs 32	0.42

* Log-rank *P* values were calculated between each combination of groups NLR and dNLR

Results

The correlation between NLR and dNLR

The reeiver-operating characteristics (ROC) curves, with overall survival (OS) as the endpoint, for NLR and dNLR are shown in Fig. 1. The ROC curves for NLR and dNLR were 0.615 ($P < 0.001$) and 0.617 ($P < 0.001$), respectively. Spearman's rank correlation between NLR and dNLR was 0.935 ($P < 0.001$).

NLR and dNLR during diagnosis

Of the 1,527 patients, mean NLR and dNLR were 2.39 ± 1.54 and 1.81 ± 1.09, respectively, while median values were 2.01 (range 0.8–18.12) and 1.54 (range 0.34–12.45), respectively. All patients were grouped according to NLR and dNLR. There was a difference regarding OS between the groups (Table 3). With these findings, the cut-off values for NLR and dNLR predicting patient prognosis were determined as 4.0 and 2.0, respectively.

The association between NLR–dNLR and the clinicopathological variables

There were 138 (9.1 %) patients with NLR above 4, and 397 (26.2 %) patients with dNLR above 2. The association between NLR–dNLR and clinicopathological variables is shown in Table 4. According to this evaluation, tumor depth (pT), nodal status, AJCC staging, and distant metastasis status were found to be statistically significant associated with high NLR and dNLR percentages (Table 4). Furthermore, dNLR had a statistically significant association with age, menopausal status, HER2 expression status [28], ki-67 proliferation index [29], and hormone receptor positivity [30] (Table 4). The relationship between the NLR and dNLR values and the AJCC stage in all patients is shown in Fig. 2. Increasing pathological stage was associated with increasing median values of NLR and dNLR. Median NLR values were 1.45 ± 0.41 for stage 0, 1.86 ± 1.32 for stage I, 1.88 ± 1.19 for stage II, 2.14 ± 1.63 for stage III, and 2.65 ± 1.82 for stage IV. Median dNLR values were 1.11 ± 0.33 for stage 0, 1.45 ± 0.97 for stage I, 1.48 ± 0.99 for stage II, 1.67 ± 1.17 for stage III, and 1.78 ± 1.34 for stage IV.

The prognostic significance of NLR and dNLR

Univariate analysis

Median follow up time was 30 months (range 2–75 months). The differences between the mean OS and mean disease-free survival (DFS) (for stage I–III) for the groups of patients with above and below NLR and dNLR cutoff values were found to be statistically significant (Figs. 3, 4, 5, 6; Table 5). Hazard ratios of baseline characteristics for OS and DFS among patients with breast cancer were determined in this study using Cox regression analysis (Table 5).The analysis showed that high NLR, high dNLR, age, histological grade, histological type, high levels of preoperative CA15-3, positive HER2 (with FISH or IHC +++), pT, pN, and advanced AJCC stage were all associated with a high mortality risk while adjuvant chemotherapy, adjuvant hormonal therapy, adjuvant radiotherapy, neo-adjuvant chemotherapy and LHRH analogs were associated with low mortality risk (Table 6). In addition, according to univariate analysis; patients with luminal B-like (HER2 positive), HER2 positive (non-luminal), and basal-like (triple negative) subtypes have inferior OS and recurrence rates when compared with patients with the luminal A-like subtype (Table 6).

Multivariate analysis

The variables found to be associated with prognosis in the univariate analysis were evaluated using the Cox proportional hazard multivariate model (Table 7). The model

