ORIGINAL RESEARCH

Association Between Neutrophil-Lymphocyte Ratio and Oncotype Dx Recurrence Score in Early-Stage Hormonal Receptor-Positive, HER2-Negative, Node-Negative Breast Cancer

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Purpose: The correlation between the preoperative neutrophil-to-lymphocyte ratio (NLR) and Oncotype DX® (ODX) recurrence score (RS) has not yet been established. We aimed to investigate the association between NLR and ODX RS in patients with hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2−) early-stage breast cancer (BC).

Patients and Methods: This retrospective study included consecutive patients with HR+/HER2−, node-negative primary BC who underwent surgical tumor resection from 2011 to 2019. Receiver operating characteristic curve analysis was used to obtain an optimal NLR cutoff value. Logistic regression analyses were used to estimate associations between various parameters and ODX RS. Furthermore, the factors significantly associated with the ODX RS in multivariable analysis were incorporated in a separate model and estimated using logistic regression.

Results: A total of 160 patients were enrolled. The optimal preoperative NLR cutoff was 2.15. Multivariable analysis revealed that NLR and tumor grade (G1/G2 vs G3) were independent predictive factors of high RS cutoff (≥26). Moreover, including the two variables yielded a stronger association; patients with low NLR and low-grade tumors were unlikely to have high RS (≥26; odds ratio [OR] = 0.03, 95% confidence interval [CI]: 0.006–0.154; p < 0.001). Conversely, the presence of any of the following factors made patients unlikely to have low RS (<16; OR = 0.34, 95% CI: 0.16–0.73; p = 0.006): high NLR, high grade, or high Ki-67 levels (>20).

Conclusion: NLR is a promising independent predictor of RS. Furthermore, in addition to tumor grade and Ki-67 level, they together are also a potential indicator of high and low RS. However, further studies are required to validate this hypothesis.

Keywords: neutrophil-to-lymphocyte ratio, Oncotype Dx recurrence score, early-stage breast cancer, hormonal receptor-positive, human epidermal growth factor receptor 2-negative, node-negative breast cancer

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women and the most frequent cause of cancer-related deaths worldwide.[1] BC accounted for an estimated 24.5% of all new cancer cases and 15.5% of all cancer-related mortalities in 2020.[1] In general, four major biological subtypes of BC are identifiable: hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2−) Luminal A, Luminal B, HER2-enriched, and triple-negative disease.[2–4] The HR+/HER2− subtype represents most common BC molecular subtypes, ranging from

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59% to 75% of cases.[2–6] Multidisciplinary approaches to reduce BC mortality include surgery, chemotherapy ± targeted therapy ± hormonal therapy, and radiation therapy.[7] The utilization of systemic adjuvant chemotherapy has aided in the decline in cause-specific recurrence and mortality, and it is now recognized as the standard of care.[8–10] Oncotype DX® (ODX) recurrence score (RS) assay is a multigene reverse transcription-polymerase chain reaction that measures the transcriptional activities of 21 genes (16 cancer-associated and 5 housekeeping genes) and uses the expression pattern to calculate RS (range: 0–100) that predicts the 10-year risk of BC recurrence; this assay has been incorporated into early BC management and is used widely to determine appropriate candidates for adjuvant chemotherapy.[11–18]

Inflammation and the immune system are major hallmarks of malignancy.[19] Neutrophil-to-lymphocyte ratio (NLR) is the ratio of absolute neutrophil counts to absolute lymphocyte counts in the peripheral blood. NLR reflects the host's inflammatory and immune responses to cancer, which plays crucial roles in carcinogenesis.[20–22] The neutrophilic response stimulates tumor growth and inhibits the immune system by suppressing the cytotoxic activity of T cells and is associated with poor prognosis.[19,23] Lymphocytopenia is associated with adverse outcomes in patients with cancer.[24,25]

Several predictive models built using clinicopathological variables have been suggested to be surrogate markers for RS.[26] NLR has not been included with these factors, and the relationship between NLR and RS has not been established yet; however, NLR has been identified as an independent prognostic factor for disease-free survival (DFS) and overall survival (OS) in a variety of solid tumors,[27–30] including BC.[25,31–34] A study reported no correlation between ODX RS and NLR,[35] although current theories support the hypothesis that such a correlation might exist. Immunological biomarkers are of high prognostic value when used in addition to other clinicopathological factors.[36] The diversity in tumor gene expression of luminal HR+[37] is related to immune cell infiltration and host immune response.[33,38] The correlation of NLR with RS could provide an additional valuable biomarker that is available for clinical utilization during BC management. We aimed to investigate the relationship between preoperative NLR and other clinicopathological factors with ODX RS in patients with HR+/HER2−, node-negative

BC who underwent surgical resection of the primary tumors.

