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RESEARCH ARTICLE

Peripheral Neutrophil-to-Lymphocyte Ratio (NLR) and Breast Cancer Distant Metastasis: Analysis of a Simple Diagnostic Biomarker

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Abstract

Introduction: Neutrophils and lymphocytes serve as easily accessible indicators representing the immune landscape in breast cancer. Combination of such variables in the form of neutrophil-to-lymphocyte ratio (NLR) allowed for extensive analysis and better interpretation. The heterogeneity in predictive role of NLR to prognosticate breast cancer sruvivalcould be attributed to metastatic spread. Methods: Data from breast cancer patients attending Oncology clinic in Sanglah Hospital, Bali was collected retrospectively. Samples were selected by simple random sampling technique from a sampling frame of patients with available preoperative complete blood count in the registry. Bivariate and multivariate analysis was performed with NLR value stratification using optimal NLR cut-off value derived from receiver operating characteristic (ROC) curve analysis. Results: A total of 51 patients diagnosed within the period of January 2016 through November 2017 were investigated. The cut-off point of NLR value in this study was 3.1 (Area under ROC curve [AUC]: 0.826). A significant association between NLR value and distant metastasis was found consistentlyin bivariate (PR 5.4, 95%CI 2.5-11.8, p=0.001) and multivariate analysis (Adjusted PR 4.9, 95%CI1.53-16.1, p 0.01). The association was not affected bybreast cancer subtype, histological grading, lymphovascular invasion, and tumor-infiltrating lymphocytes level. Conclusions: The NLRvalue was found to be an independent factor for distant metastasis in breast cancer. Its added value on diverse NLR clinical prognostication utility warrants further prospective investigation.

Keywords: *Neutrophil to lymphocyte ratio, Neoplasm metastasis, Breast neoplasms, Biomarkers.*

Introduction

Neutrophils and lymphocytes are amongst the most extensively studied cellular components of systemic inflammatory response. Multiple inflammatory mediators derived from these immune cells are known to exert distinct functions within intricate signaling cascadesof inflammation culminating in carcinogenesis. Inflammatory mechanisms were found to necessitate the whole sequence of cancer development, maintenance, and ultimate progression into metastatic spread.In fact, a vast majority of factors implicated in carcinogenesis are linked to chronic inflammation [1, 2].

Salient properties pertained to neutrophils,for instance, theirabundance andplasticityunderpin the multitude of roles they play in cancer development and progression.Prominent pro-tumor effectsexerted by neutrophils was first identified when the pretreatment absolute count elevation of these immune cells was reported to correlate with poorer survival.Findings on tumor-associated neutrophils (TAN)further extended the plausibilityof the pro-metastatic effect implicated by neutrophils [3-5].The role of lymphocytes in cancer immunosurveillance is well-established.

It encompasses throughout tumor development phasesby means of primary prevention of tumor development in pre-malignant state, tumor growth suppression, amelioration of tumor immunogenocity, and T-cell dependent immunoediting process[6].The latter involved lymphocyte infiltration into tumor microenvironment (i.e., tumor-infiltrating lymphocytes [TIL]), which act as a natural anti-cancer defense [7].The former explanations supported the observed increase of neutrophil levels and decreasedlymphocyte levelsin the circulation [1].

Exhaustive researches on prognostic implications of neutrophil-to-lymphocyte ratio (NLR) in breast cancer have been attempted. The capacity of NLR to predict overall and disease-free survival and treatment response was reported in previous studies [8-11].However, heterogeneity of results reported in these studies may be indirectly contributed by the metastatic spread thatwas not accounted for [12, 13].Therefore, this study sought to determine the association between NLR with metastasis in breast cancer because this diagnostic biomarkerconfers the benefit of being relatively cheap, widely available, and routinely assessed.

Material and Methods

This cross-sectional study was conducted retrospectively by collecting patients' data from medical records.Patients that were examined in Oncology Clinic, Sanglah General Hospital, Denpasar, Bali within the period of January 2016 to November 2017with the diagnosis code of C50 were chosen according to simple random sampling technique.

Eligible samples from the sampling frame provided by search results in the registry were subjected to simple random sampling by using random sample select case feature in SPSS® with an adequate approximate percentage to meet the minimum sample size required.