Table 4 NLR and dNLR according to clinicopathological variables

	High NLR[1] *n* (%)	Low NLR[1] *n* (%)	*P*[2]	High dNLR[3] *n* (%)	Low dNLR[3] *n* (%)	*P*[2]
Age (years)						
≤35	86 (5.6)	9 (0.6)	0.068	67 (4.4)	28 (1.8)	**0.003**
36–50	550 (35.9)	69 (4.5)		431 (28.1)	189 (12.3)	
>50	755 (49.3)	62 (4.0)		1130 (73.8)	402 (26.2)	
Menopausal status						
Premenopausal	595 (39.0)	72 (4.7)	0.144	465 (30.4)	203 (13.3)	**0.003**
Postmenopausal	784 (51.3)	67 (4.4)		657 (43.0)	194 (12.7)	
Histology						
IDC	981 (65.0)	94 (6.2)	0.502	796 (52.7)	279 (18.5)	0.243
ILC	85 (5.6)	6 (0.4)		62 (4.1)	30 (2.0)	
Mixed	107 (7.1)	15 (1.0)		86 (5.7)	36 (2.4)	
Histological grade						
G1	218 (14.2)	14 (0.9)	0.332	182 (11.9)	50 (3.3)	0.165
G2	888 (58.0)	96 (6.3)		719 (46.9)	265 (17.3)	
G3	283 (18.5)	30 (2.0)		228 (14.9)	86 (5.6)	
HER2 receptor (+)[4]						
Yes	1070 (72.7)	108 (7.3)	0.280	863 (58.6)	316 (21.5)	**<0.001**
No	270 (18.4)	23 (1.6)		221 (15.0)	72 (4.9)	
Ki-67[5]						
<14	39 (3.8)	499 (48.4)	0.257	123 (11.9)	415 (40.3)	**0.033**
≥14	42 (4.1)	450 (43.7)		138 (13.4)	354 (34.4)	
ER and/or PR[5]						
(+)	1064 (70.8)	103 (6.9)	0.376	870 (57.9)	298 (19.8)	**<0.001**
(−)	303 (20.2)	32 (2.1)		240 (16.0)	95 (6.3)	
pT						
T0, T1	430 (29.6)	25 (1.7)	*<0.001*	365 (25.2)	90 (6.2)	**<0.001**
T2	673 (46.4)	58 (4.0)		538 (37.1)	193 (13.39)	
T3	91 (6.3)	13 (0.9)		78 (5.4)	26 (1.8)	
T4	128 (8.8)	33 (2.3)		94 (6.5)	67 (4.6)	
pN						
N0	512 (35.0)	30 (2.1)	*<0.001*	429 (29.3)	113 (7.7)	**<0.001**
N1	399 (27.3)	30 (2.1)		336 (23.0)	93 (6.4)	
N2	274 (18.7)	47 (3.2)		201 (13.7)	120 (.2)	
N3	151 (10.3)	9 (1.3)		120 (8.2)	50 (3.4)	
Distant metastasis						
M0	1159 (86.4)	92 (6.4)	*<0.001*	950 (70.8)	301 (22.4)	**0.033**
M1	68 (5.1)	22(1.6)		51 (3.8)	39 (2.9)	
AJCC stage						
0–I	228 (14.9)	16 (1.0)	*<0.001*	195 (12.7)	49 (3.2)	**<0.001**
II	628 (41.0)	38 (2.5)		528 (34.5)	138 (9.0)	
III	459 (30.0)	62 (4.0)		351 (22.99)	170 (11.1)	
IV	76 (5.0)	24 (1.6)		56 (3.7)	45 (2.9)	
Ki-67[6]						
<14	39 (3.8)	499 (48.4)	0.257	123 (11.9)	415 (40.3)	**0.033**
≥14	42 (4.1)	450 (43.7)		138 (13.4)	354 (34.4)	

[1] *High NLR* the patient group with NLR values of 4.0 or higher. *Low NLR* the patient group with NLR values <4.0

[2] *P* values were calculated using the chi squared test

[3] *High dNLR* the patient group with dNLR values of 2.0 or higher. *Low dNLR* the patient group with dNLR values <2.0

[4] HER2 status was defined according to The American Society of Clinical Oncology and the College of American Pathologists expert panel [28]

[5] Ki-67 score was defined according to St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013 in which the cut-off value of Ki-67 was determined as 14 % [29]

[6] Estrogen receptor was defined according to St Gallen international consensus panel 2009 in which the ER positivity was accepted as ≥1 % [30]

