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Pre-treatment neutrophil-lymphocyte and platelet-lymphocyte ratios as additional markers for breast cancer progression: A retrospective cohort study

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ABSTRACT

Background: Breast cancer is the most prevalent cancer that causes significant morbidity and loss of productivity. Around a third of all breast cancer patients are potentially develop distant metastases albeit the current implementation of multidisciplinary treatment. A simple but effective marker to predict the risks of cancer progression is very important for clinicians to improve treatment and surveillance.
Methods: We recruited 1083 non-metastatic patients and analyzed the ratios of neutrophil to lymphocyte (NLR) and platelet to lymphocyte (PLR) in relation to progression-free survivals (PFS) and risks of distant metastases.
Results: Baseline clinicopathological variables were not significantly different in the pretreatment NLR and PLRs. Using maximum points of sensitivity and specificity of the Receiver Operating Characteristic (ROC) curve, cut-off values were determined 2.8 for NLR and 170 for PLR. Higher NLR was associated with skin and chest wall cancer infiltration (T4, $P = 0.0001$). Elevated PLR was associated with more advanced stages at diagnosis ($P = 0.03$). High NLR values were significantly associated with risks of disease progression (OR 1.555, 95% CI: 1.206–2.005, $P = 0.001$). Patients with high NLR had shorter PFS (34.9 vs 53.5 months, Log-rank test = 0.001) and shorter time to develop recurrent distant metastatic disease (66.6 vs 104.6 months, Log-rank test = 0.027).
Conclusion: High NLR is significantly associated with higher risk of disease progression and shorter time to develop metastases particularly among breast cancer patients diagnosed in the advanced stages.

1. Introduction

Breast cancer is the most frequently diagnosed cancer among females worldwide [1]. Particularly in developing countries such as Indonesia, morbidity and mortality rates as well as risk of disease progression are proportionally higher than breast cancer patients in developed countries [2,3]. Most case fatalities due to breast cancer are associated with manifestation of distant organ metastases [4]. Decreasing quality of life and productivity loss due to breast cancer have been associated with the nature of the disease progression including both locoregional and distant dissemination [5–7]. The host immune responses play a vital role to limit cancer initiation and progression by recognizing and eliminating the cancer cells through activation of cellular and humoral immune components [8].

Inflammatory responses both systemic and local microenvironment around the tumor influence the capability of tumor cells to continuously grow and migrate [9,10]. Lymphocytes and neutrophils are large constituents of systemic inflammatory response [11,12]. Lymphocytes are a key element in the cellular and humoral immune responses to selectively attack cancer cells. Chronic neutrophilia often causes depletion of cellular immune responses [13]. Inflammation-based scores, such as the neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR), have also been proposed as prognostic markers in solid cancers including colon, lung, nasopharyngeal, and breast cancer [13,14].

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Table 1
Baseline characteristics of breast cancer patients and the comparison of NLR and PLR means.

Variables	Category	N (%)	NLR mean (SD)	PLR mean (SD)	*P* value[a]	*P* value[b]
Age	Mean (SD)	50.92 (10.54)	2.9 (2.2)	169 (93.7)	0.067	0.449
	≤35	76 (7.0%)	2.8 (1.8)	172 (104)		
	36–40	111 (10.2%)	3.46 (4.2)	171 (97)		
	41–55	530 (48.9%)	2.9 (2.1)	172 (86)		
	56–65	283 (26.1%)	2.7 (1.6)	159 (103)		
	>65	83 (7.7%)	2.99 (1.8)	173 (91)		
Ethnicity	Javanese	1060 (98.0%)	2.9 (2.2)	169 (93)	0.489	0.908
	Non-Javanese	23 (2.0%)	3.2 (2.5)	171 (102)		
Residence	Rural	770 (72%)	3.0 (2.4)	168 (95)	0.043	0.860
	Urban	313 (28%)	2.7 (1.7)	169 (89)		
Menarche (years)	≤12	190 (17.5%)	2.8 (1.95)	175 (96)	0.533	0.538
	13–14	515 (47.6%)	2.9 (1.95)	167 (93)		
	≥15	378 (34.9%)	2.98 (2.8)	167 (93)		
Menopause (years)	≤50	593 (76.2%)	2.8 (1.9)	170 (95)	0.675	0.07
	>50	185 (23.8%)	2.75 (1.7)	156 (81)		
Parity	Nulliparous	118 (11%)	3.0 (2.0)	171 (94)	0.566	0.715
	Multiparous	965 (89%)	2.9 (2.3)	168 (94)		
Breastfeeding	No	220 (21.0%)	3.05 (2.3)	177 (102)	0.327	0.158
	Yes	865 (79.0%)	2.9 (2.3)	167 (91)		
BMI	≤18.5	136 (12.6%)	2.9 (2.0)	160 (84)	0.713	0.351
	18.6–25	536 (49.5%)	2.8 (1.85)	167 (99)		
	25.1–30	303 (27.9%)	3.0 (3.1)	171 (86)		
	>30	108 (10.0%)	3.0 (1.8)	181 (93)		
Family history	Yes	199 (18.0%)	3.1 (2.2)	170 (91)	0.301	0.828
	No	884 (82.0%)	2.9 (2.3)	168 (93)		
Histology grade	I	5 (0.5%)	3.9 (1.9)	253 (94)	0.599	0.09
	II	209 (19.3%)	2.9 (2.3)	163 (74.6)		
	III	869 (80.2%)	2.9 (2.3)	169.6 (98)		
Stage	I	12 (1.2%)	2.7 (1.4)	190 (102)	0.633	0.719
	II	355 (32.8%)	2.8 (2.5)	168 (101)		
	III	716 (66.0%)	2.96 (2.1)	168.5 (89.7)		
Tumor size	≤2 cm	46 (4.2%)	2.49 (1.2)	148 (73.9)	0.019	0.03
	2–5 cm	313 (28.9%)	2.7 (1.6)	156.4 (76.1)		
	>5 cm	724 (66.8%)	3.0 (2.5)	175.5 (100.8)		
Node status	N0	301 (27.8%)	3.0 (2.8)	175 (106)	0.484	0.536
	N1	548 (50.6%)	2.86 (2.1)	165 (84)		
	N2	192 (17.7%)	2.79 (1.7)	169 (94)		
	N3	42 (3.9%)	3.2 (2.0)	164 (107)		
ER	Negative	477 (44%)	3.05 (2.1)	172 (94)	0.65	0.091
	Positive	606 (56%)	2.8 (2.4)	166 (93.5)		
PR	Negative	618 (57%)	2.98 (2.1)	167.8 (90)	0.303	0.375
	Positive	465 (43%)	2.8 (2.5)	170 (98)		
HER2	Negative	789 (72.8%)	2.9 (2.4)	168 (96)	0.903	0.964
	Positive	294 (27.2%)	2.8 (1.7)	169 (87)		
Subtype	Luminal-A	497 (45.9%)	2.8 (2.5)	164.5 (96.5)	0.248	0.497
	Luminal-B	124 (11.5%)	2.76 (1.6)	172 (78.4)		
	Her2- enriched	173 (16.0%)	2.9 (1.99)	168.7 (93)		
	TNBC	289 (26.6%)	3.1 (2.2	175 (95)		