Patients and Methods
Study Patients

We retrospectively identified all patients diagnosed with HR +/HER2−, and node-negative BC who met the inclusion criteria from January 2012 to February 2020. This study was conducted in accordance with the guidelines for reporting tumor marker studies in BC research and treatment.[39,40] The study protocol was approved by the Medical Ethics Committee of the Research Advisory Council at the King Faisal Specialist Hospital & Research Centre, and was conducted according to the ethical principles of the Declaration of Helsinki (2000). The participants remained anonymous, and no identifying or protected health information was recorded; hence, the ethics committee waived the requirement for informed consent.

The inclusion criteria were as follows: 1) age, 18–65 years; 2) patients with early-stage HR+/HER2−, and node-negative BC who had undergone surgical removal of the tumors and underwent the ODX 21-gene RS assay test; and 3) the availability of a differential leukocyte count recorded before surgery. The upper age limit was 65 years based on our knowledge of underuse of ODX RS in patients older than 65 years due to chemotherapy tolerability and the association of older age with NLR.[41,42]

The exclusion criteria were as follows: 1) a lack of information regarding pathologic or laboratory results; 2) pregnancy-related BC; 3) ductal carcinoma in situ; 4) hormone receptor-negative, HER2-positive, or node-positive diseases; 5) stage IV BC or inflammatory BC; 6) systemic clinical evidence of active infection; 7) hematological disorders; 8) prior steroid therapy; 9) chronic obstructive pulmonary disease requiring treatment; 10) chronic liver disease; 11) end-stage renal disease; 12) history of a cerebrovascular accident; and/or 13) systemic lupus erythematosus.

Patient Data Collection

The data were retrieved from a prospective database at the King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia. The medical records of each patient were reviewed independently by two physicians for the baseline characteristics in terms of patient demographics and clinicopathological data, including age, menopausal status, tumor size, grade, lymph node status, Ki-67 protein levels, presence/absence of lymphovascular invasion (LVI), hormonal

receptor expression, HER2 status, comorbidities, and obesity (body mass index [BMI] ≥ 30 kg/m^2).

A specialized BC pathologist confirmed all pathology slides. HR+ BC was defined as tumor cells stained ≥1% on immunohistochemistry (IHC) for estrogen and/or progesterone receptors. However, ODX RS was only ordered based on our guidelines for patients with an ER of ≥10%. HER2− BC was defined as IHC staining of 0, 1+, or confirmed negative fluorescence in situ hybridization findings if IHC was equivocal (2+) and HER2 to CEP17 ratio was <2 with a HER2 copy number of <4. Tumor grade was determined based on the Nottingham histologic grade. Ki-67 was classified as low (≤20%) or high (>20%). White blood cells with differential diagnoses were obtained after the initial diagnosis and confirmed before curative breast surgery. Preoperative NLR was calculated as the quotient of the absolute neutrophil count divided by the absolute lymphocyte count. ODX RS was performed on paraffin-embedded tumor samples at the Exact Sciences Corp, Madison, Wisconsin. The patients were stratified based on the ODX RS into high-risk (≥26) vs intermediate/low-risk (<26), and low-risk (<16) vs intermediate/high-risk (≥16) groups. These cutoffs were determined according to the NCCN guidelines for node-negative diseases.[16] If RS is <26 or <16, there is no additional benefit of chemotherapy in post-menopausal and pre-menopausal women, respectively. Additional chemotherapy is recommended when RS is ≥26 in post-menopausal and pre-menopausal women.[43]