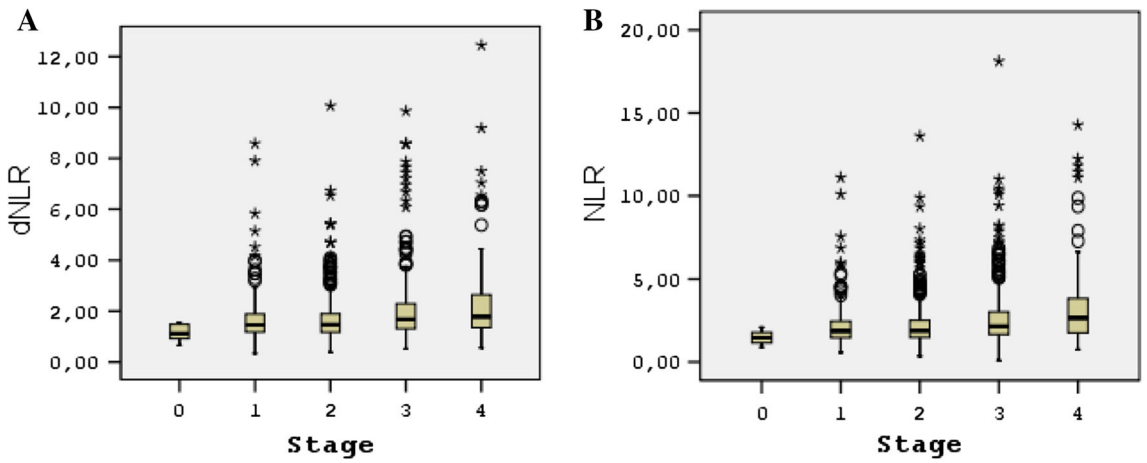


Fig. 2 The relationship between NLR, dNLR and AJCC stage in patients with breast cancer. **a** NLR (stage 0, I, II vs stage III, $P < 0.001$; stage III vs stage IV, $P < 0.001$), **b** dNLR (stage 0, I, II vs stage III, $P < 0.001$; stage III vs stage IV, $P < 0.048$)

demonstrated that high NLR (NLR ≥4) is an independent prognostic factor for OS and DFS while prognostic significance could not be shown for high dNLR (dNLR ≥2) ($P = 0.001$, $P = 0.83$, respectively). The other independent prognostic factors for OS and DFS included pN, pT, subtype, and AJCC stage.

Discussion

The present study is the first study evaluating the prognostic affects of both NLR and dNLR in breast cancer. In the present study, a high NLR value at diagnosis in patients with breast cancer was found to be associated with poor prognosis. ROC and Spearman's rank correlation analysis demonstrated similar findings for NLR and dNLR, while the multivariate analysis did not show prognostic significance for dNLR regarding OS and DFS (Figs. 1, 3; Tables 6, 7).

Cancer-related inflammation has been shown to have adverse effects on cancer prognosis. Therefore, the high NLR resulting from inflammation is considered to be potentially related to poor prognosis and this association has been shown in several studies [24–27]. Absolute neutrophil and lymphocyte counts could be affected by various physiological, pathological, and physical factors while NLR remains stable with respect to these factors. This is why the stability of NLR is superior to the leukocyte subtype [27]. The stability of dNLR is similar to that of NLR as the same parameters are used to calculate them.

In the previous study, NLR values >3.33 were shown to be an independent significant predictor for mortality in breast cancer [31]. This study reported lower 1- and 5-year mortality rates for patients with NLR >3.33 (0 vs 16 and 13 vs 44 %; $P < 0.0001$). The same study showed that patients with high NLR ratios were older, had larger tumors, and were in more advanced AJCC stages, all with statistical significance.

The cut-off value for NLR was determined as 4 in the present study, and the 1- and 5-year OS rates in patients with high (≥4) and low (<4) NLR were 98.1 vs 95.4 and 81.1 vs 63.3 %, respectively (Table 5; Fig. 5; log rank; $P < 0.001$). Over a follow-up period of 18 months, the difference in DFS between patients with high and low NLR became more significant, similar to the results for OS (Table 5; Fig. 3; Log rank; $P \leq 0.001$). Additionally, NLR significantly predicted short-term and long-term OS and DFS, as found in another study published by Basem et al. [28]. The present study also showed increasing NLR ratios with increasing AJCC stages (Fig. 2; Table 4). Furthermore, nodal involvement, larger tumors, positive HER2 (IHC 3+ or FISH+), and the presence of distant metastasis were shown to be associated with high NLR while positive ER and PR were associated with lower NLR values (Table 4). The present study demonstrates that high NLR is an independent prognostic factor in breast cancer as per the multivariate analysis (HR 1.95; $P = 0.001$). The results of the present study are consistent with the results of Basem et al. [31].