The study was approved by Sanglah Hospital Ethics Committee (2624/UN.14.2 / KEP /2017-2017.02.1.1264) and complied with ethical standards in the Declaration of Helsinki 1964 and the following amendments. The femalesamples included should have complete medical record informationregarding identity, clinical findings, complete blood count (CBC) prior to biopsy leading to initial diagnosis, immunohistochemistry result, and supporting imaging findings for cancer staging purposes. Patients with history of pregnancy-related breast cancer; cancer treatment with any modality including surgical, chemotherapy, radiation, hormonal, or targeting therapy; treatment with granulocyte-macrophage-colony-stimulating factor (GM-CSF); concurrent infection or inflammation (acute to chronic) on CBC test date; hypertension ordiabetes were excluded from participation. Breast cancer and its staging were diagnosed according to AJCC (American Joint Committee of Cancer).

Age group was divided into two based on menopausal status. Three histological grades were assigned to low (grade I-II) and high grade (grade III). Classification by TIL value into two groups (i.e., high and low).Retrospective data from preoperative complete blood count was obtained to count NLR value by dividing absolute neutrophilcount (ANC) with absolute lymphocyte count (ALC). Statistical analysis was performed usingSPSS® version 22.

The significance level was specified as $p<0.05$ for all analyses. Continuous data were presented in suitable central tendency and dispersion based on data normality analyzedwith Kolmogorov-Smirnov test. Receiver operating characteristic (ROC) curve analysis was carried out to determine NLR cut-off value with the best accuracy, area under ROC curve (AUC), and sensitivity and specificity of the cut-off value. Bivariate analysis using Chi-square test was done to select for confounding variables which would be included in multivariate analysis by cox regression.

Results

The study involved 51 patients with a mean age of 47.8±9.5 (Table1). Some of the patientsare from other areas, namely South Sumatra, East Nusa Tenggara, West Nusa Tenggara, and East Java.Sixteen patients (31.4%) experienced distant metastasis, and most of which had visceral metastatic spread (75%), while identical proportions of the remainder had bone (12.5%) and bone andvisceral (12.5%) metastatic spread. The TIL ($p = 0.28$), age ($p = 0.32$), subtype ($p =$

0.15), histological grading (p = 0.62), and lymphovascular invasion(p = 0.55) were considered as confounding factors in subsequent multivariate analysis (Table 2).

Table1: Sample baseline and clinical characteristics

Variable		n (%)
Age (years)		47.8±9.5
	≤40	41(80.4)
	>40	10(19.6)
Educational level	Primary school	16 (31.4)
	Junior highschool	16 (9.8)
	Senior highschool	5 (31.4)
	Bachelor	14 (27.5)
Occupation	Paramedic	1 (2)
	Government employees	6 (11.8)
	Entrepreneur	12 (23.5)
	Private employees	8 (15.7)
	Farmer	1 (2)
	Housewife	23 (45.1)
Address	Bali	39 (76.5)
	Others	12 (23.5)
Menopausal status	Pre-menopause	31 (60.8)
	Post menopause	20 (39.2)
Parity	0	12 (23.5)
	1	6 (11.8)
	2	18 (35.3)
	3	11 (21.6)
	4	2 (3.9)
	5	2 (3.9)
Stage	I	1 (2)
	IIA	21 (41.2)
	IIB	13 (25.5)
	IV	16 (31.4)
Subtype	Luminal A	12 (23.5)
	Luminal B	23 (45.1)
	HER2	4 (7.8)
	TNBC	12(23.5)
TIL	High	14 (27.4)
	Low	37 (72.6)
LVI	Negative	32 (62.7)
	Positive	19 (37.3)
Histological grading	High Grade	20 (39.2)
	Low Grade	31 (60.8)

HER2, human epidermal growth factor receptor 2; LVI, lymphovascular invasion; TIL,tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer

