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The Prognostic Value of Pretreatment Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio, and Systemic Immune-inflammation Index on Survival Outcomes in Non-metastatic Breast Cancer: A Retrospective Cohort Study

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ABSTRACT

Objective: Systemic inflammatory response contributes to tumor progression and may influence survival outcomes in breast cancer. Blood-based inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), have been proposed as prognostic biomarkers. This study evaluated the prognostic value of pretreatment NLR, PLR, and SII across molecular subtypes of breast cancer.

Material and Methods: This retrospective cohort study included 190 patients with invasive breast cancer who were treated between February 2012 and April 2022. Pretreatment NLR, PLR, and SII were calculated from complete blood counts. Optimal cut-off values were determined using a 60 months landmark receiver operating characteristic analysis. Overall survival (OS) and disease-free survival (DFS) were analyzed using the Kaplan-Meier method, the log-rank test, and Cox regression. Subgroup analyses were performed according to molecular subtype.

Results: After a median follow-up of 58.8 months, 30 deaths (15.8%) and 44 DFS events (23.2%) occurred. Optimal cut-offs were 1.78 for NLR, 138.08 for PLR, and 449.55 for SII. In the overall cohort, elevated NLR was associated with numerically worse OS (hazard ratio [HR]=2.05; p=0.096) and DFS (HR: 1.78; p=0.088), although these associations did not reach statistical significance. In the triple-negative breast cancer subgroup, high NLR was associated with poorer OS (log-rank p=0.019) and DFS (log-rank p=0.025); however, ridge-penalized Cox estimates were imprecise [OS: HR=1.52, 95% confidence interval (CI)=0.42-5.57; DFS: HR=1.75, 95% CI=0.50-6.11]. Multivariate analysis identified Ki-67 ≥20% and lymph node positivity as independent predictors of OS, while progesterone receptor positivity, Ki-67 ≥20%, and Stage III independently predicted DFS.

Conclusion: NLR was not independently prognostic in the overall cohort. Its association with survival outcomes in triple-negative breast cancer is preliminary and hypothesis-generating and requires validation in larger independent cohorts.

Keywords: Breast neoplasms; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; systemic immune-inflammation index; prognosis; triple-negative breast neoplasms

INTRODUCTION

Breast cancer remains the most commonly diagnosed malignancy and a leading cause of cancer-related mortality among women worldwide, accounting for approximately 2.3 million new cases and 666,000 deaths annually.[1] Despite significant advances in early detection, surgical techniques, and systemic therapies, considerable heterogeneity exists in clinical outcomes, even among patients with similar

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clinicopathological characteristics.[2] This underscores the critical need for readily accessible, cost-effective biomarkers that can refine prognostic stratification and guide therapeutic decision-making.

Over the past two decades, a growing body of evidence has established the tumor microenvironment and systemic inflammatory response as fundamental hallmarks of cancer.[3] Chronic inflammation contributes to tumorigenesis, promotes angiogenesis, facilitates immune evasion, and enhances metastatic potential through a complex interplay of cytokines, chemokines, and immune cells.[4,5] In breast cancer specifically, the inflammatory milieu has been linked to disease progression, treatment resistance, and poor survival outcomes.[6]

Peripheral blood-based inflammatory indices have attracted considerable attention as surrogate markers of the host systemic inflammatory response. The neutrophil-to-lymphocyte ratio (NLR), defined as the absolute neutrophil count divided by the absolute lymphocyte count, reflects the balance between pro-tumor neutrophil-mediated inflammation and anti-tumor lymphocyte-mediated immune surveillance.[7] Elevated NLR has been associated with adverse outcomes in multiple solid tumors, including colorectal, lung, gastric, and hepatocellular carcinomas.[8,9] In breast cancer, several meta-analyses have confirmed the prognostic relevance of NLR, although considerable heterogeneity exists regarding optimal cut-off values and the consistency of results across molecular subtypes.[10,11]

The platelet-to-lymphocyte ratio (PLR) is another composite hematological index that integrates the protumorigenic role of platelets—which facilitate tumor cell dissemination through promotion of epithelial-mesenchymal transition and shielding of circulating tumor cells from immune clearance—with lymphocyte-mediated antitumor immunity.[12,13] Elevated PLR has been reported as a negative prognostic factor in breast cancer, though its independent predictive value remains debated.[14]

More recently, the systemic immune-inflammation index (SII), calculated as SII = platelet count × neutrophil count / lymphocyte count, has been proposed as a more comprehensive marker that simultaneously captures three key components of the immune-inflammatory axis.[15] Initial studies in breast cancer have yielded promising but inconsistent results, with some demonstrating independent prognostic significance and others reporting only marginal associations.[16,17]

Breast cancer is a biologically heterogeneous disease comprising distinct molecular subtypes—luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and triple-negative—each with unique biological behavior, therapeutic responsiveness, and prognosis.[18,19] Triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, remains the most clinically challenging subtype due to its aggressive behavior, limited targeted therapy options, and high relapse rates.[20] The role of systemic inflammation may be particularly relevant in TNBC, given the established immunogenicity and higher tumor-infiltrating lymphocyte content of this subtype.[21,22] However, subtype-specific analyses of inflammatory markers remain scarce and warrant further investigation.

The aim of this study was to evaluate, in a cohort of breast cancer patients, the prognostic significance of pretreatment NLR, PLR, and SII for overall survival (OS) and disease-free survival (DFS), with specific emphasis on analyses stratified by molecular subtype. We hypothesized that inflammatory markers may exert differential prognostic effects across molecular subtypes, potentially carrying the greatest clinical utility in biologically aggressive subtypes such as TNBC.

MATERIAL AND METHODS

Study Design and Patient Selection

This retrospective cohort study was conducted at a single tertiary oncology center. The medical records of consecutive patients with histologically confirmed invasive breast cancer diagnosed between February 2012 and April 2022 were retrospectively reviewed. Patients were followed through November 2023, using hospital records and data from the national death registry. The data cut-off date for survival analyses was November 30, 2023. Of the 192 patients with available pretreatment complete blood count (CBC) data, two were subsequently excluded due to incomplete follow-up information, yielding a final analytic cohort of 190 patients. The study protocol was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee (approval number: 2023/4610, date: 3.11.2023), in accordance with the Declaration of Helsinki, and the requirement for informed consent was waived due to the retrospective nature of the study.

Inclusion criteria were: (1) histologically confirmed invasive breast carcinoma; (2) availability of pretreatment CBC obtained within 7 days prior to the initiation of any anti-cancer treatment; (3) substantially complete clinicopathological and follow-up data, with isolated missing values for individual covariates permitted; and (4) no evidence of distant metastasis at initial diagnosis. Exclusion criteria included: (1) metastatic disease (Stage IV) at presentation; (2) concurrent hematological disorders; (3) active infection or systemic inflammatory conditions at the time of blood sampling; (4)

chronic use of corticosteroids or immunosuppressive agents; (5) prior history of another malignancy; and (6) incomplete medical records. After applying these criteria, 190 patients were included in the final analysis.

