The Prognostic Value of Pretreatment Peripheral Neutrophil-Lymphocyte Ratio (NLR) and Its Correlation with Mutant P53 Expression In Indonesian Triple Negative Breast Cancer Patients

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Background

Triple-Negative Breast Cancer (TNBC) represents an aggressive phenotype among other breast cancer subtypes with worst prognosis due to abundant inflammatory process. Recent pre-clinical study suggested a correlation between p53 inactivation and systemic inflammation response in driving breast cancer progression. In this study, we evaluated the prognostic value of pre-treatment NLR and its association with mutant p53 expression.

Methods

TNBC patients treated in of Dr. Sardjito General Hospital during 2014-2017 were retrospectively analyzed. Receiver Operating Curve (ROC) was utilized to determine the NLR cut off value and Kaplan Meier survival analysis was used to evaluate the 3-year overall survival (OS). To examine the correlation of NLR and p53, chi-square and independent t-test analysis were applied. Multivariate analysis was done using Cox Proportional Hazard Regression Model with adjustment for age, BMI, clinical staging, histological grading, subtypes, and therapy.

Results

A total of 53 TNBC patients were included in this study (Table 1). The cut off value used to classify NLR into high and low NLR was 1.67 as demonstrated in Figure 1 and Table 2 (AUC: 0.720, 95%CI: 0.581-0.859, *p*: 0.007, sensitivity: 87.1%, specificity: 50.0%). Mutant p53 expression was associated with high NLR (*p*= 0.013) with significant difference (Mean difference: 0.611, 95%CI: 0.425-1.179, student's t-test *p*: 0.036). Patients with high NLR showed worse 3-year OS than patients with low NLR as shown in Figure 2 (Median OS±SE (months): 21.205±2.356, 95%CI: 16.588-25.823 vs unreached, *p*: 0.006). NLR was an independent prognostic factor of TNBC based on multivariate analysis (HR: 3.705, 95%CI: 1.176-11.666, *p*: 0.025) as demonstrated in Table 3.

Table 1. Patients' Clinicopathologic Profile According to NLR.

Characteristics		Low NLR (%)	High NLR (%)	*p* - Value
Age	<50 y.o	7 (13.2)	16 (30.2)	0.763
	≥50 y.o	8 (15.1)	22 (41.5)	
BMI	<25	7 (13.2)	21 (39.6)	0.572
	≥25	8 (15.1)	17 (32.1)	
Stage	Early	4 (7.5)	5 (9.4)	0.342
	LABC	10 (18.9)	26 (49.1)	
	Metastatic	1 (1.9)	7 (13.2)	
Tumor Size (T)	≤5 cm	6 (11.3)	8 (15.1)	0.182
	>5 cm	9 (17.0)	30 (56.6)	
Nodal Status (N)	Positive	7 (13.2)	25 (47.2)	0.200
	Negative	8 (15.1)	13 (24.5)	
Distant Metastasis (M)	Present	1 (1.9)	7 (13.2)	0.282
	Absent	14 (26.4)	31 (58.5)	
Mutant P53	Positive	3 (5.7)	22 (41.5)	**0.013**
	Negative	12 (22.6)	16 (30.2)	

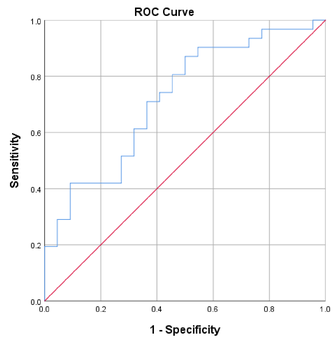

Figure 1. Area under curve (AUC 0.720, 95%CI: 0.581-0.859, *p*: 0.007, sensitivity: 87.1%, specificity: 50.0%.

Table 2. AUC value of NLR ROC.

Area	Std. Error	p value
0.720	0.071	**0.007**

95% Confidence Interval	
Lower Bound	Upper Bound
0.581	0.859

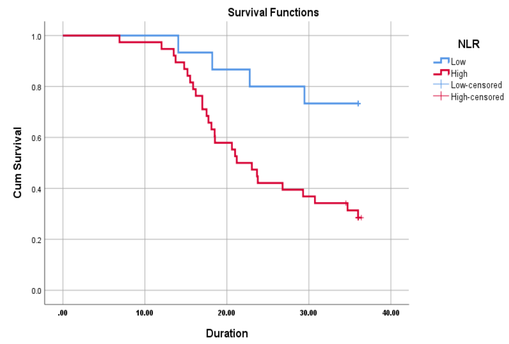

Figure 2. 3-year OS based on NLR (*p* Log Rank= 0.006)

Variables	HR	95% CI	*p*-value
Age	0.62	0.29-1.34	0.23
BMI	0.51	0.23-1.13	0.10
Stage	1.32	0.33-5.30	0.68
Tumor Size (T)	1.89	0.66-5.38	0.23
Nodal Status (N)	0.49	0.21-1.14	0.10
Distant Metastasis (M)	1.83	0.28-11.79	0.52
Mutant P53	0.91	0.43-1.93	0.80
NLR	3.70	1.18-11.67	**0.02**

Table 3. Multivariate Analysis

Discussion

The prognostic value of systemic inflammation profile, especially NLR, has been established in many solid tumors. In TNBC, NLR has a role as a negative prognostic factor. Circulating neutrophil secrets cytokines, such as VEGF, IL-18, and MMM, thus creating an optimum environment for tumor growth, progression, and metastasis.[1] Furthermore, the contribution of genetic aberration in the inflammatory response is still being investigated. Mutant p53 loses its NF-kB inhibitory action, which leads to over synthesis of proinflammatory mediators, such as Cox-2, IL-6, and iNOs. Those cytokines and chemokines recruit more inflammatory cells locally as well as systemically.[2,3] Loss of p53 expression also induces the production of WNT ligands and activates tumor-associated macrophage to produce IL-1β, which subsequently drives systemic inflammation response.[4] Our finding successfully identified the positive correlation between NLR and mutant p53 in TNBC patients. However, further studies are needed to explore the exact mechanism and types of inflammatory cells affected by the p53-deficient tumor.

Conclusion

Mutant p53 expression was associated with high NLR and, furthermore, NLR was an independent prognostic marker for TNBC. Therefore, this combination can be used to stratify TNBC patients' risk.

References

1. Pistelli, M., De Lisa, M., Ballatore, Z., Caramanti, M., Pagliacci, A., Battelli, N., et al. (2015). Pre-treatment neutrophil to lymphocyte ratio may be a useful tool in predicting survival in early triple negative breast cancer patients. *BMC cancer*, *15*, 195.
2. Uehara, I., & Tanaka, N. (2018). Role of p53 in the Regulation of the Inflammatory Tumor Microenvironment and Tumor Suppression. *Cancers*, *10*(7), 219. https://doi.org/10.3390/cancers10070219
3. Qu, X., Tang, Y., & Hua, S. (2018). Immunological Approaches Towards Cancer and Inflammation: A Cross Talk. *Frontiers in immunology*, *9*, 563.
4. Wellenstein, M. D., Coffelt, S. B., Duits, D., van Miltenburg, M. H., Slagter, M., de Rink, I., et al. (2019). Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*, *572*(7770), 538–542.

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