[a] Comparison of the NLRs using ANOVA or Independent sample T-test.
[b] Comparison of the PLRs using ANOVA or Independent sample T-test.

Increasing evidence has suggested the roles of NLR and PLR as predictor markers for therapeutic response and clinical outcome in breast cancer [12]. NLR and PLR are calculated from blood count rendering as a simple and cheaper marker for prognostication that are potentially applicable in countries with limited resources.

In this study, we analyzed the potential values of pretreatment NLR and PLR in association with intrinsic breast cancer subtypes, clinicopathological variables, and risks of disease progression as well as whether the predictive value was independent from other clinicopathological variables. We reported this study in accordance with the Strengthening the Reporting Items for Cohort Studies in Surgery (STROCSS) guidelines [15].

2. Material and methods

2.1. Patients

Breast cancer patients who were initially diagnosed and treated in our oncology unit during the period of January 2014 to December 2018 were recruited in this study. All diagnoses were confirmed pathologically both from biopsy and surgery. The protocol of this study has been reviewed by our institutional Ethical Committee (EC No 1143/2018). Information of patient's demographic characteristics as well as clinical and tumor variables were collected from the medical records. Detail tumor characteristics were extracted from the pathological report. Delivered treatment (type of surgery, prescribed chemotherapeutic drugs, anti-hormonal drugs, and radiotherapy) were summarized from the medical records. The clinical and pathological stages of breast cancer were determined using the 7th Edition of American Joint Committee on Cancer (AJCC) [16]. Type of cellular histology was classified according to guidelines from the World Health Organization (WHO) [17]. Histopathological grades of the primary tumor were classified using the modified Bloom and Richardson system (mSBR) [18]. Subgrouping of clinicopathological variables, breast cancer subtypes, and outcomes was performed as previously described [3,19]. Follow-up and patient surveillance, as well as criteria of disease progression and recurrent metastatic breast cancer were also performed as previously described [3,20].

Protein expression of estrogen receptor (ER), progesterone receptor

Table 2
Comparison of dichotomous clinicopathological variables of breast cancer patients according to the high or low NLR and high or low PLR.

