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ORIGINAL ARTICLE

Elevated Neutrophil–Lymphocyte Ratio in Luminal-Type Locally Advanced Breast Cancer to Circumvent Neo-Adjuvant Chemotherapy

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Abstract

Neutrophil–Lymphocyte Ratio (NLR) provides an understanding of the systemic inflammatory conditions. NLR plays an important role as a predictor of mortality in breast and other malignancies. The application of NLR to predict prognosis of Locally Advanced Breast Cancer (LABC) has not been well developed. In this retrospective study, we establish a relationship of pre-treatment NLR with the Pathological Complete Response (pCR) in LABC patients to enhance decision-making and treatment protocols. Data of women diagnosed with carcinoma breast between January 2015 and December 2017 was retrieved from hospital records of a tertiary medical centre in Bangalore, India, after obtaining institutional ethical clearance. LABC patients were categorized into pCR(+) and pCR(−). NLR was calculated and divided into quartiles. The cutoff NLR was determined using the Receiver Operating Characteristic (ROC) curve. Statistical analysis was performed on 119 LABC patients, of which 25 (21%) achieved pCR. Oestrogen Receptor (ER) positivity was significantly lower in pCR(+) than in pCR(−) ($p = 0.012$). NLR of 2.46 (AUC, 0.744; 95% CI [0.201–0.584]; $p = 0.056$) was considered the optimum cutoff for pCR(+). A sensitivity of 54%, specificity of 8%, positive predictive value of 1% and high Negative Predictive Value (NPV) of 84% was achieved in the study. A relationship between pCR and the pre-treatment NLR determined a significantly high NPV. Poor pCR in luminal A/B subtype presents with elevated NLR. Therefore, in luminal type A/B (ER- and PR-positive) with elevated NLR (poor outcome) and low pCR (poor response to NACT), the decision of eliminating NACT could be considered, thereby recommending surgical intervention.

Keywords Neutrophil-Lymphocyte Ratio · Locally advanced breast cancer · Luminal staging · NACT

Introduction

Inflammatory response plays a vital role in development and progression of malignancy [1] with neutrophils being associated with poor surveillance [2] while decreased lymphocyte count is considered a negative predictor of survival [3].

Breast cancer is the most commonly diagnosed malignancy worldwide [4]. Its growth is relatively influenced by chronic inflammation by creating a microenvironment augmented by reactive oxygen and nitrogen radicals released by the inflammatory cells involved in tumour pathology and hence propagating DNA alterations in the host. Thus, inflammatory cytokines and proteins influence tumour growth [5]. Pathological staging includes the size of primary tumour; lymph node involvement [6, 7]; expression of recognized biomarkers like hormone receptors, such as Oestrogen Receptor (ER) and Progesterone Receptor (PR); Human Epidermal Growth Factor Receptor 2 (Her2neu) and measure of proliferation (Ki67). These variables are essential factors determining the prognosis of breast cancer despite advanced molecular techniques and assays. A high density of lymphocytes in the stroma presents with better prognosis when compared with high neutrophils which present with poorer clinical outcomes [8–10].

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Peripheral blood-derived inflammatory markers such as neutrophil-Lymphocyte Ratio (NLR), platelet-lymphocyte ratio and lymphocyte-monocyte ratio are other prognostic indicators of malignancy [1]. The NLR is calculated using the absolute neutrophil count to absolute lymphocyte count ratio from the peripheral blood. Advanced stage of malignancy and poor prognosis is found to be significant with elevated levels of NLR [11].

With results from various trials around the world, Preoperative Systemic Treatment (PST) or Neo-Adjuvant Chemotherapy (NACT) is increasingly accepted not only for primary breast cancer of a locally advanced stage but also to lower the stage of breast cancer [12]; PST is responsible for downsizing the tumour to enhance the likelihood of Breast Conservation Surgery (BCS). The response to PST provides prognostic information for ongoing patient management with Pathological Complete Response (pCR) as an alternative marker for survival [13].

In this study, we intend to determine a relationship between the pre-treatment peripheral blood NLR in Locally Advanced Breast Cancer (LABC) patients with response to NACT using pCR as the indicator of effect on tumour cells and define various characteristics of LABC at different NLR quartiles. We establish the cutoff NLR for the study to define elevated NLR and hence determine a relationship between pCR and NLR within various molecular subtypes of LABC to improve decisions on treatment protocols in LABC.

Methodology

A retrospective analysis of 119 LABC patients diagnosed between January 2015 and December 2017 at a tertiary medical college hospital in Bangalore, South India, were included in this study for retrospective analysis. The patients were staged as stage IIB (T3 and beyond) and stage IIIA, B and C according to the American Joint Committee on Cancer (AJCC) [14]. The study was approved by the Institutional Ethical Committee (IEC). The hospital's Medical Records Department (MRD) and the cancer registry were used to obtain relevant patient details. All clinical data retrieved were anonymized and compiled ensuring patients' confidentiality.