Data Collection

The following data were extracted from medical records: demographic characteristics [age, menopausal status, body mass index (BMI)]; histopathological features (histological type, grade, ER status, PR status, HER2 status, Ki-67 proliferation index); staging parameters [tumor size, lymph node status, TNM stage according to the American Joint Committee on Cancer (AJCC) 8th edition]; lymphovascular invasion (LVI); perineural invasion (PNI); treatment modalities (surgery, chemotherapy, radiotherapy, and/or hormonal therapy as clinically indicated, including neoadjuvant chemotherapy where applicable); and survival outcomes. For patients who received neoadjuvant chemotherapy, disease stage was recorded as the pre-treatment clinical stage according to the AJCC 8th edition; for patients who underwent primary surgery, disease stage was recorded using pathological staging.

Molecular subtype classification was performed based on immunohistochemical surrogates according to the St. Gallen International Expert Consensus:[23] luminal A (ER^+ and/or PR^+, $HER2^-$, Ki-67 < 20%), luminal B (ER^+ and/or PR^+, $HER2^-$ with Ki-67 ≥20%, or ER^+ and/or PR^+, $HER2^+$), HER2-enriched (ER^-, PR^-, $HER2^+$), and triple-negative (ER^-, PR^-, $HER2^-$).

Inflammatory Marker Definitions

Pretreatment CBC parameters—including absolute neutrophil count, absolute lymphocyte count, and platelet count—were obtained from venous blood samples collected within 7 days prior to any anti-cancer treatment (surgery or neoadjuvant chemotherapy). The following inflammatory indices were calculated:

NLR = Absolute neutrophil count / absolute lymphocyte count

PLR = Platelet count / absolute lymphocyte count

SII = Platelet count × neutrophil count / absolute lymphocyte count

The lymphocyte-to-monocyte ratio (LMR) could not be evaluated because monocyte counts were not routinely documented in the institutional records during the study period.

Survival Endpoints

The primary endpoints were OS and DFS. OS was defined as the time interval from the date of histological diagnosis to the date of death from any cause or to the date of last follow-up. DFS was defined as the time from the date of diagnosis to the first documented locoregional recurrence, distant metastasis, or death from any cause. Patients who were alive and event-free at the last follow-up were censored on that date. The data cut-off date was November 30, 2023.

Statistical Analysis

Descriptive statistics were presented as the median with interquartile range (IQR) for continuous variables with a non-normal distribution, and as frequencies (percentages) for categorical variables. Optimal cut-off values for NLR, PLR, and SII were determined using 60 months landmark receiver operating characteristic (ROC) curve analysis with the Youden index (J = sensitivity + specificity - 1). The area under the curve (AUC) was calculated to assess discriminatory capacity. Specifically, a time-dependent ROC analysis using a 60 months (5 years) landmark time point was performed to define the binary outcome for cut-off determination: patients who experienced the event (death for OS; recurrence, metastasis, or death for DFS) before 60 months were classified as event-positive, whereas patients who remained alive and event-free at 60 months were classified as event-negative. Patients who were censored before the 60 months landmark (i.e., lost to follow-up without experiencing an event) were excluded from the ROC analysis because their 60 months survival status could not be determined. A landmark approach was chosen because it converts the time-to-event outcome into a well-defined binary status at a clinically meaningful horizon (5 years survival), allowing the application of conventional ROC methodology and the Youden index. Under this rule, 114 of the 190 patients contributed to the OS landmark ROC analysis (23 who died within 60 months and 91 known to be alive at the 60 months landmark), and 116 patients contributed to the DFS landmark ROC analysis (42 with a DFS event within 60 months and 74 known to be event-free at the landmark); the same patients contributed to all three marker analyses because NLR, PLR, and SII were available for the entire cohort. The cut-off values obtained in this landmark subset (NLR=1.78; PLR=138.08; SII=449.55) were subsequently applied unchanged to the complete cohort of 190 patients, and all Kaplan-Meier, log-rank, and Cox analyses reported below were performed.

Associations between dichotomized inflammatory markers and clinicopathological features were evaluated using the chi-square test or Fisher's exact test, as appropriate. Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify prognostic factors; variables with $p<0.25$ in univariate analysis were entered into the multivariate model.

Specifically, multivariable Cox proportional hazards models were fitted using L2 (Ridge) penalization to improve the stability of coefficient estimates, given the relatively limited number of events; a fixed penalty of λ=0.01 was specified a priori (lifelines, CoxPHFitter, penalizer=0.01) and not tuned on the data. The hazard ratios (HRs), 95% confidence intervals (CIs) and p-values reported in Table 1 are the Wald-type quantities returned by this penalized fit: coefficients were obtained by maximizing the penalized log partial likelihood, standard errors were taken from the inverse of the negative Hessian at the penalized solution, confidence limits were calculated as coefficient ±1.96x standard error and then exponentiated, and p-values were derived from the corresponding Wald z statistics. Because these standard errors are conditional on λ and are not adjusted for penalization, the intervals in Table 1 are reported as measures of the estimates' precision rather than intervals with exact nominal coverage. For the TNBC subgroup, in which the observed events were confined to the high-NLR group, the same implementation was used with a larger penalty (lifelines, CoxPHFitter, penalizer=0.5) to obtain finite parameter estimates under near-complete separation. This is an L2 (Ridge) penalty rather than Firth's penalized partial likelihood, which lifelines does not implement. Subgroup analyses were performed by molecular subtype. All statistical tests were two-sided; $p<0.05$ was considered statistically significant. Statistical analyses were performed using Python (version 3.11) with the following packages: lifelines (v0.30.3), scikit-learn (v1.9.0), scipy (v1.14.1), pandas (v2.2.3), numpy (v1.26.4), and matplotlib (v3.9.2). This study was conducted and reported in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines where applicable.[24]

A complete-case analysis was employed; patients with missing values for specific covariates were excluded from the corresponding multivariable models.

In addition to the dichotomized analysis using ROC-derived cutoffs, inflammatory markers (NLR, PLR, SII) were also analyzed as continuous variables using Cox proportional hazards regression. HRs were reported both per unit increase and, after z-score standardization [mean=0, standard deviation (SD)=1], per one SD increase, to facilitate comparison across markers with different scales. Multivariate models were adjusted for patient age (continuous), Stage III disease, and Ki-67 status (≥20%). To address the potential optimism bias inherent in ROC-derived cut-offs obtained from the same dataset, internal bootstrap validation was performed with 1000 resamples. In each iteration, the complete analytic pipeline was repeated within the resample: the 60 months landmark ROC analysis was re-run, a new optimal Youden cut-off was derived, the marker was dichotomized at that bootstrap-derived cut-off, and the Kaplan-Meier (log-rank) and Cox proportional hazards analyses were repeated. The bootstrap-derived cutoff, HR, and log-rank p-value were recorded at each iteration and summarized as medians with 2.5^{th}-97.5^{th} percentile intervals, together with the proportion of resamples yielding both a HR greater than 1 and a log-rank p-value below 0.05 (Supplementary Table S1).

RESULTS

Patient Characteristics

A total of 190 patients with non-metastatic invasive breast cancer who met the inclusion criteria were analyzed.

TABLE 1: Multivariate Cox proportional hazards regression analysis for OS and DFS.