Apart from the study by Basem et al., there are no large studies in the literature demonstrating the

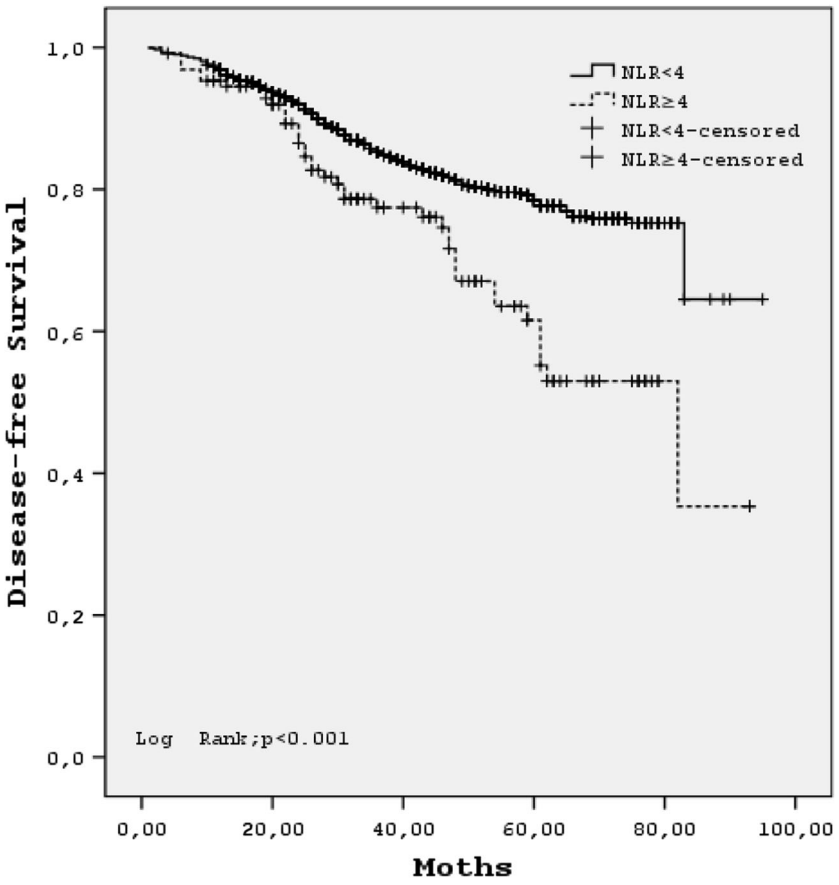


Fig. 3 Disease-free survival curves stratified by neutrophil–lymphocyte ratio during diagnosis