Inclusion and Exclusion Criteria

All patients with a histopathologically confirmed carcinoma breast and clinically staged LABC were included in the study. pCR(+) is defined as no residual tumour in primary site/lymph nodes at the completion of NACT [15]. The histopathology reports lacking hormonal status and pCR status were excluded. Patients without a reported absolute neutrophil/lymphocyte counts at the time of diagnosis before NACT or surgery were excluded. All patients diagnosed with metastatic stage IV or inflammatory carcinoma of the breast (T4d) were excluded from the study. Breast cancers due to secondaries were an exclusion to the study. Chronic disease states such as Systemic Lupus Erythematosus (SLE), liver disease, end-stage renal disease, active infections, haematological disorders, autoimmune conditions with or without steroid therapy and acute febrile conditions were excluded. We also excluded all male breast cancers in this study.

The variables determining the outcome of the study such as age, absolute neutrophil and lymphocyte count along with components of pathological status such as tumour size, histology, lymph node status and nuclear grade (based on Scarff-Bloom-Richardson scheme) [16] were obtained and statistically analysed. The NLR was calculated using the absolute differential leucocyte counts reported using the Coulter counter technique from a peripheral blood sample obtained by venepuncture at the time of diagnosing breast malignancy and before NACT or surgery (either modified radical mastectomy or BCS) was offered.

Hormonal status of ER, PR and Her2neu was evaluated using Immunohistochemistry (IHC). Tumours with nuclear expression levels $\geq 1\%$ were considered positive. Her2neu status was assessed either using IHC or Fluorescence In Situ Hybridisation (FISH) and was considered positive if the score was greater than 3 or if the expression was two-fold stronger than the CEP-17 signal of tumour cells. Molecular subtypes are divided into four groups based on ER, PR, Her2neu and Ki67 according to the guidelines [17] described in Table 1. Reported pCR from all cases was used for statistical analysis.

Statistical Analysis

The data was analysed in Statistical Package for the Social Sciences version 24 (SPSS 24). The (NLR) was calculated using the odds absolute neutrophil count to the absolute lymphocyte count. Based on the histopathological reports, all patients were categorized into pCR(+)

Table 1 Molecular subtypes of breast cancer with receptor status [17]

Molecular subtype	Clinico-pathological definition			
	ER	PR	Her2neu	Ki67
Luminal A	+	±	–	< 14%
Luminal B				
Her2neu-negative	+	±	–	≥ 14%
Her2neu-positive	+	±	+	Any
Her2neu overexpression	–	–	Overexpression	
Triple-negative/basal like	–	–	–	

Table 2 Characteristics of patients with Pathological Complete Response (pCR)

	Pathological complete response (+) ($n = 25$)	Pathological complete response (−) ($n = 94$)	p value
Age (mean in years)	47.0 ± 10.2	49.4 ± 8.6	0.872
Histology type			0.823
Infiltrating ductal carcinoma	84.7%	83.5%	
Infiltrating lobular carcinoma	11.6%	10.5%	
Mixed histology (IDC+ILC)	3.7%	6%	
Clinical T stage			0.632
T0	1.3%	1.78%	
T1	4.92%	7.8%	
T2	71.7%	64.8%	
T3	14%	14.56%	
T4	8.08%	11.06%	
Clinical N stage			0.853
N0	12.56%	16.7%	
N1	43.6%	40.3%	
N2	36.67%	33.5%	
N3	7.17%	9.5%	
Oestrogen receptor (+)	28.5%	71.42%	0.012
Progesterone receptor (+)	31.94%	68.06%	0.017
Her2neu (+)	62.18%	37.82%	0.092
Grade			0.932
Grade I	2.7%	4.1%	
Grade II	57%	60%	
Grade III	34.74%	31.85%	
NLR (mean)	1.79 ± 0.25	3.23 ± 0.673	0.012

and pCR(−). The calculated NLR was categorized into equal quartiles according to the 25th, 50th and 75th NLR percentile based on the 4th or highest NLR quartile that included the patients within the uppermost 25% NLR values. Frequency analysis, independent sample t test and chi-square test were performed. Specificity, sensitivity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were obtained. Receiver Operating Characteristic (ROC) curve was constructed to assess the ability of NLR to predict the pCR in patients with breast cancer and define the optimal cutoff value. A 5% type-1 error was accepted as statistically significant predictive value for the variables used in the study and a p value of < 0.05 was considered significant.