	OS model			DFS model		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Ki-67 ≥20%	3.20	1.31-7.80	**0.011**	2.52	1.26-5.07	**0.009**
Lymph node positive[a]	3.09	1.07-8.93	**0.037**	—	—	—
Stage III disease	2.08	0.97-4.47	0.059	2.27	1.26-4.09	**0.007**
Neoadjuvant CT[a]	2.35	0.96-5.73	0.060	—	—	—
PR positive	0.41	0.14-1.16	0.094	0.33	0.15-0.76	**0.009**
High NLR (≥1.78)	1.92	0.83-4.46	0.128	1.76	0.88-3.54	0.113
ER positive	0.65	0.23-1.84	0.418	0.94	0.41-2.15	0.880
Grade 3 histology	0.90	0.40-2.03	0.802	1.22	0.63-2.35	0.559
High PLR (≥138.08)[a]	—	—	—	1.10	0.59-2.08	0.761

Variables with $p<0.25$ in univariate analysis were entered into the multivariate model. Bold values indicate statistical significance ($p<0.05$).
[a]: Lymph node positivity and neoadjuvant chemotherapy were entered into the OS model only, and high PLR was entered into the DFS model only; each met the $p<0.25$ threshold for one endpoint but not the other.
—: Not included in model. Complete-case analysis: OS model n=183 (30 deaths); DFS model n=184 (44 events). Models were fitted with L2 (Ridge) penalization (λ=0.01; lifelines, CoxPHFitter, penalizer=0.01). HRs are exponentiated penalized coefficients; 95% CIs and p-values are Wald-type quantities calculated from the standard errors of the same penalized fit (coefficient ± 1.96 × standard error, exponentiated) and are therefore conditional on λ.
HR: Hazard ratio; CI: Confidence interval; CT: Chemotherapy; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; ER: Estrogen receptor; PR: Progesterone receptor OS: Overall survival; DFS: Disease-free survival.

TABLE 2: Baseline demographic, clinicopathological, and laboratory characteristics of the study cohort (n=190).

Variable	Value
Demographics	
Age (years), median (range)	51 (26-76)
<50 years	92 (48.4%)
≥50 years	98 (51.6%)
Menopausal status	
Premenopausal	93 (50.5%)
Postmenopausal	91 (49.5%)
BMI (kg/m^2), median (range)	29.4 (18.8-46.4)
Normal (<25)	38 (20.0%)
Overweight (25-29.9)	66 (34.7%)
Obese class I (30-34.9)	51 (26.8%)
Obese class II (35-39.9)	27 (14.2%)
Obese class III (≥40)	8 (4.2%)
Histopathological features	
Histological type	
Invasive ductal carcinoma	145 (78.0%)
Invasive lobular carcinoma	7 (3.8%)
Other	34 (18.3%)
Histological grade	
Grade 1	14 (7.8%)
Grade 2	116 (64.4%)
Grade 3	50 (27.8%)
Receptor status	
ER positive	131 (71.2%)
PR positive	114 (62.0%)
HER2 positive	40 (21.7%)
Ki-67 index (%), median (range)	20 (0–95)
Ki-67 <20%	80 (43.5%)
Ki-67 ≥20%	104 (56.5%)
Molecular subtype	
Luminal A	52 (28.3%)
Luminal B	81 (44.0%)
HER2-enriched	10 (5.4%)
Triple-negative (TNBC)	41 (22.3%)
Disease staging	
Stage I	25 (14.0%)
Stage IIA	45 (25.1%)
Stage IIB	50 (27.9%)
Stage IIIA	41 (22.9%)
Stage IIIB	4 (2.2%)
Stage IIIC	14 (7.8%)
Lymph node positive	125 (66.1%)
LVI positive	104 (55.9%)
PNI positive	70 (37.6%)
Treatment	
Neoadjuvant chemotherapy	46 (24.3%)
Inflammatory markers	
NLR, median (IQR)	2.06 (1.52-2.55)
PLR, median (IQR)	132.86 (104.68-169.40)
SII, median (IQR)	589.24 (449.66-811.76)
Survival outcomes	
Median follow-up (months)	58.8
Deaths (OS events)	30 (15.8%)
DFS events	44 (23.2%)

Data are presented as n (%) for categorical variables and as median (range) or median (IQR) for continuous variables. Percentages are calculated using the number of patients with an evaluable value for the variable concerned as the denominator (valid-case analysis). The same rule applies to Table 2, Table 3, and Section Patient Characteristics, and no imputation was performed. Evaluable patients per variable (missing): menopausal status, 184 (6); histological type, 186 (4); histological grade, 180 (10); ER/PR/HER2/Ki-67 status, 184 (6); molecular subtype, 184 (6); pathological stage, 179 (11); lymph node status, 189 (1); LVI/PNI, 186 (4); neoadjuvant chemotherapy, 189 (1). All remaining variables were evaluable in all 190 patients. Records that did not contain an interpretable value for a given variable were counted as missing for that variable only and were retained in the cohort for every other analysis. Of the 192 patients with available pretreatment CBC data, two were excluded due to incomplete follow-up information. Therefore, the final analytic cohort consisted of 190 patients, and all analyses were performed using this cohort. Molecular subtypes were classified based on IHC surrogates, in accordance with the St. Gallen International Expert Consensus.

BMI: Body mass index; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; TNBC: Triple-negative breast cancer; LVI: Lymphovascular invasion; PNI: Perineural invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; IQR: Interquartile range; OS: Overall survival; DFS: Disease-free survival; IHC: Immunohistochemical; CBC: Complete blood count.

The baseline demographic, clinicopathological, and laboratory characteristics of the study cohort are summarized in Table 2. Unless otherwise stated, percentages are calculated using the number of patients with an evaluable value for the variable concerned as the denominator; the number of evaluable patients for each variable is listed in the footnote to Table 2; the same rule applies to Table 3.

The median age at diagnosis was 51 years (range: 26-76 years), with 98 patients (51.6%) aged ≥50 years. Menopausal status was almost equally distributed (premenopausal: 50.5%; postmenopausal: 49.5%). The median BMI was 29.4 kg/m^2 (range: 18.8-46.4), indicating that the study population was predominantly overweight or obese. The predominant histological type was invasive ductal carcinoma (78.0%). The majority of tumors were Grade 2 (64.4%). ER and PR positivity were detected in 71.2% and 62.0% of cases, respectively. HER2 overexpression was present in 21.7% of tumors. The median Ki-67 index was 20% (range: 0-95%), with 56.5% of patients having Ki-67 ≥20%.

TABLE 3: Association between dichotomized inflammatory markers and clinicopathological features.