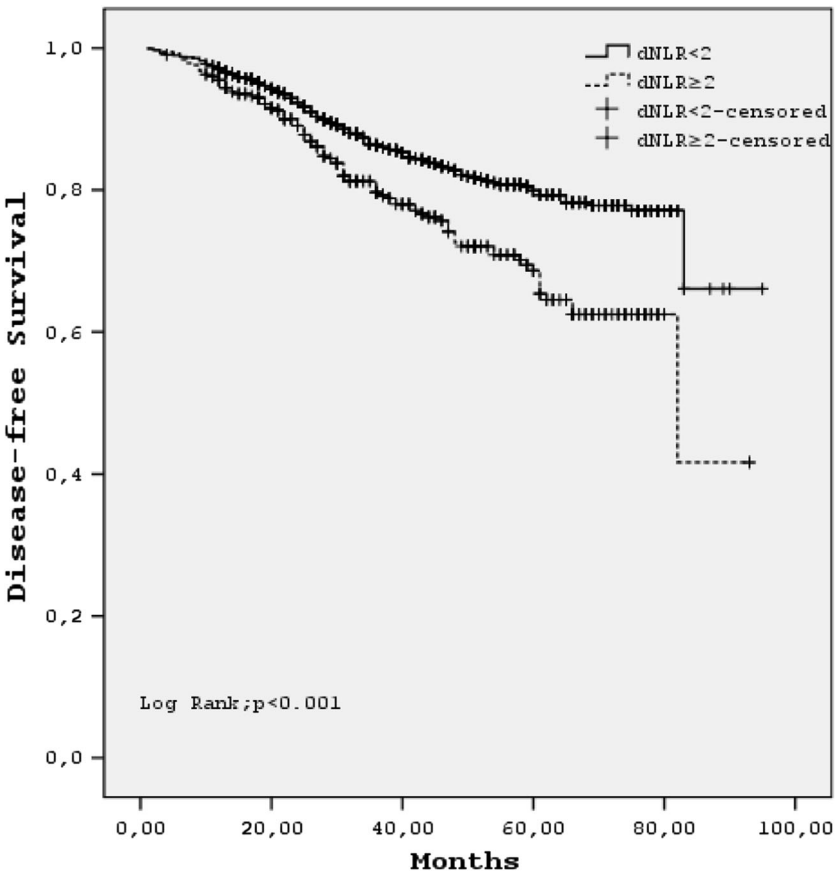


Fig. 4 Disease-free survival curves stratified by derived neutrophil–lymphocyte ratio during diagnosis

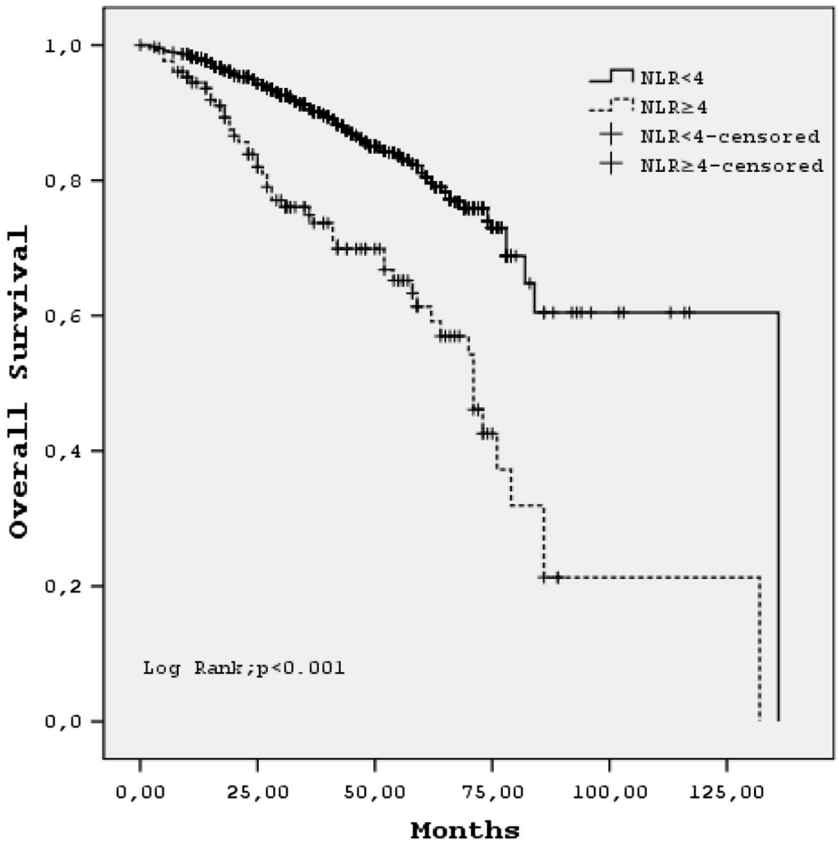


Fig. 5 Overall survival curves stratified by neutrophil–lymphocyte ratio during diagnosis

prognostic significance of NLR in breast cancer. In a study showing that NLR is a prognostic factor in unselected cancer patients, the proportion of patients with breast cancer was 29 % ($n = 621$) [27]. However, there was no subgroup analysis for patients with breast cancer. A subgroup of the same study showed the association between NLR and survival in patients with advanced (Dukes C and D) colorectal cancer. Another study demonstrating the significance of NLR in colorectal cancer showed that NLR >5 predicted poor overall survival in these patients ($P = 0.002$) [32]. The same study also showed improved PFS following normalization of NLR after chemotherapy ($P = 0.012$). Similarly, a difference in 5-year survival was found between patients with high NLR and low NLR in gastric cancer (57 vs 82 %, $P < 0.001$). That study reported that preoperative high NLR may be a marker of poor prognosis in gastric cancer. Pre-treatment NLR values have been associated with OS and PFS in patients with metastatic renal cell carcinoma receiving sunitinib therapy [33].