Statistical Analysis

Categorical variables are described as frequencies and were compared using the Chi-square test and continuous variables as median values with interquartile range (IQR) and compared using the Mann–Whitney *U*-test. Pearson's correlation coefficient was used to assess the correlation between continuous variables. The optimal NLR cutoff was obtained by a receiver operating characteristic curve
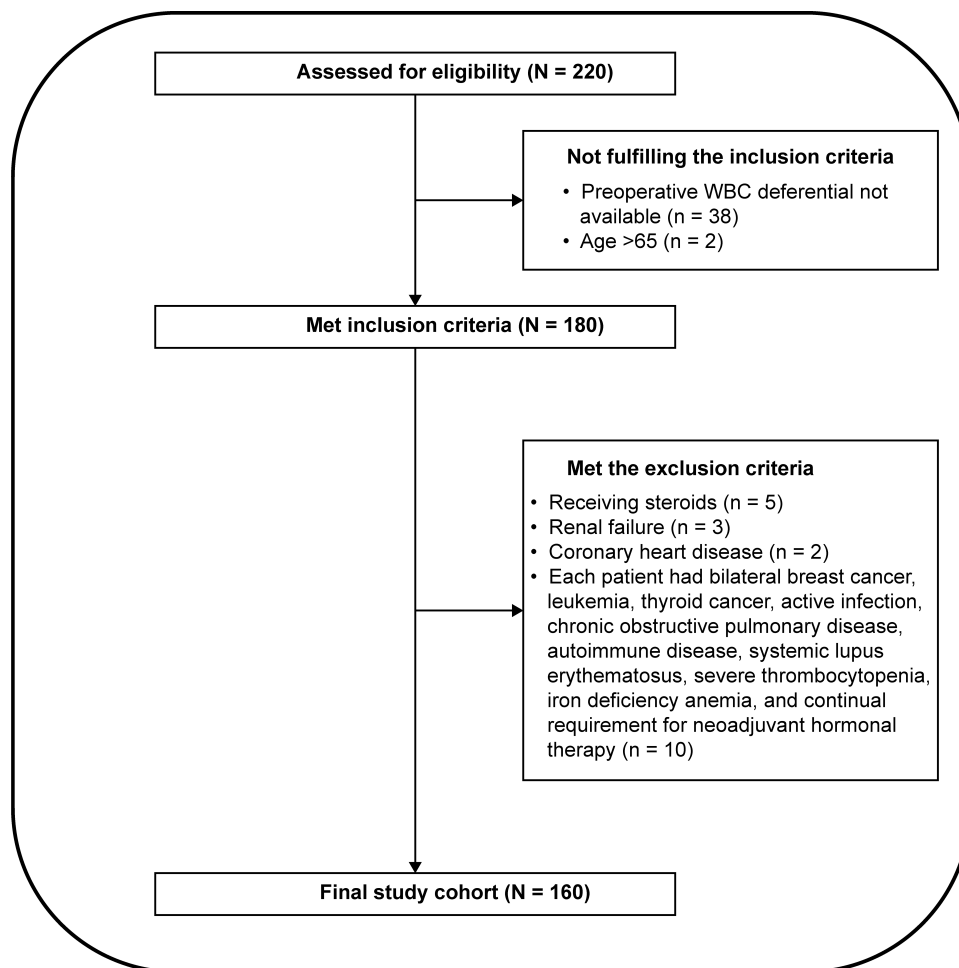

Figure 1 Flow diagram for selection criteria.

(ROC).[33,44] Associations between various parameters and the RS cutoff values of 16 and 26 were estimated using univariable and multivariable binary logistic regression analyses. The variables included were age, menopausal status, tumor grade, Ki-67 level (≥20% or ≤20%), LVI, T stage, NLR, platelet-to-lymphocyte ratio (PLR), BMI, and comorbidities. The clinicopathological factors that remained significant in multivariable analysis were incorporated in separate models and evaluated to predict RS using logistic regression. DFS was estimated using the Kaplan–Meier method. Statistical significance was set at a p-value of <0.05, and all statistical analyses were performed using SPSS for Mac, v27 (IBM Corp, Armonk, NY, USA).