Clinical feature	NLR high (≥1.78) n=121	NLR low (<1.78) n=69	p-value	PLR high (≥138.08) n=92	PLR low (<138.08) n=98	p-value	SII high (≥449.55) n=143	SII low (<449.55) n=47	p-value
Age ≥50 years	55/121 (45.5%)	43/69 (62.3%)	**0.037**	39/92 (42.4%)	59/98 (60.2%)	**0.021**	64/143 (44.8%)	34/47 (72.3%)	**0.002**
Postmenopausal	51/118 (43.2%)	40/66 (60.6%)	—	36/89 (40.4%)	55/95 (57.9%)	—	59/139 (42.4%)	32/45 (71.1%)	—
Grade 3	35/114 (30.7%)	15/66 (22.7%)	0.328	27/86 (31.4%)	23/94 (24.5%)	0.384	42/137 (30.7%)	8/43 (18.6%)	0.179
ER positive	77/118 (65.3%)	54/66 (81.8%)	**0.027**	59/89 (66.3%)	72/95 (75.8%)	0.208	96/140 (68.6%)	35/44 (79.5%)	0.226
PR positive	68/118 (57.6%)	46/66 (69.7%)	0.145	52/89 (58.4%)	62/95 (65.3%)	0.422	84/140 (60.0%)	30/44 (68.2%)	0.425
HER2 positive	23/118 (19.5%)	17/66 (25.8%)	0.423	22/89 (24.7%)	18/95 (18.9%)	0.441	28/140 (20.0%)	12/44 (27.3%)	0.418
Ki-67 ≥20%	65/117 (55.6%)	39/67 (58.2%)	0.846	52/89 (58.4%)	52/95 (54.7%)	0.722	76/140 (54.3%)	28/44 (63.6%)	0.359
LN positive	77/121 (63.6%)	48/68 (70.6%)	0.418	56/92 (60.9%)	69/97 (71.1%)	0.181	94/143 (65.7%)	31/46 (67.4%)	0.978
LVI positive	66/120 (55.0%)	38/66 (57.6%)	0.854	46/91 (50.5%)	58/95 (61.1%)	0.195	78/142 (54.9%)	26/44 (59.1%)	0.755
PNI positive	43/120 (35.8%)	27/66 (40.9%)	0.599	30/91 (33.0%)	40/95 (42.1%)	0.257	50/142 (35.2%)	20/44 (45.5%)	0.295
Stage III	39/112 (34.8%)	20/67 (29.9%)	0.603	28/84 (33.3%)	31/95 (32.6%)	1.000	48/135 (35.6%)	11/44 (25.0%)	0.267
Neoadjuvant CT	35/120 (29.2%)	11/69 (15.9%)	0.062	28/92 (30.4%)	18/97 (18.6%)	0.083	38/142 (26.8%)	8/47 (17.0%)	0.249

Inflammatory markers were dichotomized using optimal cut-off values derived from a 60 months landmark ROC analysis (NLR=1.78, PLR=138.08, and SII=449.55). All analyses were performed in the final study cohort of 190 patients. P-values were calculated using the chi-square test or Fisher's exact test, as appropriate. Bold p-values indicate statistical significance ($p<0.05$). Denominators are the numbers of patients with an evaluable value for the corresponding variable within each marker subgroup, applying the same valid-case rule used in Table 2 and Section Patient Characteristics; therefore, subgroup totals differ between rows according to variable-specific data availability. ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; LVI: Lymphovascular invasion; PNI: Perineural invasion; CT: Chemotherapy; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; ROC: Receiver operating characteristic.

Luminal B was the most prevalent molecular subtype (44.0%), followed by luminal A (28.3%), TNBC (22.3%), and HER2-enriched (5.4%). The majority presented with Stage II disease (53.1%), while 33.0% presented with Stage III disease. Lymph node involvement was present in 66.1% of patients. LVI and PNI were observed in 55.9% and 37.6%, respectively. Neoadjuvant chemotherapy was administered to 24.3% of patients. The median values of inflammatory markers were NLR 2.06 (IQR: 1.52-2.55), PLR 132.86 (IQR: 104.68-169.40), and SII 589.24 (IQR: 449.66-811.76). Patients were followed through November 2023. After a median follow-up of 58.8 months, 30 patients (15.8%) died, and 44 DFS events (23.2%) were recorded.

Determination of Optimal Cut-off Values

The 60 months landmark ROC analysis yielded the following optimal cut-off values: NLR=1.78, PLR=138.08, and SII=449.55 (Table 4). The AUC values for 5-year OS prediction were 0.566 (NLR), 0.560 (PLR), and 0.543 (SII). For 5 years DFS prediction, the AUC values were 0.581 (NLR), 0.605 (PLR), and 0.570 (SII). These modest AUC values suggest limited discriminatory power at the individual level, which is consistent with the established role of inflammatory markers as supplementary rather than stand-alone prognostic tools.

Based on the optimal NLR cut-off of 1.78, 121 patients (63.7%) were classified into the high NLR group (≥1.78) and 69 patients (36.3%) into the low NLR group (<1.78). Using the PLR cut-off of 138.08, 92 patients (48.4%) had high PLR, and 98 patients (51.6%) had low PLR. The SII cut-off of 449.55 categorized 143 patients (75.3%) as high SII and 47 (24.7%) as low SII.

Association Between Inflammatory Markers and Clinicopathological Features

The relationships between dichotomized inflammatory markers and clinicopathological features are presented in Table 3. High NLR (≥1.78) was significantly associated with younger age (<50 years) (p=0.037) and ER negativity (p=0.027). High PLR (≥138.08) was significantly associated with younger age (p=0.021). High SII (≥449.55) was significantly

TABLE 4: ROC analysis results and Kaplan-Meier survival estimates stratified by optimal inflammatory marker cut-offs.

Marker	Cut-off	AUC (OS)	AUC (DFS)	Group	n (events)	5 years OS (%)	5 years DFS (%)	p-value (OS)	p-value (DFS)
NLR	1.78	0.566	0.581	High (≥1.78)	121 (23 OS/32 DFS)	79.4	65.9	0.089	0.084
				Low (<1.78)	69 (7 OS/12 DFS)	93.8	83.1		
PLR	138.08	0.560	0.605	High (≥138.08)	92 (16 OS/24 DFS)	79.4	65.4	0.323	0.149
				Low (<138.08)	98 (14 OS/20 DFS)	89.1	78.1		
SII	449.55	0.543	0.570	High (≥449.55)	143 (22 OS/32 DFS)	82.4	71.5	0.871	0.917
				Low (<449.55)	47 (8 OS/12 DFS)	90.9	75.1		

Optimal cut-off values were determined by 60 months landmark ROC analysis using the Youden index. Five years survival rates were estimated by the Kaplan-Meier method. P-values were calculated using the log-rank test.
AUC: Area under the curve; OS: Overall survival; DFS: Disease-free survival; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; ROC: Receiver operating characteristic.

associated with older age (≥50 years) (p=0.002). None of the inflammatory markers showed significant associations with tumor grade, lymph node status, disease stage, LVI, or PNI.

Kaplan-Meier Survival Analysis

Kaplan-Meier survival curves stratified by inflammatory marker cut-offs are summarized in Table 4. For OS, patients with high NLR had a 5 years OS rate of 79.4%, compared with 93.8% in the low NLR group (log-rank p=0.089). High PLR was associated with a 5 years OS rate of 79.4% vs. 89.1% for low PLR (p=0.323). SII showed no meaningful separation between the survival curves (high SII: 82.4% vs. low SII: 90.9%; p=0.871). For DFS, patients with high NLR had a 5 years DFS rate of 65.9%, compared with 83.1% in the low NLR group (log-rank p=0.084). NLR consistently demonstrated the strongest prognostic signal among the three inflammatory markers (Figures 1 and 2).