dNLR was initially defined by Proctor et al. [27]. This ratio employed the WBC–neutrophil value instead of the lymphocyte value as the denominator. According to this study, dNLR and NLR were similar regarding cancer-specific death, and were independent risk factors for survival across all cancer types. That study reported the cut-off values for NLR and dNLR as 4:1 and 2:1, respectively, and similar cut-off values were determined for NLR and dNLR in the present study. The present study also demonstrates the similarity between dNLR and NLR regarding cancer-specific death, and the OS and DFS were found to be significantly different between patients with high dNLR and those with low dNLR (HR 1.8, $P < 0.001$ for OS; HR 1.71 $P < 0.001$ for DFS). However, the multivariate analysis does not demonstrate that dNLR is an independent risk factor. The use of WBC–neutrophil value instead of the lymphocyte value results in the inclusion of the effect of the monocyte value together with the lymphocyte value. The peripheral monocyte count is known to increase in cancer [17, 18, 23]. The increased number of monocytes

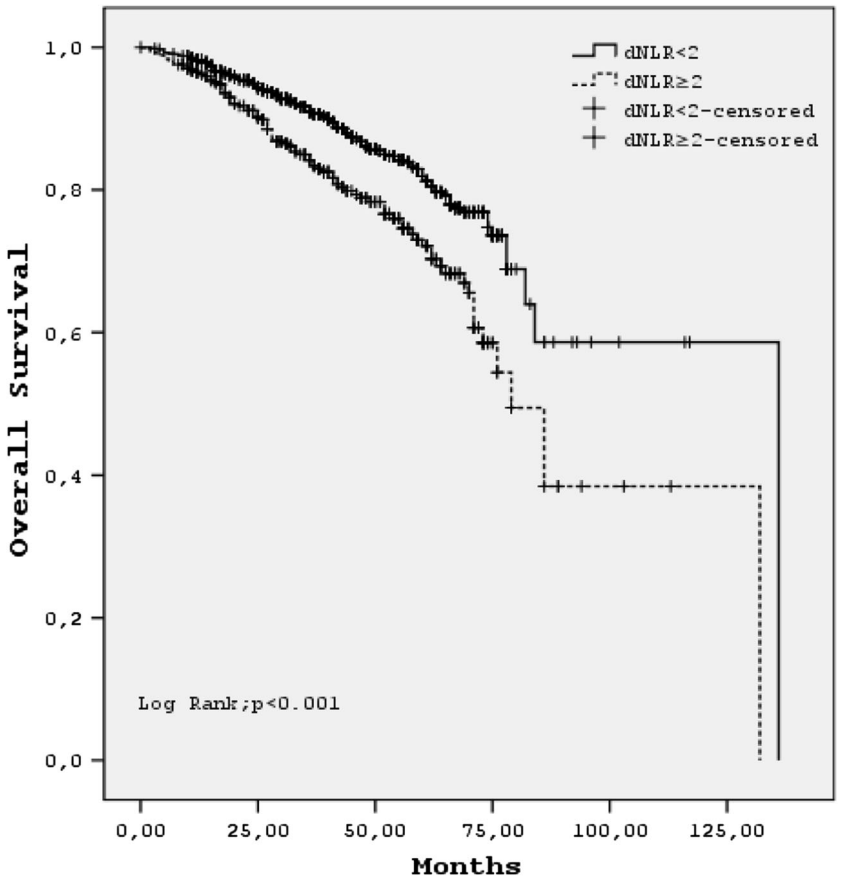


Fig. 6 Overall survival curves stratified by derived neutrophil–lymphocyte ratio during diagnosis

Table 5 Overall survival and disease-free survival according to cutoff values (NLR:4, dNLR:2)