Results

A total of 160 patients were included in the final analysis (Figure 1). The median age at diagnosis was 49 years (IQR: 42–56 years). Patient demographics and disease characteristics are shown in Table 1. The median NLR was 1.61 (IQR: 1.21–2.15), and there was a significant association between NLR and RS (r = 0.29, p < 0.001). There was no association between RS and PLR (p = 0.59), lymphopenia (p = 0.74), or BMI (p = 0.59). The ROC curve revealed an optimal NLR cutoff point of 2.15 (Figure 2). Patients were stratified into the low- and high-NLR groups based on the NLR cutoff point of 2.15, as shown in Table 2. The variables tested in the univariate analyses were age, menopausal status, tumor grade, LVI, T stage, Ki-67 status, obesity, and comorbidities. Multivariable analysis showed that NLR and tumor grade remained significant predictors of ODX RS of ≥26. The NLR, tumor grade, and Ki-67 status remained significant predictor of ODX RS of <16 (Table 3).

The significant predictive factors of ODX RS of >26 were combined, and patients were classified into four groups: low NLR and low grade, low NLR and high grade, high NLR and low grade, and high NLR and high grade. There was a significant difference between the groups as measured by the Pearson's Chi-square test (p < 0.001; Figure 3A). Logistic regression analysis revealed that compared with high NLR and high grade (G3), all other groups showed lower odds of RS of ≥26 (odds ratio [OR] = 0.3, 95% CI: 0.006–0.154, p < 0.001; OR = 0.14, 95% CI: 0.023–0.85, p < 0.03; and OR = 0.05, 95% CI: 0.01–0.34, p < 0.002 for low NLR and low grade, low

Table 1 Patients Demographics and Disease Characteristics (n = 160)

Characteristics	Frequency (%)
Menopausal	
Pre	99 (61.9)
Post	61 (38.1)
Tumor Grade	
1	22 (13.8)
2	109 (68.1)
3	29 (18.1)
ER	
Positive	159 (99.4)
Negative	1 (0.6)
PR	
Positive	151 (94.4)
Negative	9 (5.6)
LVI	
Positive	38 (23.8)
Negative	120 (75)
Missing	2
T stage	
1	84 (52.5)
2	72 (45)
3	4
Ki-67	
(≤20)	95 (59.4)
(>20)	62 (38.8)
Missing Oncotype Dx	3
<16	62 (38.8)
≥16	98 (61.3)
≥26	34 (21.3)
<26	126 (78.8)
BMI	
≥30	81 (50.9)
<30	78 (49)
Missing	1
Comorbidities[a]	
Present	44 (27)
Absent	116 (72.5)

Notes: [a]Comorbidities not under exclusion criteria: diabetes mellitus, hypertension, dyslipidemia, depression, hypothyroidism, and obesity.
Abbreviations: BMI, body mass index; ER, estrogen receptor; LVI, lymphovascular invasion; PR progesterone receptor.
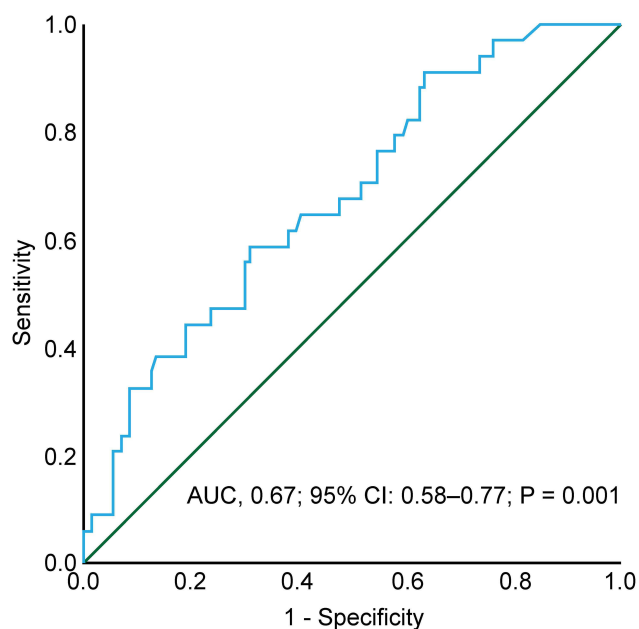
Figure 2 ROC curve showed that neutrophil-to-lymphocyte ratio associated with Oncotype Dx and the cutoff point of 2.15 revealed 0.81 and 0.60 sensitivity and specificity, respectively.