Variables	Category	Low NLR (<2.8)	High NLR (≥2.8)	*P* value[a]	Low PLR (<170)	High PLR (≥170)	*P* value[a]
Age	≤40 years	114 (10.5%)	73 (6.7%)	0.225	115 (10.6%)	72 (6.6%)	0.668
	>40 years	588 (51.5%)	308 (28.4%)		565 (52.2%)	331 (30.6%)	
Ethnicity	Javanese	688 (63.5%)	372 (34.3%)	0.689	665 (61.4%)	395 (36.5%)	0.808
	Non-Javanese	14 (1.3%)	9 (0.8%)		15 (1.4%)	8 (0.7%)	
Residence	Urban	212 (1.96%)	101 (9.3%)	0.201	197 (18.2%)	116 (10.7%)	0.948
	Rural	490 (45.2%)	280 (25.8%)		483 (44.6%)	287 (26.5%)	
Menarche	≤12 years	133 (12.3%)	57 (5.2%)	0.100	111 (10.2%)	79 (7.3%)	0.170
	>12 years	569 (52.5%)	324 (29.9%)		569 (52.5%)	324 (29.9%)	
Menopause (years)	≤50 years	389 (50.0%)	204 (26.2%)	0.367	367 (48.3%)	226 (29.0%)	0.028
	>50 years	128 (16.4%)	57 (7.3%)		131 (16.8%)	54 (6.9%)	
Parity	Nulliparous	73 (6.7%)	45 (4.2%)	0.477	73 (6.7%)	45 (4.2%)	0.826
	Multiparous	629 (58.1%)	336 (31%))		607 (56.0%)	358 (33.1%)	
Breastfeeding	No	142 (13.1%)	78 (7.2%)	0.924	132 (12.2%)	88 (8.1%)	0.338
	Yes	560 (51.7%)	303 (27.9%)		548 (50.6%)	315 (29.1%)	
BMI	≤25	439 (12.6%)	233 (21.5%)	0.655	435 (40.2%)	237 (21.9%)	0.091
	>25	263 (24.3%)	148 (13.6%)		245 (22.6%)	166 (15.3%)	
Family history	Yes	128 (11.8%)	71 (6.6%)	0.871	126 (11.6%)	73 (6.7%)	0.865
	No	574 (53%)	310 (28.6%)		554 (51.1%)	330 (30.5%)	
Histology grade	I-II	141 (13.0%)	75 (6.9%)	0.875	134 (12.4%)	82 (7.6%)	0.798
	III	561 (51.8%)	306 (28.3%)		546 (50.4%)	321 (29.6%)	
Histology type	Lobular	59 (5.4%)	31 (2.9%)	0.879	55 (5.1%)	35 (3.2%)	0.721
	Ductal and others	643 (59.4%)	350 (32.3%)		625 (57.7%)	368 (34.0%)	
Stage	I-II	245 (22.6%)	124 (11.4%)	0.435	233 (21.5%)	136 (12.6%)	0.862
	III	457 (42.2%)	257 (23.7%)		447 (41.3%)	267 (24.6%)	
Tumor size	≤5 cm	242 (22.3%)	116 (10.7%)	0.179	241 (22.2%)	117 (10.8%)	0.030
	>5 cm	460 (42.4%%)	265 (24.5%)		439 (40.5%)	286 (26.4%)	
Tumor status	T1-3	580 (53.5%)	274 (25.3%)	0.0001	540 (49.8%)	314 (29.0%)	0.560
	T4	122 (11.2%)	107 (10.0%)		140 (12.9%)	89 (8.2%)	
Node status	N0	186 (17.2%)	120 (11.1%)	0.081	183 (16.9%)	123 (11.4%)	0.202
	N1-3	516 (47.6%)	261 (24.1%)		497 (45.9%)	280 (25.8%)	
ER	Negative	294 (27.1%)	183 (16.9%)	0.052	295 (27.2%)	182 (16.8%)	0.569
	Positive	408 (37.7%)	198 (18.3%)		385 (35.5%)	221 (20.4%)	
PR	Negative	386 (35.6%)	232 (21.4%)	0.061	390 (36.0%)	228 (21.1%)	0.803
	Positive	316 (29.3%)	149 (13.7%)		290 (26.8%)	175 (16.1%)	
HER2	Negative	516 (47.7%)	273 (25.2%)	0.513	496 (45.8%)	293 (27.1%)	0.933
	Positive	186 (17.3%)	108 (9.8%)		184 (17.0%)	110 (10.1%)	
Subtype	Luminal	415 (38.3%)	206 (19.0%)	0.109	395 (36.5%)	226 20.9%)	0.518
	Non-Luminal	287 (26.5%)	175 (16.2%)		285 (26.3%)	177 (16.3%)	

[b] Chi-square tests of PLRs.
[a] Chi-square tests of NLRs.

(PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 were analyzed using immunohistochemistry in the Pathology Department as previously explained [21]. Subclassification of breast cancer was determined according to the St Gallen Consensus 2013 [22,23] into Luminal A-like (ER^+ or PR^+, $HER2^-$, and Ki67 less than 20% or low grade), Luminal B-like (ER^+ or PR^+, $HER2^+$, and Ki67 more than 20% or high grade), HER2-enriched ($ER^-/PR^-/HER2^+$), and triple negative ($ER^-/PR^-/HER2^-$).

2.2. Blood samples and data collection

Complete blood count from peripheral venous samples was analyzed before surgery or biopsy. NLR was calculated as a ratio between the absolute count of neutrophils and lymphocytes and PLR was calculated as a ratio between the absolute number of platelets and lymphocytes. Complete blood count analysis was performed in the central clinical pathology lab following the standardized procedures [24].