	DFS (%)	*P**	OS (%)	*P**
NLR (months)	<4 vs ≥4	***<0.001***	<4 vs ≥4	***<0.001***
12	96.9 vs 94.3		98.1 vs 95.4	
18	95.6 vs 93.8		96.5 vs 90.2	
24	92.5 vs 86.8		95.1 vs 85.2	
36	85.3 vs 77.4		90.5 vs 76.1	
60	79.2 vs 61.6		81.1 vs 63.3	
dNLR (months)	<2 vs ≥ 2	***<0.001***	<2 vs ≥ 2	***<0.001***
12	97.2 vs 95.5		98.4 vs 96.5	
18	95.2 vs 94.0		96.4 vs 94.0	
24	92.7 vs 89.8		95.2 vs 92.3	
36	86.3 vs 79.7		90.9 vs 85	
60	79.9 vs 65.4		81.9 vs 71.9	

* Log rank *P* value

has been associated with poor prognosis in cancer [22]. The increased number of monocytes and the reduced number of lymphocytes have inverse effects on the ratio outcome. Therefore, NLR may be considered a better marker to predict the outcome of inflammation than dNLR. Evaluation of the results obtained in the present study shows that NLR was superior to dNLR in predicting the prognosis. The study by M.J. Proctor and colleagues also reported the superiority of NLR in predicting prognosis in cancer [27].

The major limitations of the present study are the retrospective nature of the study and the single center experience. We are unable to exclude the possibility that unequal distribution of unidentified clinicopathological parameters in our patient cohort may have biased the results observed in the present study. Despite these limitations, our clinical observation shows that the neutrophil-to-lymphocyte ratio may be associated with DFS and OS in patients with breast cancer. The neutrophil-to-lymphocyte ratio is an easy to determine, reproducible, and inexpensive test. It can be easily implanted into routine use as a prognostic factor. NLR is obviously better than dNLR in terms of predicting prognosis. Despite all these results, prospective studies evaluating both NLR and dNLR in large series are required in this field.

Table 6 Hazard ratios of baseline characteristics for overall survival and disease-free survival among patients with breast cancer (univariate analysis)

	Overall survival			Disease-free survival		
	n	Hazard ratio (95 % CI)	*P* value	*n*	Hazard ratio (95 % CI)	*P* value
NLR ≥4	138	2.82 (2.10–3.79)	**<0.001**	116	2.18 (1.58–3.01)	**<0.001**
dNLR >2	397	1.80 (1.40–2.32)	**<0.001**	356	1.71 (1.33–2.19)	**<0.001**
Age	1529	1.02 (1.01–1.04)	**<0.001**	1433	0.99 (0.98–1.0)	NS
Menopausal status	853	0.93 (0.63–1.38)	NS	781	2.13 (0.68–6.71)	NS
Histological grade (reference: Grade1)						
Grade 2	981	2.58 (1.49–4.40)	**<0.001**	910	1.91(1.29–2.83)	**0.01**
Grade 3	318	3.05 (1.85–5.02)	**<0.001**	297	1.57 (1.0–2.47)	**0.048**
Histology (reference: other)						
IDC	1076	1.55 (0.95–2.53)	NS	1013	1.60 (1.06–2.41)	**0.024**
ILC	91	0.84 (0.51–1.39)	NS	84	2.19 (1.21–3.97)	**0.01**
Mixed	122	1.40 (1.01–1.96)	**0.042**	117	1.69 (0.98–2.92)	NS
High CA 15-3 (preoperative)	53	3.10 (1.56–6.14)	**0.001**	37	1.011 (1–1.015)	**<0.001**
Luminal A-like* (reference)	516		**0.001**	491		**<0.001**
Luminal B-like (HER2–)*	345	1.27 (0.87–1.83)	0.203	324	1.22 (0.86–1.74)	0.261
Luminal B-like (HER2+)*	143	1.87 (1.18–2.95)	**0.007**	126	1.77 (1.13–2.77)	**0.013**
HER2+(non-luminal)*	117	1.65 (1.03–2.64)	**0.037**	111	1.91 (1.24–2.94)	**0.003**
Basal-like (triple negative)*	214	2.29 (1.52–3.46)	**<0.01**	198	2.37 (1.57–3.56)	**<0.001**
Tumor depth (reference: pT0–T1)						
pT2	730	1.76 (1.21–2.57)	**0.003**	717	1.85 (1.33–2.57)	**<0.001**
pT3–pT4	264	4.98 (3.40–7.31)	**<0.001**	222	4.14 (2.89–5.92)	**<0.001**
N factor (reference: pN0)						
pN1	429	1.88 (1.23–2.88)	**0.003**	422	1.41 (0.99–2.01)	0.055
pN2–pN3	490	4.55 (3.18–6.51)	**<0.001**	441	3.30 (2.44–4.45)	**<0.001**
AJCC stage (reference 0–1)						
Stage 2	665	1.93 (1.08–3.46)	**0.026**	665	1.50 (0.95–2.37)	NS
Stage 3–4 (only stage 3 for DFS)	620	6.25 (3.62–10.77)	**<0.001**	522	3.98 (2.59–6.11)	**<0.001**
Adjuvant chemotherapy (reference: no chemo)						
Taxane-based	380	0.48 (0.34–0.68)	**<0.001**	365	0.62 (0.47–0.82)	**0.001**
Anthracycline-based	894	0.33 (0.23–0.41)	**<0.001**	872	0.90 (0.61–1.31)	NS
Hormonal therapy	1077	0.52 (0.40–0.67)	**<0.001**	1022	1.30 (1.01–1.67)	**0.038**
Adjuvant radiotherapy	1218	0.41 (0.31–0.53)	**<0.001**	1183	0.071 (0.50–0.9)	**0.043**
Trastuzumab therapy	170	0.99 (0.61–1.61)	NS	159	0.57 (0.39–0.83)	**0.004**
Goserelin or leuprolide	167	0.37 (0.21–0.67)	**<0.001**	164	1.13 (0.77–1.67)	NS
Neo-adjuvant chemotherapy (only for stage III)	112	0.44 (029–0.67)	**<0.001**	112	0.63 (0.44–0.90)	**0.013**