Univariate Cox Regression Analysis

The results of univariate Cox proportional hazards regression for OS and DFS are presented in Table 5. For OS, the following variables were identified as significant prognostic factors (p<0.05): Ki-67 ≥20% (HR: 3.63; 95% CI: 1.48-8.88; p=0.005), PR positivity (HR: 0.30; 95% CI: 0.14-0.63; p=0.002), ER positivity (HR: 0.33; 95% CI: 0.16-0.67; p=0.002), Stage III disease (HR: 2.76; 95% CI: 1.34-5.69; p=0.006), lymph node positivity (HR: 2.90; 95% CI: 1.01-8.33; p=0.048), and Grade 3 histology (HR: 2.30; 95% CI: 1.08-4.87; p=0.030). High NLR was associated with a numerically inferior OS (HR: 2.05; 95% CI: 0.88-4.79; p=0.096), although this did not reach statistical significance. For DFS, significant prognostic factors included PR positivity, ER positivity, Ki-67 ≥20%, Stage III disease, and Grade 3 histology. High NLR was associated with a numerically elevated risk of DFS (HR: 1.78; 95% CI: 0.92-3.46; p=0.088), which did not reach statistical significance. PLR and SII did not reach statistical significance for either endpoint (Figure 3).

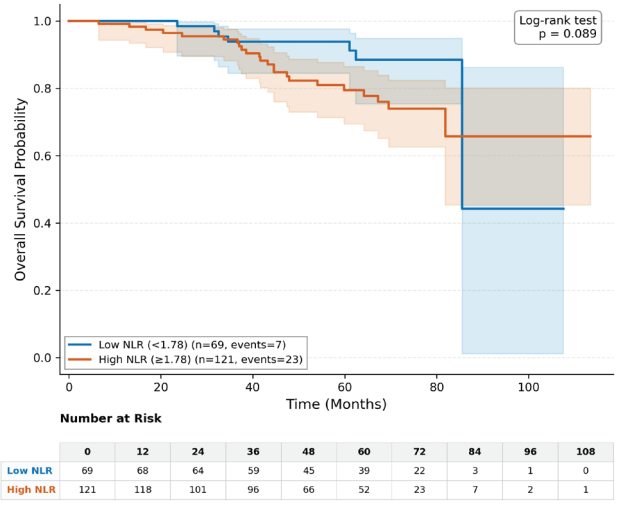

FIGURE 1: Kaplan-Meier overall survival curves according to pretreatment NLR. Patients were stratified into low NLR (<1.78; n=69) and high NLR (≥1.78; n=121) groups. The p-value was obtained from the log-rank test.

NLR: Neutrophil-to-lymphocyte ratio

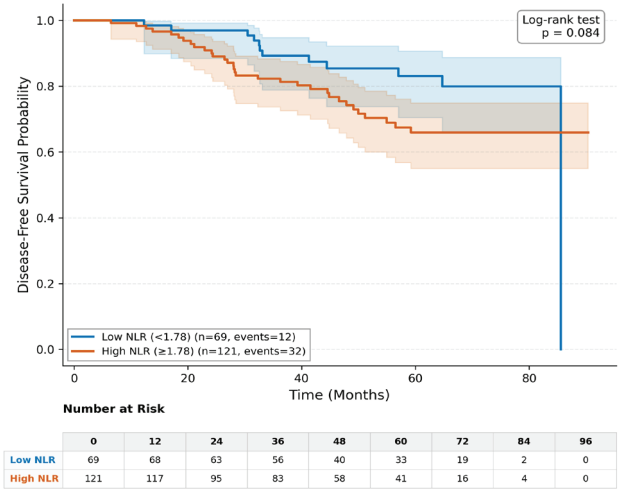

FIGURE 2: Kaplan-Meier disease-free survival curves according to pretreatment NLR. Patients were stratified into low NLR (<1.78; n=69) and high NLR (≥1.78; n=121) groups. The p-value was obtained from the log-rank test.

NLR: Neutrophil-to-lymphocyte ratio

TABLE 5: Univariate Cox proportional hazards regression analysis for overall survival and disease-free survival.

	Overall survival			Disease-free survival		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Age ≥50 years	1.29	0.62-2.69	0.492	0.94	0.51-1.70	0.826
Postmenopausal status	1.10	0.59-2.05	0.758	0.87	0.51-1.48	0.610
Grade 3 histology	2.30	1.08-4.87	**0.030**	2.14	1.13-4.04	**0.019**
ER positive	0.33	0.16-0.67	**0.002**	0.36	0.20-0.66	**<0.001**
PR positive	0.30	0.14-0.63	**0.002**	0.30	0.16-0.55	**<0.001**
HER2 positive	0.94	0.40-2.20	0.884	0.79	0.37-1.65	0.524
Ki-67 ≥20%	3.63	1.48-8.88	**0.005**	2.91	1.47-5.77	**0.002**
Lymph node positive	2.90	1.01-8.33	**0.048**	1.43	0.71-2.91	0.320
LVI positive	1.55	0.71-3.42	0.274	1.32	0.71-2.48	0.380
PNI positive	1.12	0.53-2.37	0.758	1.29	0.70-2.37	0.408
Neoadjuvant CT	1.73	0.74-4.06	0.206	1.47	0.70-3.08	0.310
Stage III disease	2.76	1.34-5.69	**0.006**	2.17	1.20-3.93	**0.011**
High NLR (≥1.78)	2.05	0.88-4.79	0.096	1.78	0.92-3.46	0.088
High PLR (≥138.08)	1.44	0.70-2.95	0.326	1.54	0.85-2.80	0.152
High SII (≥449.55)	1.07	0.48-2.41	0.870	1.04	0.53-2.01	0.917

Bold values indicate statistical significance (p<0.05).
HR: Hazard ratio; CI: Confidence interval; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; LVI: Lymphovascular invasion; PNI: Perineural invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index.

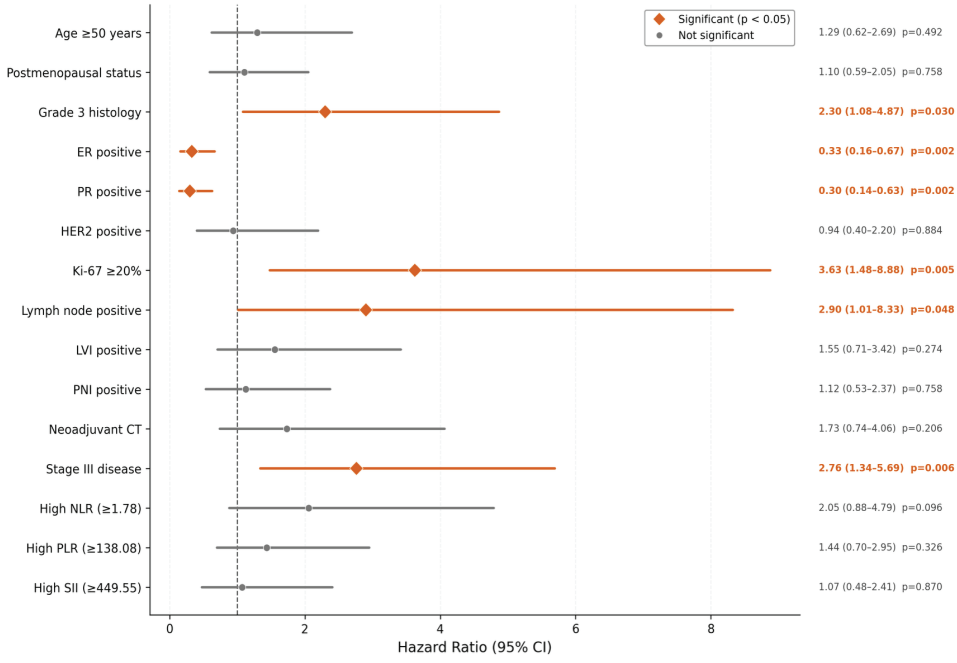

FIGURE 3: Forest plot of univariate Cox regression analysis for OS. HR with 95% CI are shown for each clinical and laboratory variable. The vertical dashed line represents HR: 1.0 (no effect).