2.3. Statistical analysis

Cut-off values of NLR and PLR were calculated using maximum point (sensitivity + specificity) of the Receiver Operating Characteristic (ROC) curve for the prediction of distant metastasis. The area under curves (AUCs) were 0.688 (0.648–0.728) and 0.638 (0.598–0.678) for NLR and PLR, respectively. Using this method, cut-off values for NLR and PLR were 2.8 and 170, respectively (Supplementary Figure 1).

The associations of NLR, PLR, distant metastasis and other clinicopathological variables were evaluated using Chi-square or Fisher's exact tests. Univariate and multivariate tests were performed using a logistic regression model. Odds ratio (ORs) and the corresponding 95% confidence intervals (95% CIs) were reported with *P* value < 0.05 considered as statistically significant. All statistical tests were performed using SPSS 19.0 software (SPSS Inc., Chicago, Ill).

3. Results

3.1. Patients and baseline characteristics

We included a cohort of 1083 breast cancer patients in which pretreatment complete blood counts were available before surgery or biopsy. Baseline clinicopathological variables and the comparison of NLRs and PLRs are summarized in Table 1. Mean age at diagnosis of breast cancer was 51 years (range 24–91). Means of NLRs and PLRs were not significantly different according to age at breast cancer diagnosis, ethnicity, menarche, breast feeding practice, menopause, parity, family history, and body mass index (BMI) statuses (Table 1). Also, no significant PLR and NLR differences were found according to axillary node status and expression of ER, PR, and HER2. Bigger tumor sizes had significantly higher NLRs and PLRs ($P = 0.019$ and $P = 0.030$, respectively). NLRs were also higher in breast cancer patients living in rural than those in urban areas ($P = 0.043$). NLR and PLRs were not significantly different among intrinsic breast cancer subtypes (Luminal A, Luminal B, Her2-enriched, and triple-negative breast cancer subtypes).

Table 3
Odd ratios and 95% Confidence intervals of high NLR and PLR values to the risks of disease progression and recurrent metastatic disease using multivariable logistic regression.

Variables	Category	Reference	Disease progression (OR, 95%CI)	Recurrent metastatic disease (OR, 95%CI)
Ethnicity	Javanese	Non Javanese	1.208 (0.483–3.018), $P = 0.686$	1.076 (0.407–2.845), $P = 0.882$
Residence	Rural	Urban	1.580 (1.158–2.115), $P = 0.004$	1.326 (0.928–1.895), $P = 0.122$
Menarche	≤12 years	>12 years	1.022 (0.725–1.441), $P = 0.900$	1.027 (0.706–1.492), $P = 0.890$
Menopause	>50 years	≤50 years	0.910 (0.635–1.303), $P = 0.606$	0.570 (0.464–1.007), $P = 0.060$
Parity	Multiparity	Nulliparity	0.958 (0.545–1.684), $P = 0.881$	1.385 (0.735–2.608), $P = 0.314$
Breastfeeding practice	Yes	No	1.127 (0.730–1.739), $P = 0.589$	0.961 (0.599–1.541), $P = 0.867$
BMI	>25	≤25	0.814 (0.622–1.066), $P = 0.136$	0.883 (0.656–1.280), $P = 0.414$
Family history	Yes	No	1.213 (0.864–1.703), $P = 0.263$	0.892 (0.622–1.280), $P = 0.536$
Stage	III (Advance)	I-II (Early)	2.342 (1.599–3.430), $P = 0.0001$	1.545 (1.000–2.390), $P = 0.050$
Tumor size	>5 cm	≤5 cm	0.889 (0.652–1.213), $P = 0.459$	0.894 (0.638–1.253), $P = 0.515$
Axillary node	Positive	Negative	1.698 (1.185–2.433), $P = 0.004$	1.381 (0.910–2.097), $P = 0.130$
Estrogen receptor	Positive	Negative	0.968 (0.661–1.419), $P = 0.869$	1.202 (0.799–1.807), $P = 0.378$
Progesterone receptor	Positive	Negative	0.610 (0.420–0.885), $P = 0.009$	0.759 (0.507–1.137), $P = 0.181$
HER2 expression	Positive	Negative	0.751 (0.557–1.013), $P = 0.060$	0.890 (0.642–1.235), $P = 0.487$
Age	≤40 years	>40 years	1.789 (1.081–2.958), $P = 0.024$	1.960 (1.146–3.356), $P = 0.014$
NLR	>2.8	≤2.8	1.966 (1.450–2.665), $P = 0.0001$	1.245 (0.904–1.713), $P = 0.179$
PLR	>170	≤170	0.784 (0.612–1.040), $P = 0.060$	1.082 (0.787–1.486), $P = 0.629$

3.2. Associations of NLR and PLR with baseline clinicopathological variables

Using cut-off values of 2.8 and 170 for NLR and PLR, we found that tumors with skin and chest wall infiltration were significantly associated with high NLR values ($P = 0.0001$, Table 2). In addition, larger tumor sizes (>5 cm) were associated with high PLR values ($P = 0.030$, Table 2). Patients with age of menopause less than 50 years were associated with high PLR values ($P = 0.028$). High NLR and PLR values were not associated with age, ethnicity, menarche, parity, breastfeeding practice, BMI, family history, histology, and hormonal status (Table 2).