Results

Out of 237 patients diagnosed with breast cancer in the hospital during the study period, 119 (50.21%) patients met the inclusion criteria to be part of the study. The mean age for pCR(+) and pCR(−) was 47.0 ± 10.2 and 49.4 ± 8.6 respectively with a p value of 0.872. Of the total, only 25 (21%) patients were pCR(+).

Invasive Ductal Carcinoma (IDC) was the most common diagnosed histology in 89 (74.78%) patients followed by Invasive Lobular Carcinoma (ILC) in 20 (16.8%), and mixed histology was present in 10 (8.4%). There was no significant difference between pCR(+) and pCR(-) with regard to histology ($p = 0.823$), clinical T ($p = 0.632$), clinical N ($p = 0.853$) and grade ($p = 0.932$). Luminal type A, type B and triple-negative cases estimated to 69, 38 and 12, respectively. There existed significant difference ($p = 0.012$) in the ER status among the two groups with ER(+) seen in 85 patients (71.42%) having pCR(*) along with a significant difference ($p = 0.017$) in the progesterone receptor status seen in 81 (68.06%) patients, with positivity in pCR(-). pCR(+) showed more positivity in 74 patients (62.18%) for Her2neu than pCR(-), but was not statistically significant ($p = 0.092$). There existed a significant difference in the mean NLR of 1.79 ± 0.25 for pCR(+) and 3.23 ± 0.673 for pCR(-) with a p value of 0.012. The characteristics of the pCR are described in Table 2.

Table 3 Baseline characteristics of patients with NLR quartiles

Variables	1st quartile (NLR < 1.80) $n = 29$	2nd quartile (NLR = 1.80–2.44) $n = 30$	3rd quartile (NLR = 2.45–3.32) $n = 29$	4th quartile (NLR ≥ 3.33) $n = 31$	p value
Age (mean in years)	44.3 ± 13.1	48.3 ± 14.1	44 ± 13.6	54 ± 12.1	0.0045
Tumour biology					
Size of tumour					0.0115
Stages T0 and T1	6 (20.7)	9 (31.2)	8 (28)	10 (33.8)	
Stage T2	21 (72.4)	20 (68.5)	19 (68)	16 (55)	
Stages T3 and T4	2 (6.9)	1 (0.3)	2 (4)	5 (11.3)	
Lymph node status					0.723
N0	3 (9.1)	2 (6.6)	3 (9.1)	4 (12.9)	
N1	6 (20.8)	8 (26.6)	6 (20.8)	7 (22.6)	
N2 and N3	20 (70.1)	20 (66.6)	20 (70.1)	19 (61.2)	
AJCC stage					0.0312
Stages 0 and 1	13 (44.8)	14 (46.66)	6 (20.69)	3 (9.67)	
Stage 2	12 (41.37)	11 (36.66)	12 (41.38)	15 (48.3)	
Stage 3	2 (6.9)	5 (16.66)	11 (37.93)	13 (41.9)	
Histology					0.623
Infiltrating ductal carcinoma	22 (76)	21 (70)	24 (82.6)	20 (64.5)	
Infiltrating lobular carcinoma	5 (17)	2 (6.66)	3 (10.34)	6 (19.4)	
Mixed histology (IDC+ILC)	2 (7)	7 (23.33)	2 (7)	5 (16.12)	
Tumour grade (SBR)					0.0485
Grade I	3 (10.3)	14 (46.6)	1 (3.4)	12 (38.7)	
Grate II	16 (55.1)	12 (40)	19 (65.5)	18 (58)	
Grade III	10 (34.4)	4 (13.3)	9 (31)	1 (3.2)	
Oestrogen receptor (+)	18 (62)	16 (53.3)	18 (62.06)	17 (61.8)	0.672
Progesterone receptor (+)	9 (31)	10 (33.3)	10 (33.3)	11 (35)	0.519
Her2neu (+)	2 (7)	4 (13.33)	1 (3.4)	1 (3.2)	0.423
Laboratory					
Neutrophil count	3.1 ± 0.9	3.2 ± 1.4	4.9 ± 1.3	5.8 ± 1.5	< 0.0001
Lymphocyte count	2.2 ± 0.5	2.0 ± 0.5	1.3 ± 0.6	1.2 ± 0.4	< 0.0001

AJCC, American Joint Committee on Cancer

There were 3 cases presenting with leucocyte count > 11,000/cm^3 with the highest being 12,500/cm^3. There were six cases with a lymphocyte count < 1000/cm^3 with the lowest being 400/cm^3. The baseline characteristics of the cases compared with the NLR quartiles have been represented in Table 3. The NLR quartile > 75th percentile had patients with larger tumours and more advanced stages when compared with the NLR < 25th percentile. The analysis also showed patients in the lowest NLR quartile having lower total leucocyte count compared with those in the highest (> 75th) NLR quartile.