HR: Hazard ratio; CI: Confidence interval; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; OS: Overall survival; CT: Chemotherapy; HER2: Human epidermal growth factor receptor 2; ER: Estrogen receptor; PR: Progesterone receptor; LVI: Lymphovascular invasion; PNI: Perineural invasion

The ROC curves for the inflammatory markers predicting OS and DFS are presented in Figure 4.

Multivariate Cox Regression Analysis

Variables with p<0.25 in univariate analysis were included in the multivariate model. The results are presented in Table 1. For OS, two variables retained independent prognostic significance: Ki-67 ≥20% (HR: 3.20; 95% CI: 1.31-7.80; p=0.011) and lymph node positivity (HR: 3.09; 95% CI: 1.07-8.93; p=0.037). Stage III disease (HR: 2.08; 95% CI: 0.97-4.47; p=0.059) and neoadjuvant chemotherapy (HR: 2.35; 95% CI: 0.96-5.73; p=0.060) were associated with effects of comparable magnitude, which narrowly missed statistical significance. PR positivity showed a numerically protective effect that did not reach statistical significance (HR: 0.41; 95% CI: 0.14-1.16; p=0.094). High NLR was not independently associated with OS (HR: 1.92; 95% CI: 0.83-4.46; p=0.128), though the direction and magnitude of the HR remained clinically noteworthy.

For DFS, three independent prognostic factors were identified: PR positivity (HR: 0.33; 95% CI: 0.15-0.76; p=0.009) as a protective factor, Ki-67 ≥20% (HR: 2.52; 95% CI: 1.26-5.07; p=0.009), and Stage III disease (HR: 2.27; 95% CI: 1.26-4.09; p=0.007) as independent risk factors. High NLR was not statistically significant for DFS (HR: 1.76; 95% CI: 0.88-3.54; p=0.113), although the direction and magnitude of the HR remained consistent with the univariate estimate.

Subgroup Analysis by Molecular Subtype

To evaluate whether the prognostic significance of inflammatory markers differed across molecular subtypes, subgroup survival analyses were performed (Table 6).

The most striking finding was observed in the TNBC subgroup (n=41). Among TNBC patients, elevated NLR (≥1.78) was significantly associated with both inferior OS (p=0.019) and inferior DFS (p=0.025). In the high NLR TNBC group (n=29), 12 OS events and 15 DFS events were observed, compared with 1 OS event and 2 DFS events among the 12 patients with low NLR. PLR did not show significant prognostic value in the TNBC subgroup for OS (p=0.446) and DFS (p=0.340). Because the observed survival events in the TNBC subgroup were concentrated within the high-NLR group, standard Cox regression could not be reliably estimated because of near-complete separation; ridge-penalized Cox regression (L2 penalty, λ=0.5; lifelines CoxPHFitter) was therefore applied, yielding a HR of 1.52 (95% CI: 0.42-5.57) for OS and 1.75 (95% CI: 0.50-6.11) for DFS. Given the very small subgroup size and the limited number of events, these effect estimates are unstable and should be interpreted with considerable caution.

In contrast, NLR showed no prognostic significance in the luminal B subgroup (OS: p=0.506; DFS: p=0.416) or the luminal A subgroup (DFS: p=0.560). PLR and SII similarly failed to achieve significance in the luminal subtypes (all p>0.30). The HER2-enriched subgroup (n=10) was considered too small for meaningful survival analysis. These findings demonstrate a subtype-specific prognostic role of NLR, with its predictive value confined primarily to the TNBC.

Continuous Variable Analysis and Internal Validation

When analyzed as a continuous variable, NLR was not significantly associated with OS (HR: 0.984; 95% CI: 0.729-1.328; p=0.915) or DFS (HR: 1.117; 95% CI: 0.880-1.418; p=0.362).

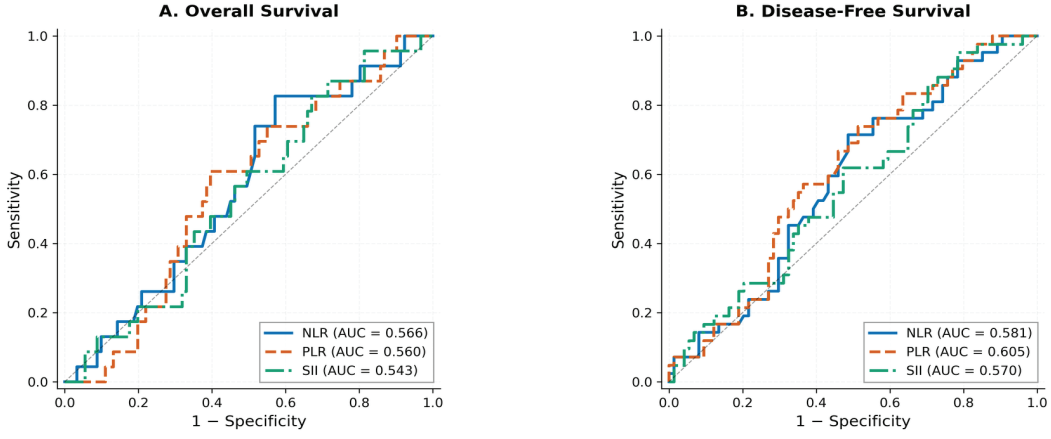

FIGURE 4: ROC curves for NLR, PLR, and SII in predicting OS **(A)** and DFS **(B)**. AUC values are shown for each marker. OS AUCs: NLR=0.566, PLR=0.560, SII=0.543; DFS AUCs: NLR=0.581, PLR=0.605, SII=0.570.

ROC: Receiver operating characteristic; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; OS: Overall survival; DFS: Disease-free survival; AUC: Area under the curve

TABLE 6: Subgroup analysis: Kaplan-Meier survival estimates by molecular subtype.