3.3. Association of high NLR and PLR values with disease progression and recurrent metastatic diseases

After a mean follow-up of 48 months, we observed 592 events of disease progression including 273 events of distant organ metastases. Using multivariable logistic regression, high NLR values were significantly associated with risk of breast cancer progression (OR = 1.966, 95%CI:1.450–2.665, $P = 0.0001$, Table 3). Other variables including residence in rural areas (OR = 1.580, 95%CI:1.158–2.115, $P = 0.004$), advanced stages (OR = 2.342, 95%CI = 1.599–3.430, $P = 0.0001$), positive axillary lymph nodes (OR = 1.698, 95%CI:1.185–2.433, $P = 0.004$), younger age at diagnosis (OR = 1.789, 95%CI:1.081–2.958, $P = 0.024$) were also associated with risks of breast cancer progression (Table 3). Using multivariable logistic regression, high NLR and PLR values were not specifically associated with elevated risks of distant metastases. Advanced stages and young age at diagnosis were significantly associated with risks of progression into distant metastases (OR = 1.545, 95%CI:1.000–2.390, $P = 0.050$ and OR = 1.960, 95% CI:1.146–3.356, $P = 0.014$; respectively, Table 3).

Comparison of pre-treatment NLR and PLR values of breast cancer patients with progression-free survival and time to develop distant metastases were then performed using Kaplan-Meier survival curves. In comparison to low NLR values, patients with high NLR values at diagnosis were significantly associated with shorter progression-free survival (means were 34.9 vs 53.5 months, Log-rank Mantel-Cox test $P = 0.001$, Fig. 1) and time to develop distant organ metastases (means were 66.7 vs 104.6 months, Log-rank Mantel-Cox test $P = 0.027$, Fig. 1). However, high PLR values were not significantly associated with shorter progression-free survival (means were 41.9 vs 46.6 months, Log-rank Mantel-Cox test $P = 0.070$, Fig. 1) and time to develop distant organ metastases (means were 69.5 vs 102.8 months, Log-rank Mantel-Cox test $P = 0.551$, Fig. 1).

After stratification according to breast cancer stages, associations of higher NLR values with shorter PFS and time to develop metastases were found only in the population of breast cancer patients diagnosed in the late stages (Stage III). High NLRs were associated with shorter PFS time among breast cancer patients in advanced stages (means PFS were 25.1 vs 43.6 months, Log-rank Mantel-Cox test $P = 0.0001$, Fig. 2). High NLRs were also associated with shorter time to develop recurrent metastatic diseases among breast cancer patients in advanced stages (means were 50.7 vs 96.1 months, Log-rank Mantel-Cox test $P = 0.025$, Fig. 2). However, among patients in early stages, high NLRs were not significantly associated with shorter PFS and time to develop recurrent metastatic diseases. High PLR values were also not significantly associated with shorter time to PFS and development of distant metastases both in early and advanced stages of breast cancer patients.

4. Discussion

During the past decade, the major advances in cancer treatment have highlighted the acceptance of immune-based treatment in several hypermutable cancers [25]. Although breast cancer has long been considered as a cold tumor because of the limited ability to induce immune responses, a subset of breast cancer has been shown to benefit from immune checkpoint inhibitors [26]. Activation of innate and adaptive immune responses has also been associated with better therapeutic outcomes and breast cancer long-term survival [27]. In addition, host immune responses both tissue- and circulating cells has been translated into biomarkers to predict and monitor therapy [28]. Because chronic inflammation characterizes cancer development, identifying easily and robust metrics of systemic inflammation will additionally improve disease stratification and prediction of therapeutic response [12]. Systemic inflammation is partially represented with NLR and PLR. In our study, pretreatment high PLRs and NLRs were associated with larger tumor sizes and infiltration to the skin/chest wall. Previous studies using retrospective cohort design (N = 442 and N = 437,