The optimum NLR cutoff point for pCR(+) is 2.46 (AUC, 0.744; 95% CI [0.201–0.584]; $p = 0.056$) depicted in the ROC curve (Fig. 1). The study estimated a sensitivity of 54%, specificity of 8%, positive predictive value of 1% and negative predictive value of 84%.

Discussion

Inflammatory markers such as neutrophils and lymphocytes have different responses to malignancy [18]. Oncostatin M is produced by neutrophils which signal human breast cancer cells to increase the production of Vascular Endothelial Growth Factor (VEGF), thereby increasing the detachment of malignant cells and promoting invasiveness [19]. Neutrophils are also responsible to produce circulating angiogenetic and fibroblastic growth factors which promote tumour progression. However, lymphocytes govern host immune response through the production of cytotoxic cell death and cytokines which inhibit the proliferation of malignant cells [18]. Neutrophil-derived reactive oxygen species further decrease the adhesion, thereby promoting properties of extracellular matrix and activate Nuclear Factor (NF)-kB which inhibit apoptosis in malignant cells [20].

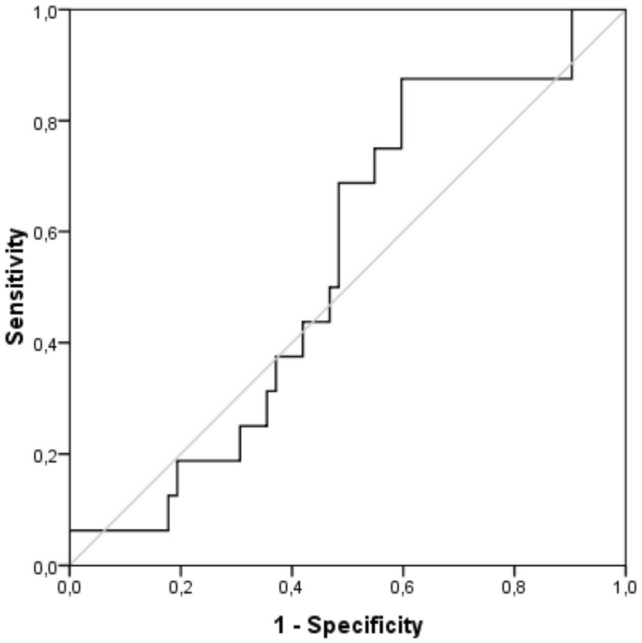

Fig. 1 Receiver operating characteristic (ROC) curve representing the optimal cutoff NLR value

The optimum cutoff NLR for our study was set at 2.46. NLR was elevated in pCR(-) patients which reflects poorer prognosis, when compared with pCR(+) which shows good response to NACT and hence with better prognosis. Elevated NLR has been correlated with advanced stage of breast cancer [11] specifically in luminal subtype A/B [21] and an adverse Overall Survival (OS) rate in most solid tumours [22]. It is, however, a challenge to validate if an advanced stage of cancer induced more inflammatory response or whether inflammation itself as reflected by the elevated NLR resulted in tumour progression and spread.

Pre-treatment NLR results of our study showed significantly high NPV with pCR implying that a poor response to NACT {pCR(-)} correlates with elevated NLR. A poor pCR suggests that the malignant cells were resistant to chemotherapy. In addition, higher Ki-67 expression (differentiation) in breast malignancy is largely associated with higher pCR rates [23]. Prognosis in LABC is dependent on pCR and other pathological responses as they are known surrogate termination factors in Her2neu-positive (Her2neu overexpression), triple-negative and luminal B Her2neu–negative subtypes with a clear exception in Luminal B Her2neu–positive subtype [24].

Heterogenicity of breast cancer and other factors can influence NLR and therefore the role of systemic inflammation parameters in breast cancer should be further evaluated. The sample size was a limitation to the study and we hope to include a greater sample size to further strengthen the relationship between NLR and pCR in LABC patients. The advent of newer neo-adjuvant chemotherapeutic drugs could possibly be effective even in poor pCR pathology which could possibly not be in favour of our results. Avoidance of NACT in LABC is still debatable in clinical practice.

Conclusion

The significantly high NPV can be used to enhance clinical decisions to circumvent NACT in luminal types A and B (ER and PR +) LABC patients with elevated pre-treatment NLR (poor pCR to NACT), thereby offering surgery as the first modality of treatment. Further studies with congruent significant results are essential for clinical implementation. However, in patients with T4 lesions, NACT would considerably reduce the size of tumour, pre-operatively aiding better reconstructive options.

Compliance with Ethical Standards

The study was approved by the Institutional Ethical Committee (IEC).

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

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