Molecular subtype	Marker	Endpoint	High group n (events)	Low group n (events)	Log-rank p-value
Luminal A (n=52)	NLR	DFS	26 (2)	26 (3)	0.560
	PLR	DFS	22 (2)	30 (3)	0.920
	SII	DFS	48 (5)	4 (0)	0.480
Luminal B (n=81)	NLR	OS	44 (8)	37 (5)	0.506
	NLR	DFS	44 (12)	37 (8)	0.416
	PLR	OS	37 (7)	44 (6)	0.410
	PLR	DFS	37 (10)	44 (10)	0.398
	SII	OS	73 (12)	8 (1)	0.817
	SII	DFS	73 (19)	8 (1)	0.387
TNBC (n=41)	NLR	OS	29 (12)	12 (1)	**0.019**
	NLR	DFS	29 (15)	12 (2)	**0.025**
	PLR	OS	24 (8)	17 (5)	0.446
	PLR	DFS	24 (11)	17 (6)	0.340

Kaplan-Meier log-rank test results for inflammatory markers stratified by molecular subtype. The HER2-enriched subgroup (n=10) was considered too small for meaningful survival analysis and was excluded. Bold values indicate statistical significance ($p<0.05$). For the luminal A subgroup, only DFS comparisons are presented, because the number of deaths observed in this subgroup was too small for a meaningful OS comparison. For SII, the cut-off value classified the majority of patients within each subtype as high-SII, resulting in low-SII comparison groups that included only 4 patients (luminal A) and 8 patients (luminal B). Consequently, the SII rows are based on markedly unbalanced groups and should be regarded as exploratory. SII comparisons are not presented for the TNBC subgroup because the low-SII group was too small for meaningful comparison.
NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SII: Systemic immune-inflammation index; OS: Overall survival; DFS: Disease-free survival; HER2: Human epidermal growth factor receptor 2; TNBC: Triple-negative breast cancer.

For a one-SD increase, the HRs were 0.98 (OS) and 1.13 (DFS) for NLR; 0.94 (OS) and 1.06 (DFS) for PLR; and 0.97 (OS) and 1.08 (DFS) for SII. After adjustment for age, stage, and Ki-67 status, NLR retained a per-SD HR of 1.06 (95% CI: 0.76-1.48; p=0.728) for OS. Bootstrap internal validation of the complete cut-off selection procedure (1000 resamples), in which the optimal cut-off was re-derived in every resample, yielded median NLR cutoffs of 1.78 for OS (95% percentile interval, 1.29-2.41) and 1.95 for DFS (95% percentile interval, 1.29-2.30), which were close to the primary estimates. The median bootstrap HRs were 2.25 (95% percentile interval, 1.04-8.24) for OS and 2.15 (95% percentile interval, 1.28-5.99) for DFS, with a HR greater than 1 in 98.1% of resamples for OS and in 100% of resamples for DFS and a log-rank p-value below 0.05 in 47.7% of resamples for OS and in 62.9% of resamples for DFS. These findings indicate sampling variability in the ROC-derived cut-off values and should be interpreted with appropriate caution (Supplementary Table S1). Overall, the continuous-variable analyses should be interpreted as complementary to the ROC-derived categorical analyses rather than as confirmatory.

DISCUSSION

The present study, with a median follow-up of 58.8 months, evaluated the prognostic significance of three pretreatment inflammatory indices—NLR, PLR, and SII—in 190 patients with non-metastatic breast cancer. The principal findings are as follows: (1) NLR showed a consistent direction of association with both OS and DFS in the overall cohort, although statistical significance was not reached; (2) Ki-67 ≥20% was confirmed as an independent risk factor for both OS and DFS, lymph node positivity for OS and Stage III disease for DFS, while PR positivity was independently protective for DFS; (3) NLR was a statistically significant prognostic marker in the TNBC subgroup for both OS and DFS but not in Luminal subtypes, indicating a subtype-specific effect; and (4) PLR and SII did not achieve significant prognostic value in this cohort.

NLR as a Prognostic Marker

In the overall cohort, NLR showed the strongest prognostic signal among the three inflammatory markers evaluated, with HRs of 2.05 for OS and 1.78 for DFS in univariate analysis; however, statistical significance was not achieved (p=0.096 and p=0.088; respectively). This finding is consistent with a meta-analysis by Ethier et al.[10], which included 15 studies and 8,563 patients and reported that elevated NLR was associated with worse OS (HR: 2.56; 95% CI: 1.96-3.35) and DFS (HR: 1.74; 95% CI: 1.47-2.07). However, the same meta-analysis highlighted substantial heterogeneity in cut-off values (ranging from 1.9 to 5.0), which may explain the variable significance across individual studies.

Our NLR cut-off of 1.78, derived from ROC analysis, is comparable to the cut-off of 1.34 reported by Cho et al.[25] in a cohort of 661 Korean breast cancer patients. The modest AUC of 0.566 observed in our study is also consistent with their

finding (AUC=0.58), reinforcing the notion that NLR alone has limited discriminatory capacity but may serve as a useful adjunct within a broader prognostic framework.

Subtype-specific Prognostic Significance of NLR in TNBC

A notable hypothesis-generating observation in our study was an apparent association between elevated NLR and inferior survival outcomes in the TNBC subgroup (OS: p=0.019; DFS: p=0.025); no such association was observed in luminal subtypes. This subtype-specific pattern has several important implications. From a biological perspective, TNBC is characterized by higher mutational burden, greater genomic instability, and a more immunogenic tumor microenvironment compared to hormone receptor-positive subtypes.[20,21] Neutrophils, which constitute the numerator of NLR, promote tumor progression through the release of reactive oxygen species, matrix metalloproteinases, and vascular endothelial growth factor, while also suppressing T-cell function.[26,27] An elevated NLR, therefore, reflects a pro-tumorigenic, immunosuppressive systemic state that may disproportionately impact outcomes in an inherently immunogenic subtype, such as TNBC.

This finding aligns with the study by Pistelli et al.[28], who reported that NLR >3 was independently associated with inferior DFS in early TNBC (HR: 5.15; 95% CI: 1.11-23.88; p=0.03). Similarly, Jia et al.[29] found that elevated NLR was associated with worse OS and DFS in the overall breast cancer cohort and in the TNBC subgroup, but not in the other molecular subtypes. This observation is particularly relevant given the recognized molecular heterogeneity within TNBC itself.[30] The clinical relevance of this finding is substantial: TNBC lacks established therapeutic targets; NLR—a universally accessible, inexpensive marker derived from routine blood tests—warrants further investigation as a potential supplementary prognostic tool; its role in informing clinical decision-making remains to be established through prospective validation. Immunotherapy-based regimens have shown particular promise in TNBC.[31] It must be emphasized, however, that the TNBC subgroup was small and included few events, resulting in near-complete separation, necessitating ridge-penalized (L2) Cox regression to obtain finite parameter estimates; consequently, these findings should be regarded as hypothesis-generating and require validation in larger, prospective TNBC cohorts.

PLR and SII: Limited Prognostic Value

In contrast to NLR, neither PLR nor SII demonstrated statistically significant prognostic value in this cohort. PLR showed a modest, non-significant trend for DFS (HR: 1.54; p=0.152), but not for OS (p=0.326). This contrasts with the findings of Cho et al.[25], who identified PLR as an independent predictor of both disease-specific survival and DFS, with PLR >185.5 conferring a substantially increased risk (HR: 3.23 for DSS). Their larger sample size (n=661) provided greater statistical power to detect associations, and they used a considerably higher PLR cut-off (185.5 vs. 138.08 in our study). SII, despite its theoretical advantage of integrating three hematological components, failed to show any prognostic signal in our cohort (OS: p=0.871; DFS: p=0.917) (Supplementary Figures S1-S4). A possible explanation is that adding platelet count to the SII formula may introduce noise rather than signal in this patient population.