Table 2: Bivariate analysis of variables and metastasic spread

Variable	Metastatic		Prevalence Ratio (PR)	95% CI	P
	Yes	No			
NLR ≥ 3.1	10 (83.3%)	2 (16.7%)	5.4	2.5-11.8	0.001
NLR < 3.1	6 (15.4%)	33 (84.6%)			
TIL Low	13 (35.1%)	24 (64.9%)	1.6	0.55-4.9	0.28
TIL High	3 (21.4%)	11 (78.6%)			
Age >40	14 (34.1%)	27 (65.9%)	1.7	0.46-6.33	0.32
Age ≤ 40	2 (20%)	8 (80%)			
Luminal A	6 (50%)	6 (50%)	6.4	0.25-3.64	0.15
Luminal B	5 (21.7%)	18 (78.3%)			
HER2	0 (0%)	4 (100%)			
TNBC	5 (41.7%)	7 (58.3%)			
Grade 3	14 (31.1%)	31 (68.9%)	0.9	0.28-3.13	0.62
Grade 1 and 2	2 (33.3%)	4 (66.7%)			
LVI positive	5 (26.3%)	14 (73.7%)	0.8	0.31-1.86	0.55
LVI Negative	11 (34.4%)	21 (65.6%)			

*p-value by Chi-square test. HER2, human epidermal growth factor receptor 2; LVI, lymphovascular invasion; TIL, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer

The mean NLR value was 2.573±0.96. Cut-off value of 3.1 was established with ROC curve analysis (AUC= 0.826, p=0.000)by selecting the value with the highest sensitivity (62.5%) and specificity (94.3%). High NLR value was a significant predictive factor of metastasis (p=0.001, PR 5.4, 95%CI 2.5-11.8).

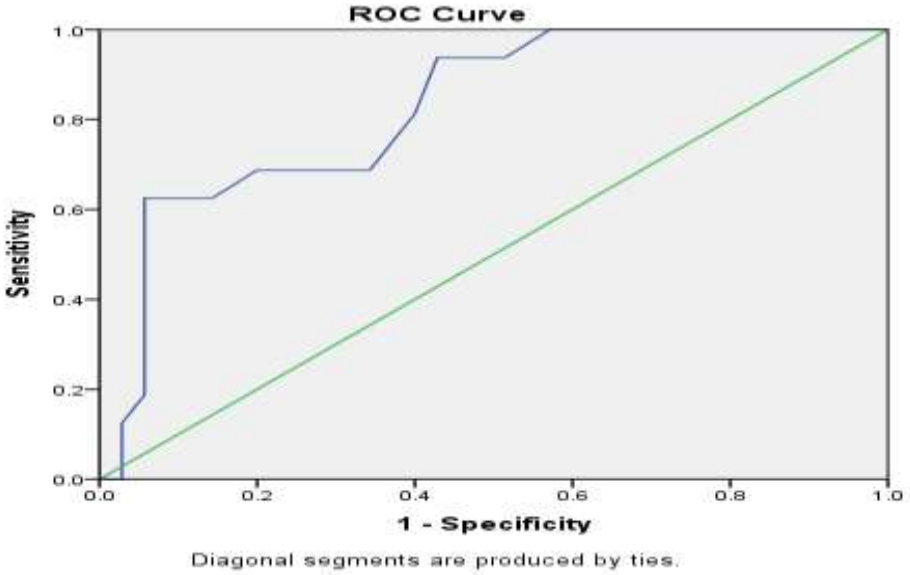

Figure 1: ROC analysis of NLR (AUC= 0.826, p=0.000)

In Table 3, the final model included TIL, age, subtype, histological grading, and lymphovascular invasion as independen covariates and NLR value still showed statistically significant impact (p=0.01, PR 4.9, 95%CI 1.53-16.1).

Table 3: Multivariate analysis of the factors affecting metastasis

Variable	Adjusted Prevalence Ratio	95%CI	p
NLR	4.9	1.53-16.1	0.01
TIL	1.2	0.31-4.8	0.78
Age	1.6	0.59-4.52	0.34
Subtype	0.9	0.25-3.64	0.94
Histological grading	1.3	0.25-6.58	0.76
LVI	1.0	0.36-3.17	0.90

LVI, lymphovascular invasion; NLR, neutrophil-to-lymphocyte ratio; TIL, tumor-infiltrating lymphocytes.

Discussion

The demand for biological exposition to substantiate incessant growth of evidence linking NLR to cancer prognostic factors should be raised proportionately.Despite many unequivocal proofs of the predictive role of NLR being reported, the current development of its mechanistic aspect was limited and still lacking overall coherence. The rationale behind the link was often narrowed down to inflammation concept in general, although it was unclear how such systemic process could represent specific tumor microenvironment well [7, 14].