Table 2 Patients and Disease Characteristics Stratified Based on Low and High NLR

Characteristics	High NLR ≥2.15 (n = 40)	Low NLR <2.15 (n = 120)
	n (%)	n (%)
Median age (IQR)	48.5 (42–56.5)	49 (42–56)
Pre-menopausal	24 (60)	75 (62.5)
Tumor Grade		
1	7 (17.5)	15 (12.5)
2	22 (55)	87 (72.5)
3	11 (27.5)	18 (15)
ER-positive	40 (100)	119 (99.2)
PR-positive	36 (90)	115 (95.8)
LVI-positive	9 (22.5)	29 (24.6)
T stage		
1	21 (52)	63 (52.5)
2	18 (45)	54 (45)
3	1 (2.5)	3 (2.5)
Ki-67 level		
≤20	24 (60)	71 (59.2)
>20	16 (40)	46 (38.3)
Oncotype Dx		
<16 (62/160)	9 (22.5)	53 (44.2)
≥16 (98/160)	31 (77.5)	67 (55.8)
≥26 (34/160)	15 (37.5)	19 (15.8)
<26 (126/160)	25 (62.5)	101 (84.2)
BMI ≥ 30 kg/m²	62 (52.1)	19 (47.5)
BMI < 30 kg/m²	57 (47.9)	21 (52.5)
Presence of Comorbidities	12 (30)	32 (26.7)

Abbreviations: ER, estrogen receptor; IQR, interquartile range; LVI, lymphovascular invasion; NLR, neutrophil-to-lymphocyte ratio; PR progesterone receptor.

NLR and high grade, and high NLR and low grade groups, respectively).

The significant predictive factors of ODX RS of <16 were combined, and the patients were classified into four groups according to the number of risk factors (high category: Ki-67 > 20, G3 or NLR ≥ 2.15). There was a significant difference between the groups (p < 0.001; Figure 3B). Logistic regression revealed that compared with patients without any risk factors in high categories, the presence of any of the factors in the high category was associated with a lower odds of ODX RS of <16 (OR = 0.34; 95% CI: 0.16–0.73; p = 0.006). The presence of two factors in the high category was associated with a lower odds of ODX RS of <16 (OR = 0.12; 95% CI: 0.03–0.38; p < 0.001); the presence of all three factors in the high category was associated with an OR of 0.10 (95% CI: 0.01–0.92; p = 0.04).

The median follow-up duration was 30 months (IQR 16–57 months). There were 12 recurrence cases and four deaths reported in the analysis. The 5-year DFS of the low NLR group (<2.15) and high-NLR group was 88.8% and 86.1%, respectively (p = 0.49), whereas for patients with NLR in the first quartile (<1.22), the 5-year DFS rate was 100% and 82.7% (p = 0.02), respectively. The 5-year DFS for ODX ≥26 and < 26 groups was 77.4% and 90.2% (p = 0.09), respectively, and for the ODX <16 and ≥16 groups was 97.4% and 81.9%, respectively (p = 0.13; Figure 4A–D).

Discussion

Early-stage HR+, HER2−, and node-negative BC is generally considered to have a low risk of recurrence. However, highly useful tools might enable the distinction of patients who would not require postoperative chemotherapy from those likely to be at high risk requiring adjuvant postoperative chemotherapy; this information would prove critical for strategic planning regarding monitoring and management of patients. ODX RS is a test that predicts the benefits of chemotherapy and the need

Table 3 Multivariate Logistic Regression Analysis for ODX RS Cutoffs

	Oncotype Dx $\geq$ 26			Oncotype Dx < 16		
	OR	95% CI	p-value	OR	95% CI	p-value
Menopausal status						
Pre-menopausal	1			1		
Post-menopausal	1.25	0.49–3.13	0.63	1.4	0.69–2.86	0.34
NLR						
<2.15	1			1		
≥2.15	5.4	1.2–8.05	0.01	0.39	0.16–0.92	0.03
Tumor Grade						
G1/G2	1			1		
G3	5.12	1.89–15.38	<0.01	0.25	0.06–0.95	0.04
Ki-67 level						
≤20	1			1		
>20	1.86	0.03–6.5	0.57	0.43	0.19–0.95	0.03