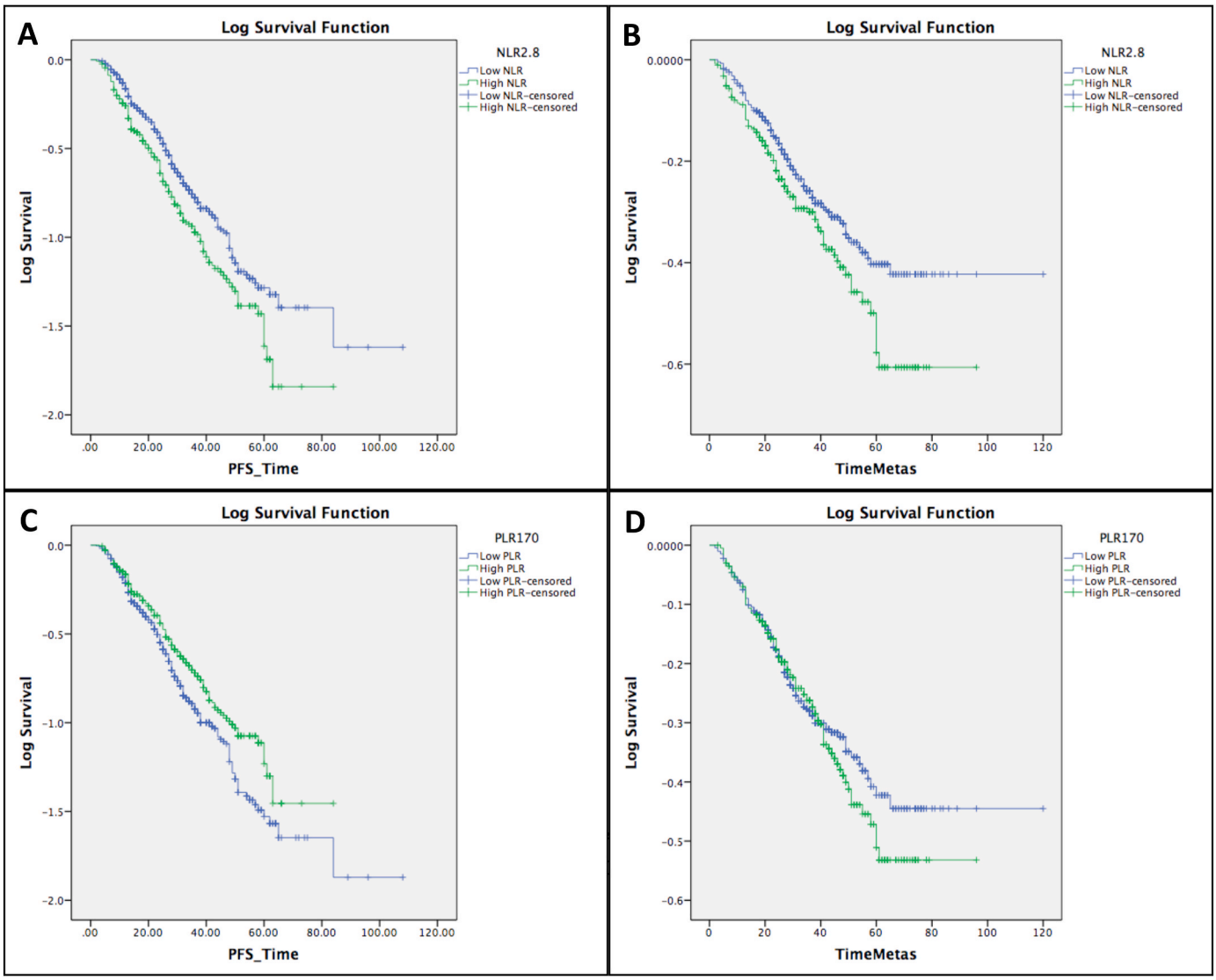

Fig. 1. Association of NLR and PLR values with disease-free progression and time to distant metastases. (A) High NLR values were associated with shorter PFS (means were 34.9 and 53.5 months in high and low NLRs, respectively; Log-rank Mantel-Cox test, $P = 0.001$). (B) High NLR values were associated with shorter time to develop distant metastases (means were 66.7 and 104.6 months in high and low NLR, respectively; Log-rank Mantel-Cox test, $P = 0.027$). (C) PLR values were not significantly associated with PFS (means were 46.6 and 41.9 months in high and low PLRs, respectively; Log-rank Mantel-Cox test, $P = 0.070$). (D) High PLR values were also not significantly associated with time to develop distant metastases (means were 69.5 and 102.8 months in high and low PLRs, respectively; Log-rank Mantel-Cox test, $P = 0.551$).

respectively) showed that higher NLRs were associated with bigger tumor size, younger age, and HER2 positivity (NLR cut-off value = 2.5) [29,30]. Elevated NLRs have also been associated with axillary lymph node infiltration, and distant metastases [30]. However, other reports also using retrospective design (N = 1527 and N = 187, respectively) did not find association of high NLR with TNM staging, distant metastasis and survival of breast cancer patients [31,32]. In addition, NLR and PLR might serve as independent from grades, Ki67 and molecular subtypes as a predictive marker of distant metastasis in breast cancer [29]. Higher pretreatment NLR has been associated as worse prognosis in a prospective study involving 177 TNBC patients [33]. However, NLRs and PLRs in our study were not associated with hormonal receptors, HER2 expression, and molecular subtypes. No significant different of NLR and PLR values across intrinsic subtypes were also reported by Yersal et al. in a retrospective cohort involving 255 breast cancer patients [34].

One of the principal functions of host immune responses is the ability of immune surveillance for pathogens including cancer cells [8]. The immune surveillance is mainly enforced by dendritic cells, natural killer cells, and lymphocytes [8]. Activated lymphocytes are the most important component in the immune surveillance against cancer. Whether NLR and PLR can partially represent immune surveillance activity in cancer patients is still debatable. However, lymphopenia has been associated with breast cancer worse prognosis independently of breast cancer stage [35,36].