*Breast cancer subtypes were categorized according to the 2013 St Gallen International Expert Consensus. *IDC* invasive ductal carcinoma; *ILC* invasive lobular carcinoma

Table 7 Cox proportional multivariate hazard model for overall survival and disease free survival among patients with breast cancer

Variable	Overall survival Hazard ratio (95 % CI)	*P* value	Disease-free survival Hazard ratio (95 % CI)	*P* value
NLR ≥4.0	1.91 (1.31–2.79)	**0.001**	1.46 (1.04–2.04)	**0.028**
Histological grade (reference: Grade1)				
Grade 2	1.55 (0.76–3.14)	0.224	1.43 (0.87–2.34)	NS
Grade 3	1.23 (1.09–2.32)	**0.009**	0.85 (0.47–1.54)	NS
Luminal A-like (reference)		**<0.001**		**<0.001**
Luminal B-like (HER2 −)	1.19 (0.78–1.81)	NS	1.04 (0.70–1.53)	**0.834**
Luminal B-like (HER2 +)	2.48 (1.46–4.23)	**0.001**	1.96 (1.21–3.16)	**0.006**
HER2 +(non-luminal)	1.03 (0.51–2.06)	NS	2.20 (1.35–3.58)	**0.001**
Basal-like (Triple negative)	1.75 (1.02–3.31)	**0.045**	3.26 (2.07–5.11)	**<0.001**
Tumor depth (reference: pT0–T1)				
pT2	1.22 (0.76–1.95)	NS	1.40 (0.93–2.10)	NS
pT3-pT4	2.06 (1.19–3.37)	**0.009**	2.34 (1.46–3.76)	**<0.001**
N Factor (reference: pN0)				
pN1	1.21 (0.71–2.06)	**NS**	1.38 (0.92–2.07)	**NS**
pN2–pN3	3.19 (1.96–4.95)	**<0.001**	2.52 (1.80–3.55)	**<0.001**
AJCC Stage (reference: 0–1)				
Stage 2	1.50 (0.95–2.37)	**NS**	1.42 (0.01–2.12)	**NS**
Stage 3–4(only stage 3 for DFS)	3.98 (2.59–6.11)	**0.032**	3.92 (1.98–4.25)	**0.001**
Adjuvant chemotherapy (reference: no chemo)				
Taxane-based	0.26 (0.17–0.39)	**<0.001**	0.52 (0.33–0.83)	**0.007**
Anthracycline-based	0.31 (0.19–0.50)	**<0.001**	0.59 (0.36–0.98)	**0.044**
Metastases	2.18 (1.23–3.16)	**<0.001**	–	–

Conflict of interest The authors declare that they have no conflict of interest.

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