Abbreviations: CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; ODX RS, Oncotype DX recurrence score; OR, odds ratio.

for more aggressive treatment. In real-world clinical practice, the use of ODX RS is found to be beneficial in the management of more than one-third of the patients with BC.[45] The patients treated based on ODX RS had better BC-specific survival and OS; however, limited healthcare access for social or ethnic minorities in developed countries and the unavailability of the test in some parts of the world remain a - challenge.[46] Researchers have attempted to identify a link between the histopathological factors associated with BC recurrence to build a tool that can provide information similar to ODX RS. The modified Magee equations 1–3[47] and Gage et al[48] showed a significant correlation between histopathological factors and ODX RS; in particular, in patients with clearly high or low predicted risk, RS is unlikely to be different.[47] Furthermore, combining clinicopathological factors and RS did provide more prognostic information.[49] Thus, it is recommended to consider this tool when selecting patients for endocrine therapy alone.[49–51] Moreover, recently, a new prognostic tool that combined clinicopathological factors and ODX RS was able to provide more predictive information to
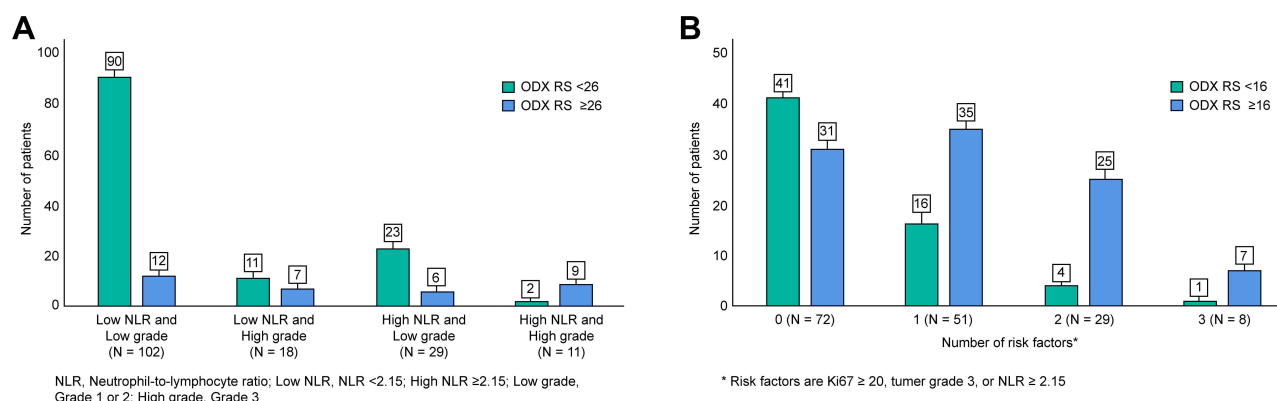

NLR, Neutrophil-to-lymphocyte ratio; Low NLR, NLR <2.15; High NLR ≥2.15; Low grade, Grade 1 or 2; High grade, Grade 3