Excess of neutrophil counts can promote tumor cell proliferation, growth, and cell migration by releasing signal transducers, transcription factors of *STAT*3, and matrix metalloproteases [37–39]. Chronic inflammation has a significant role in all stages of cancer development from initiation to invasion and distant spread. Inflammation causes higher levels of growth factors and cytokines that potentially induce cancer stem cell progenitors [40]. Chronic inflammation also induces angiogenic switch to further support tumor progression [37]. In addition, cytokines and other inflammatory mediators are abundant sources of reactive oxygen and nitrogen radicals that adversely cause DNA damages and genomic instability [38].

In association with disease progression, we found that high NLR was

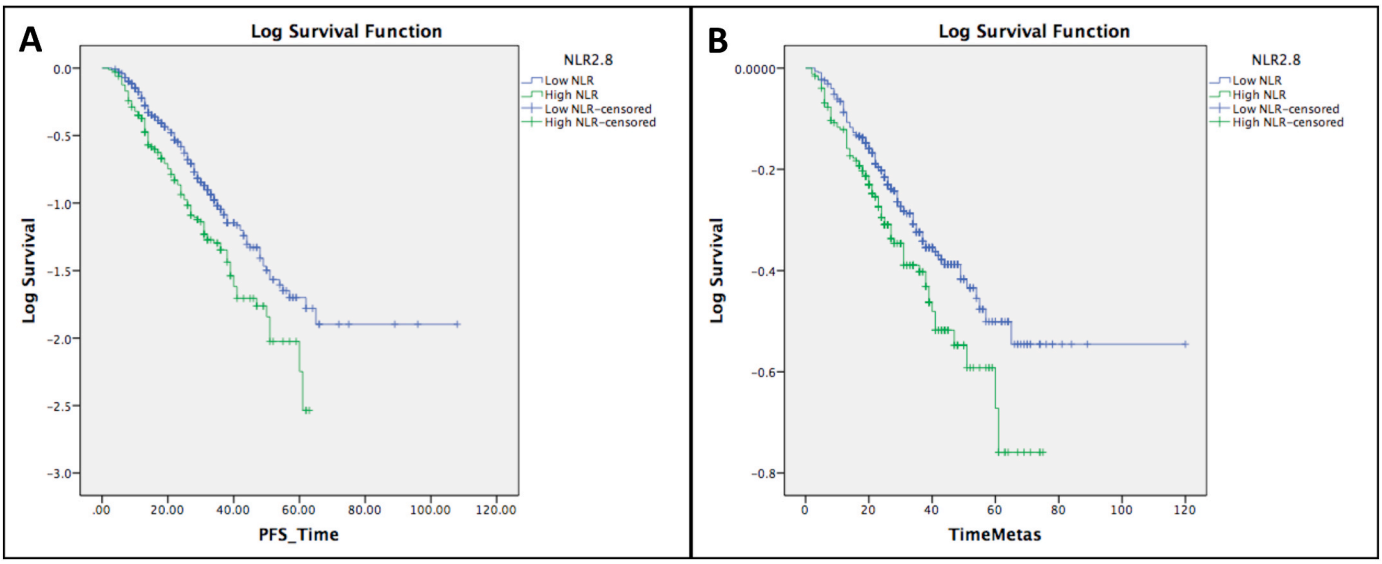

Fig. 2. Association of NLR values with disease-free progression and time to distant metastases among breast cancer patients diagnosed in late stages (Stage III, N = 714). (A) High NLR values were associated with shorter PFS (means were 22.5 and 37.2 months in high and low NLRs, respectively; Log-rank Mantel-Cox test, $P = 0.0001$). (B) High NLR values were associated with shorter time to develop distant metastases (means were 46.5 and 87.7 months in high and low NLR, respectively; Log-rank Mantel-Cox test, $P = 0.025$).

a significant risk factor using multivariate analysis (OR = 1.966, 95% CI:1.450–2.665, $P = 0.0001$) although both high NLR and PLR were not associated with risks of progression into distant metastases (Table 3). A meta-analysis has documented the relation between higher neutrophil levels with an elevated risk of distant organ metastases [41]. Multiple studies using retrospective cohort and metanalysis across different types of solid tumors found peripheral markers of immune response and inflammation, including NLR and PLR, were associated with survival and therapeutic responses [13,14,42] but the direct relation to distant metastasis is not yet previously described. In this study, we found that high NLRs and PLRs were associated with shorter PFS and time to distant metastases (Fig. 1). The association of high NLR values was found more striking among patients diagnosed in late stages (Stage III, Fig. 2). Some studies show the relation of NLRs and PLRs to mortality rates of breast cancer [29,30], although the clear association is not completely clear due to the lack of universal cut-off values.

In addition to systemic response, local immune responses are also important factors in the immune-mediated cancer attacks. The local responses are mainly executed by a specific population of T cells with selective reactivity to antigenic cancer proteins known as tumor infiltrating lymphocytes (TILs). These lymphocytes are able to recognize cancer cells and initiated adaptive immune responses to eliminate them. Although breast cancer frequently presents as lower antigenic burden, TILs has been observed in the tumor microenvironment [43]. In addition, increasing levels of TILs are associated with better response to anthracycline-based neoadjuvant chemotherapy and better survival particularly in triple negative and Her-2 enriched breast cancer [43]. However, high levels of TILs have also been found in breast cancer patients with resistance to chemotherapy and shorter overall survival [44]. Negative immune regulators including PD-1 and CTLA-4 as well as cytokines, oxidative-antioxidative, ribosomal, metabolic and systemic inflammatory factors have been associated with overall immune response against cancer cells [11].