One of the clue driving factors for pro-metastatic inflammation in breast cancer lied within the cancer cells' genome, specifically in tumor-suppressor protein p53. In vivo study showed that the loss of p53 in cancer cells up-regulated Wnt ligands which stimulate IL-1ß as the promotor of neutrophilic inflammation [15, 16].Alternatively, cancer cells could also hijack NLRP3/IL-1ß pathway in cancer-associated fibroblasts to provide tumor-supporting milieu,enhance invasiveness, and facilitate inflammation instead of its physiological function of sensing tissue damage [17].Moreover, cancer cells are capable of attenuating immune responses through programmed cell death-1 and its ligands (PD-1/PD-L1) pathway, thus downregulating T-cell activity, diminishing cytokine production, and inducing antigen tolerance [18].

From a cellular immunity point of view, TAN released NET, enzymes, growth factors, cytokines, and chemokines, which were presumed topromote tumor cell growth and migration synergistically.[4]Conversely, infiltration of lymphocytes in tumor lesion as measured quantitatively and qualitatively by the phenotype, played an essential role in tumor destruction [19].

Single-cell atlas mapping using mass cytometry found that both neutrophils and lymphocytes combined made up for the greatest portion of the immune landscape in breast cancer [20].These factors combined altogether supported the association between

peripheral blood counts with tumor immune-microenvironment not only in high ANC or NLR settings but also in the opposite settings where low ANC was accompanied with high TIL [21].The clinical utility of NLR outside of survival prediction remains an exploratory research area [3]. There were two interesting studiesinvestigating presurgical NLR predictive role in earlier stage breast cancer patients who underwent sentinel lymph node evaluation.

Both studies confirmed a statistically significant relationship between NLR and non-sentinel lymph node metastasis [22, 23].Conflicting results were demonstrated in studies focusing on NLR potential to predict prognosisin which distant metastasis was analyzed as one of the covariates. The NLR value was not associated with the presence of distant metastasis, metastasis at diagnosis, and metastasis location (including central nervous system and visceral metastasis) in multivariate analysis [24, 25].

In anotherstudy aiming to identify NLR as an independent prognostic predictor in patients diagnosed with bone metastasis from variable primary carcinomas, where one of which was breast cancer, the NLR value failed to predict existing metastasis other than bone[26].However, other usefulness of NLR for other disease, fir instances deep vein thrombosis had been explored.[27]To the best of our knowledge, this is the first study demonstrating the preoperative NLR role in predicting distant metastasis.

Cut-off values were derived from similar analyses in the former two studies concerning non-sentinel lymph node metastasis. The cut-off values were lower (1.8-2.6) than this study, probably showing a proportional increment of NLR and breast cancer metastatic staging [22, 23]. The other studies on breast cancer prognosis, however, applied a pre-defined NLR cut-off from previous study and median value (2.32) [24, 25].

Difference in methods to determine the cut-off values hindered proper comparison of these values with the current study. Our work has several limitations. The limited sample size should be considered when generalizing the result interpretation. Various subgroups in each clinical characteristics may not have been represented optimally given that not all of the variables were accounted for when deriving the minimum sample size requirement.We attempted to address some limitations due to the nature of NLR as a sensitive measurementby incorporating extensive clinical parameters in the analysis to better represent immune landscape of the tumor, viz: including TIL in analysis to account for TNBC subtypes and excluding other causes of NLR fluctuation whenever possible.

Conclusions

In conclusion, our study provided a novel insight on the role of NLR value in predicting distant metastasis. This association was not affected by breast cancer subtype, histological grading, lymphovascular invasion, andtumor-infiltrating lymphocytes level.Considering the feasibility and availability of this biomarker, further research endeavorson the clinical application of NLR as a biomarker in predicting breast cancer metastasis would markedly improve management strategy.

Ethical Clearance

This study was approved by Sanglah Hospital Ethics Committeewith a waiver of consent (2624/UN.14.2/KEP/2017-2017.02.1.1264).

Author Contributions

Conception and design: N.P.Y.K., I.B.T.W.M., I.G.B.S., and I.W.S. Data collection: N.P.Y.K. and I.G.B.S. Statistical analysis: N.P.Y.K. Drafting of the manuscript: N.P.Y.K., I.B.T.W.M.,I.G.B.S and P.A.T.A. All authors reviewed and approved the final version of the manuscript.

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