* Risk factors are Ki67 ≥ 20, tumer grade 3, or NLR ≥ 2.15

Figure 3 Illustration showing the patients' Oncotype Dx (ODX RS) recurrence score distribution <26 (green) vs ≥26 (blue) in the following four groups: low neutrophil-to-lymphocyte ratio (NLR; < 2.15) and low grade (G1/2), low NLR and high grade (G3), high NLR (≥2.15), and low grade, and high NLR and high grade (**A**). Illustration showing the patients' ODX RS <16 (green) vs ≥16 (blue) distribution in the four groups: absent (0), presence of any one (1), two (2), or all (3) of the following: high grade (G3), high NLR ≥ 2.15, or high Ki-67 (>20) (**B**).
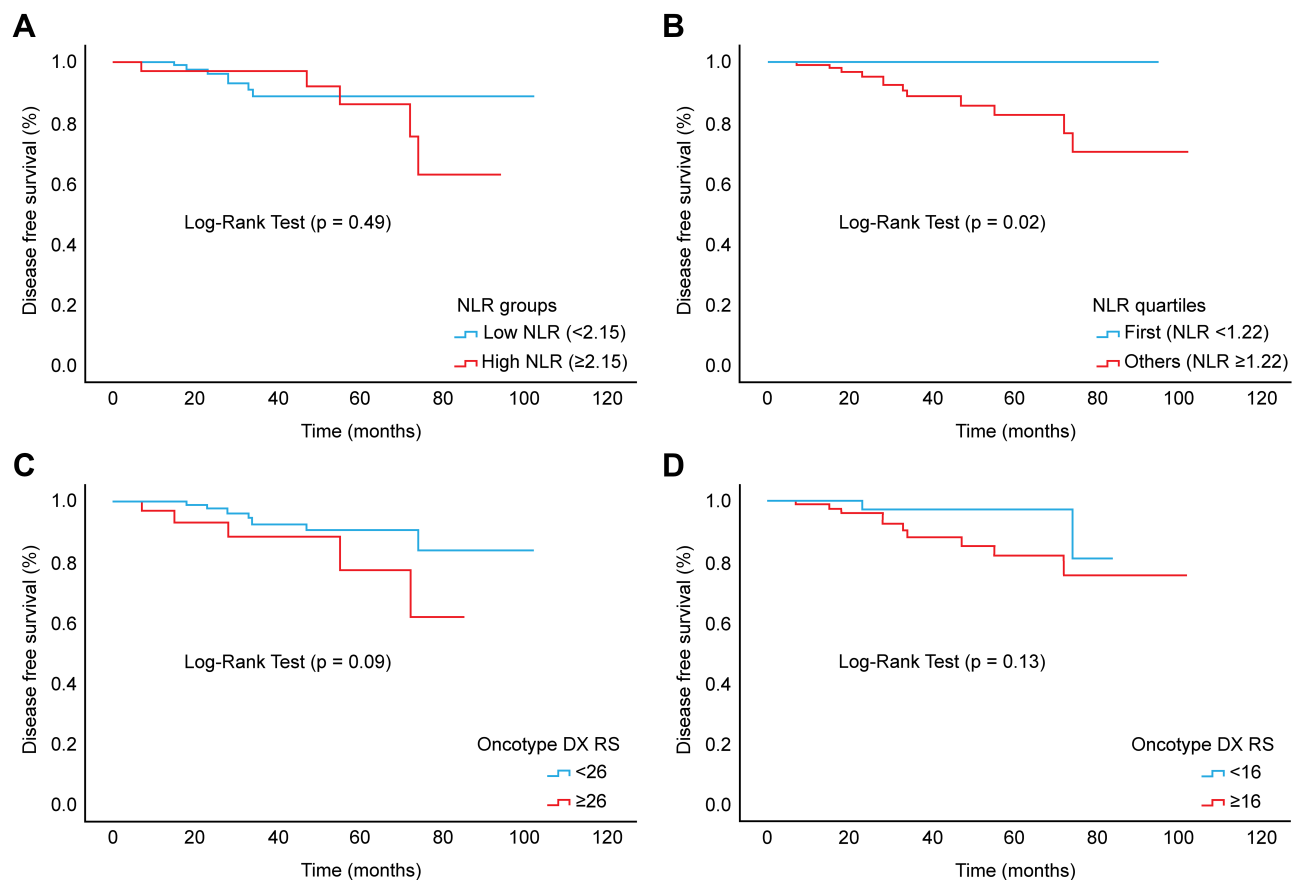
Figure 4 Disease-free survival of patients with neutrophil-to-lymphocyte ratio (NLR) of <2.15 vs ≥2.15 (**A**), NLR <1.22 vs ≥1.22 (**B**), Oncotype Dx (ODX RS) <26 vs ≥26 (**C**), and ODX RS <16 vs ≥16 (**D**).

guide adjuvant chemotherapy than either of the two alone.[52] However, none of these models included NLR. As shown earlier, NLR has been linked to recurrence and survival in patients with BC from a clinical standpoint and play major roles in malignancy.[19] We envisaged that pretreatment NLR could be an additive prognostic clinical factor for ODX RS. To investigate this possibility, we tested the pretreatment NLR value against actual ODX RS in our patients and then included NLR with other clinicopathological factors to predict RS.