Lymphocytes particularly cytotoxic T-cells and B-cell are the arsenal of the host immune system to attack cancer cells through cell-mediated and humoral immune responses. The circulating balance between neutrophils and lymphocytes represents host inflammatory responses and the activation of antitumor immune reactions. However, increased neutrophils count might also be a secondary effect of cancer-associated inflammatory response induced by tumor necrosis factor (TNF)-α, and granulocyte colony-stimulating factor [38]. Chronic inflammation can cause exhausted systemic immune response and remodeling of microenvironment to facilitate tumor growth. Neutrophils are able to inhibit activity of T-cells and natural killer cells by secreting arginase-1 and hydrogen peroxide [37,45]. Neutrophils have a key role in the stimulating and suppressing carcinogenesis by mediating immune response and inducing tumor-promoting leucocytes, angiogenesis, and tumor endothelial cell release into circulation [46]. Several immunocytes including neutrophils are able to produce vascular endothelial growth factor (VEGF) to facilitate tumor growth [46,47]. Therefore, higher neutrophil counts are associated with angiogenesis activation as well as disease progression.

In cancer, it is likely that prognosis is not only determined by clinical and histological characteristics but also by host immune responses to cancer cells. Circulating lymphocytes play a major actor in the immune surveillance and responses. In the tissues, lymphocytes are stationed in the tumor microenvironment as TILs that are associated with response to chemotherapy, immunotherapy, and survivals. In breast cancer, elevated neutrophil counts are associated with metastasis-related survival [29]. Using interventions to deplete neutrophils, Wculec et al. showed that neutrophils facilitated breast cancer cell colonization in the lung through leukotriene-generation enzyme arachidonate 5-lopoxygenase (Alox5) [48]. Breast cancer cells also induce IL-1β, IL-17, and granulocyte colony-stimulating factors (G-CSF) to further induce neutrophil production, suppress $CD8^{+}$T-cells, and promote metastasis [49].

Identification of patients with higher risks for recurrence, metastatic disease, and response to specific treatment has emerged as a rapid developing area in breast cancer. Several factors influencing mammary oncogenesis including tumor size, axillary lymph node involvement, histological grades, expression of hormone receptors, and mutations of specific tumor suppressor genes (*BRCA1/2*) have been incorporated in the prognostication of breast cancer. High NLR and PLR that partially indicate systemic inflammatory response, have been reported as an independent predictor of worse prognosis in solid tumors including breast cancer. NLR and PLR are considered as a simple non-invasive test to evaluate grossly interaction between tumor activity with microcirculation and inflammatory response. Using multivariate analysis, we found that conventional prognostic factors including age at diagnosis, tumor size, axillary node involvement, age at menopause were associated with

risks of disease progression or recurrent metastatic diseases. Adding to the previously described prognostic and predictive markers in breast cancer [50], therefore, NLR and PLR are potentially valuable as a reliable auxiliary prognostic marker in breast cancer.

Identification of several clinicopathological variables including greater tumor sizes and skin or chest wall infiltration with higher NLR or PLR as well as the association of elevated NLR with higher risks of breast cancer progression and poor survival became the major strength of this study. NLR and the competing clinic-pathological variables were analyzed using multivariate regression analysis. Limitations of this study were associated with determination of rigid cut-off values of NLR and PLR and relatively shorter time of follow up. Future study with prospective design, longer time of surveillance, and comparison with other inflammatory biomarkers as well as stratification according to preexisting comorbidities such as diabetes and obesity is required.

5. Conclusions

Using maximum sensitivity and specificity of ROC curve as cut-off NLR and PLR values, we found that higher NLR values were associated with risks of disease progression and shorter time to develop distant organ metastases in breast cancer. Pretreatment NLR might be used as a simple additional prognostic biomarker in breast cancers to help clinicians stratify patients with higher risks of disease progression. Future studies are required to further validate the potential application in combination with existing biomarkers for prognostic determination or predictive clinical outcome.

Declaration of competing interest

We declare that no potential conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.01.092.

Provenance and peer review

Not commissioned, externally peer reviewed.

Consent

Written informed consent for was acquired from the patients. Patient identifying related material was not used in this manuscript.

Ethical approval

The study has been conducted following the universal ethical standards in the Declaration of Helsinki 1964. The study protocol has been reviewed by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing - Universitas Gadjah Mada Yogyakarta (EC/1143/2018).

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Author contribution

SLA conceived the study and wrote the first draft. SLA, RC, WSA, and HYB collected the raw data. WAH and TA revised the manuscript. The final version of the manuscript draft has been approved by all authors.

Registration of research studies

1 Name of the registry: ISRCTN registry
2 Unique Identifying number or registration ID: ISRCTN13788093
3 Hyperlink to the registration (must be publicly accessible): http://www.isrctn.com/ISRCTN13788093

Guarantor

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