Independent Prognostic Factors

The multivariate analysis confirmed Ki-67 ≥20% and lymph node positivity as independent predictors of OS, and PR positivity, Ki-67 ≥20%, and Stage III disease as independent predictors of DFS. Stage III disease showed an effect of comparable magnitude on OS; this effect narrowly missed conventional significance (HR: 2.08; p=0.059). These findings are well-established in the breast cancer prognostic literature.[32,33] Ki-67, a nuclear protein associated with cellular proliferation, has been consistently identified as a prognostic marker across molecular subtypes and is incorporated into clinical decision-making frameworks, including the St. Gallen Consensus.[23]

The protective effect of PR positivity on DFS (HR: 0.33; p=0.009) is consistent with published data indicating that PR expression carries independent prognostic significance. PR-positive tumors are generally more differentiated, have lower proliferative activity, and are more responsive to endocrine therapy.[34] The observation that PR did not achieve independent significance for OS (HR: 0.41; p=0.094) may reflect the limited number of OS events (n=30) and may warrant validation in larger cohorts.

Associations Between Inflammatory Markers and Clinical Characteristics

Our study revealed that elevated NLR and PLR were significantly associated with age <50 years, whereas elevated SII was associated with older age. The association between high NLR and ER negativity (p=0.027) is biologically plausible, as ER-negative tumors—particularly TNBC—tend to elicit a stronger inflammatory response.[35] None of the inflammatory markers showed significant associations with established adverse clinicopathological features such as lymph node metastasis, advanced stage, or LVI, suggesting that these markers reflect a distinct biological dimension independent of traditional prognostic factors.

Study Limitations

This study has several strengths. First, it simultaneously evaluated three inflammatory indices and their association with survival outcomes in a homogeneous, non-metastatic breast cancer cohort. Second, the subgroup analysis by molecular subtype adds clinically relevant information to the existing literature. Third, a penalized Cox regression approach mitigated overfitting concerns inherent to studies with a limited number of events. Fourth, the optimism associated with the data-driven selection of cut-off values was quantified using a bootstrap procedure that re-derived the cut-off in each resample.

However, several limitations must be acknowledged. First, the retrospective single-center design introduces potential selection bias and limits generalizability. Second, the relatively modest sample size (n=190) with 30 OS events and 44 DFS events restricts statistical power. Third, the TNBC subgroup comprised only 41 patients, and these findings require validation in larger, multicenter cohorts. Fourth, LMR could not be assessed due to the absence of monocyte count data. Fifth, other established inflammatory markers, such as C-reactive protein and albumin levels, were not available for analysis. Sixth, the association between NLR and OS/DFS in TNBC did not undergo formal multivariate adjustment within the subgroup due to limited events and should therefore be interpreted as hypothesis-generating. Additionally, the relatively small number of events (30 deaths for OS and 44 events for DFS), relative to the number of covariates included in the multivariable models may limit the stability of the regression estimates; therefore, these findings should be interpreted with caution. The late portions of the Kaplan-Meier survival curves should be interpreted with caution, as the number of patients at risk decreases over time and CIs widen accordingly. The imbalance in SII subgroup sizes (high SII, n=143 vs. low SII, n=47) may have reduced statistical power for detecting differences, particularly in subgroup analyses, and this should be considered when interpreting SII-related findings. ER and PR status are biologically correlated; some multicollinearity between these covariates in the multivariable models may have influenced the precision of individual coefficient estimates. Although inclusion criteria required substantially complete clinicopathological data, some variables had missing values (as detailed in Table 2), and complete-case analysis may have introduced selection bias if the data were not missing completely at random. Multiple comparisons were performed to analyze associations between inflammatory marker groups and clinicopathological variables (Table 3) without formal adjustment for multiplicity. Therefore, some statistically significant associations may have arisen by chance; these findings should be interpreted with caution and confirmed in independent studies.

To address the concern of optimism bias inherent in using data-derived cut-offs for prognosis, we performed three complementary analyses. First, inflammatory markers were analyzed as continuous variables in Cox regression models, making the analyses independent of cut-off selection. Analysis of continuous variables did not confirm the direction or magnitude of the associations observed using dichotomized variables. Therefore, these analyses should be considered complementary rather than confirmatory. Second, bootstrap internal validation with 1000 resamples was performed in a manner that incorporated the cut-off selection step itself, with the landmark ROC analysis, the derivation of the optimal Youden cut-off, and the subsequent Kaplan-Meier and Cox analyses repeated within each resample. Third, this procedure yielded a median bootstrap NLR cut-off of 1.78 for OS, corresponding to the value identified in the primary analysis, with a 95% percentile interval of 1.29-2.41; the direction of the association was consistent across resamples (HR greater than 1 in 98.1%), whereas the log-rank p-value was below 0.05 in 47.7% of resamples, indicating sampling variability and uncertainty regarding the precision of the cut-off. Nevertheless, these cut-offs should be considered hypothesis-generating and require external validation in independent cohorts before clinical implementation. Additionally, the landmark ROC approach excluded patients censored before 60 months, such that the cut-offs were derived from 114 patients for OS and 116 patients for DFS, and then applied to the complete cohort of 190. Although the median follow-up of 58.8 months meant that the majority of patients contributed to this analysis, the reduced effective sample size for cut-off determination means that the thresholds reflect only the subset of patients whose 60 months status was observable. An inverse-probability-of-censoring-weighted time-dependent ROC analysis would retain all 190 patients by weighting for censoring and would represent a methodologically attractive alternative. However, it was outside the predefined analysis plan of the present study and therefore was not undertaken; we regard it as the preferred approach for future validation work.

CONCLUSION

In this retrospective analysis of 190 non-metastatic breast cancer patients, pretreatment NLR showed a consistent direction of association with both OS and DFS in the overall cohort, although statistical significance was not reached. Of particular interest, NLR showed a preliminary, hypothesis-generating association with survival outcomes specifically in TNBC, while no such association was observed in luminal subtypes; however, given the small TNBC subgroup and the few events, this observation requires confirmation.

This subtype-specific finding suggests that the prognostic relevance of systemic inflammation in breast cancer is modulated by the molecular context of the tumor.

Ki-67 proliferation index, lymph node status, and disease stage were confirmed as the strongest independent predictors of survival, with PR positivity serving as a protective factor for DFS. Inflammatory markers such as NLR may serve as readily accessible, cost-effective supplementary prognostic tools, particularly in TNBC, where conventional hormone receptor-based stratification is unavailable. Prospective, multicenter studies with larger TNBC subgroups are warranted to validate these findings and explore whether inflammatory indices may have a supplementary role in the prognostic assessment of TNBC.

Ethics

Ethics Committee Approval: The study protocol was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee (approval number: 2023/4610, date: 3.11.2023).

Informed Consent: The requirement for written informed consent was waived due to the retrospective design of the study.

Footnotes

Authorship Contributions

Concept: E.H., Me.A., Design: E.H., Me.A., Data Collection or Processing: E.H., M.K.E., M.A., Analysis or Interpretation: E.H., Me.A., Literature Search: E.H., M.K.E., Writing: E.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Figures S1-S4: https://d2v96fxpocvxx.cloudfront.net/a5223c9c-50f0-490d-b293-6a74c2af8d3b/content-images/b3a65465-2546-438d-8b81-41ce32cfead0.pdf

Supplementary Table S1: https://d2v96fxpocvxx.cloudfront.net/a5223c9c-50f0-490d-b293-6a74c2af8d3b/content-images/463ea7c8-82fc-4690-af93-6a2dea780f0e.pdf

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