To the best our knowledge, this is the first report of such a relationship between RS and preoperative NLR. However, Grenader et al found no association between the preoperative NLR as a predictor of ODX RS.[35] A possible explanation for the differences could be attributed to the following factors: i) the NLR cutoff value of 2.5, which was obtained from a heterogeneous group of Asians with all BC subtypes (28.9% HER2-positive and 22.3% triple-negative), with node positivity (35%)[33] and ii) the exclusion criteria for factors affecting the NLR were not specified in the previous study.[35] These factors might

have influenced the negative outcome as the median NLR/ optimal cutoff varies by disease characteristics, ethnicity,[53,54] and systemic factors that are known to alter white blood cell counts. We obtained optimal cutoff values from the ROC curve and followed prespecified exclusion criteria, such as active infection, autoimmune diseases, and medications such as steroids (Figure 1).

We found that the tumor grade was strongly associated with RS, in accordance with previous studies.[55,56] However, the tumor grade should be evaluated in the context of histology subtypes.[56] In this cohort, most (94.4%) patients had invasive ductal carcinoma and 5.6% had invasive lobular carcinoma (ILC). In addition, the data of all patients with ILC had ODX RS of <16, except one with an RS of 23, are in agreement with those of previous studies, which have shown that ILC is not commonly associated with high ODX RS.[57,58]

The leading strength of this study is that it included consecutive early-stage BC patients from a prospective database, following prespecified exclusion criteria and

driven NLR from the same sample. The main limitations of this study include its single-center design, limited number of patients, and follow-up. The nature of early-stage BC necessitates a longer follow-up period to assess the secondary outcomes, DFS, and OS. However, these data encourage future research to determine NLR within one ethnicity and consider other factors affecting the NLR.

In summary, NLR as a continuous variable was associated with ODX RS and a cutoff point of 2.15 was an independent predictive factor of high RS (≥26) and low RS (<16). In particular, patients with a low NLR and G1/G2 (n = 102) were more likely to have an RS of <26 (90 patients, 88%). Moreover, the presence of any factor in the high category (Ki-67 status ≥ 20, NLR ≥ 2.15) or tumor G3 makes the patient unlikely to have low RS (<16), and this chance further decreases as the number of these factors in the high category increases. Therefore, these findings suggest that NLR, tumor grade, and Ki-67 status are potential predictive biomarkers for RS. Nevertheless, the optimal NLR cutoff is a major limitation for utilization in clinical practice. Further larger studies are needed to determine the optimal cutoff point for HR+ early-stage BC considering ethnicity and systemic factors.

Conclusion

These observations suggest that preoperative NLR is a promising prognostic factor and could be additive to other clinicopathological variables in early-stage BC management. However, further studies are required to validate this hypothesis.

Abbreviations

BC, Breast cancer; BMI, Body mass index; DFS, Disease-free survival; HR+, Hormone receptor-positive; HER2-, Human epidermal growth factor receptor 2-negative; IHC, Immunohistochemistry; ILC, Invasive lobular carcinoma; IQR, Interquartile range; LVI, Lymphovascular invasion; NLR, Neutrophil-to-lymphocyte ratio; ODX, Oncotype DX®; OR, Odds ratio; OS, Overall survival; PLR, Platelet-to-lymphocyte ratio; RS, Recurrence score; ROC, Receiver operating characteristic.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Statement of Ethics

This study was approved by the Research Advisory Council of King Faisal Specialist Hospital & Research Centre (RAC number 2051-029, and conducted following the ethical principles of the Declaration of Helsinki (2000). The participants remained anonymous, and no identifying or protected health information was recorded.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

Dr. Taher Al-Tweigeri has received speaking honoraria from Roche, Novartis, and Lilly and has served as an advisory committee for these companies. The other authors declare no conflicts of interest.

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