

Original article

A high neutrophil-lymphocyte ratio and platelet-lymphocyte ratio are associated with a worse outcome in inflammatory breast cancer

C. Van Berckelaer^{a,b,*}, M. Van Geyt^{b,1}, S. Linders^{b,1}, C. Rypens^a, X.B. Trinh^b,
W.A.A. Tjalma^b, S. Van Laere^a, C. Colpaert^d, L. Dirix^{a,c}, P.A. van Dam^b

^a Translational Cancer Research Unit, GZA Hospitals & CORE, MIPRO, University of Antwerp, Antwerp, Belgium

^b Multidisciplinary Breast Clinic, Unit Gynaecologic Oncology, Antwerp University Hospital (UZA) (Belgium), Molecular Imaging, Pathology, Radiotherapy, Oncology (MIPRO), Faculty of Medicine and Health Sciences, University of Antwerp, Belgium

^c Department of Oncology, GZA Hospitals Sint-Augustinus, Antwerp, Belgium

^d Department of Pathology, UZA, Antwerp University Hospital, Edegem, Belgium



ARTICLE INFO

Article history:

Received 17 March 2020

Received in revised form

5 July 2020

Accepted 10 August 2020

Available online 17 August 2020

Keywords:

Inflammatory breast cancer (IBC)

Neutrophil-to-lymphocyte ratio (NLR)

Platelet-lymphocyte ratio (PLR)

Lymphocyte-monocyte ratio (LMR)

Stromal tumor infiltrating lymphocytes

(sTIL)

Immune response

ABSTRACT

Introduction: Inflammatory breast cancer (IBC) is an uncommon, but aggressive form of breast cancer that accounts for a disproportionately high fraction of breast cancer related mortality. The aim of this study was to explore the peripheral immune response and the prognostic value of blood-based biomarkers, such as the neutrophil-to-lymphocyte ratio (NLR), in a large IBC cohort.

Patients & methods: We retrospectively identified 127 IBC patients and collected lab results from in-hospital medical records. The differential count of leukocytes was determined at the moment of diagnosis, before any therapeutic intervention. A cohort of early stage (n = 108), locally advanced (n = 74) and metastatic breast cancer patients (n = 41) served as a control population.

Results: The NLR was significantly higher in IBC compared to an early stage breast cancer cohort, but no difference between IBC patients and locally advanced breast cancer patients was noted. In the metastatic setting, there was also no significant difference between IBC and nIBC. However, a high NLR (>4.0) remained a significant predictor of worse outcome in IBC patients (HR: 0.49; 95% CI: 0.24–1.00; P = .05) and a lower platelet-lymphocyte ratio (PLR) (≤210) correlated with a better disease-free survival (DFS) (HR: 0.51; 95% CI: 0.28–0.93; P = .03).

Conclusion: Patients with a high NLR (>4.0) have a worse overall prognosis in IBC, while the PLR correlated with relapse free survival (RFS). Since NLR and PLR were not specifically associated with IBC disease, they can be seen as markers of more extensive disease.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Inflammatory breast cancer (IBC) is an uncommon, but aggressive form of breast cancer characterized by rapid local progression and fast dissemination of the cancer cells. Therefore, almost all patients have lymph node metastases at the moment of diagnosis and one-third of patients present with 'de novo' distant disease [1]. Despite the aggressive and multidisciplinary treatment with chemotherapeutics, radiation and surgery, the overall survival (OS) remains lower than in non-inflammatory breast cancer (nIBC) [2].

Since IBC also tends to affect younger women, there is a disproportionately high loss in life-years, far greater than one could expect based on the observed incidence (approximately 3% of all breast cancers) [3] and specific targeted (immuno)therapies are not available. Therefore, further research to unravel the molecular biology of IBC is warranted.

In 2013, the World IBC consortium (WIBCC) reported a gene signature that discriminates between IBC and non-IBC (nIBC) patient samples. Translating this gene signature into molecular concepts implies inflammation-related processes and an altered TGF-β pathway in the IBC tumor phenotype [4,5]. Other research groups also underlined the importance of the tumor stroma with infiltrating immune cells like lymphocytes and tumor-associated macrophages (TAMs) in IBC [6]. Together with the high frequency of PD-L1 positivity seen in IBC [7], this might indicate that the

* Corresponding author. Translational Cancer Research Unit, CORE, University of Antwerp Oosterveldlaan 24 2610, Antwerp, Belgium.

E-mail address: Christophe.vanberckelaer@uantwerpen.be (C. Van Berckelaer).

¹ Both authors contributed equally.

aberrant biological behavior associated with IBC can be at least partly attributed to a specific but suppressed immune microenvironment. In addition, there is mounting evidence that a good local immune response, for example by a higher infiltration with stromal tumor-infiltrating lymphocytes (sTIL), plays an essential role in the chemotherapy responsiveness and long-term outcome in both IBC [8] and proliferative subtypes of nIBC [9,10].

The role of peripheral systemic immunity on (inflammatory) breast cancer is less clear. An elevated peripheral neutrophil-to-lymphocyte (NLR) ratio, an indicator of systemic inflammation, was associated with a worse prognosis in several types of solid tumors [11]. In a breast cancer-specific meta-analysis including 8563 patients, a significant prognostic effect for NLR on both OS and RFS was found [12]. Therefore, the NLR might be promising as a prognostic marker. While there is no established cut-off value, Templeton et al. defined a value of 4.0 in a study of 40,559 different solid tumor samples [11], which was later also used in breast cancer studies reporting a significant association between prognosis and NLR in both early [13,14] and advanced breast cancer patients [15,16]. Although less studied, the platelet-lymphocyte ratio (PLR) [16,17] and the lymphocyte-monocyte ratio (LMR) [18] are also peripheral blood-derived prognostic inflammation markers showing prognostic significance in breast cancer. Cho et al. even suggested that the preoperative PLR is superior to the NLR in predicting clinical outcomes [19]. Conversely, other authors suggested that the NLR is superior [20]. Finally, these inflammatory markers were also related to chemosensitivity in breast and other solid cancers. Both a low PLR [21] and a low NLR [22] were associated with a complete pathological response (pCR) after neoadjuvant chemotherapy (NACT) in several studies. Although it seems that there is a negative effect of systemic inflammation on breast cancer prognosis, the relationship between the peripheral inflammatory indices and the local immune response remains unclear. To the best of our knowledge, these markers were never looked at in IBC. In this study we have investigated the prognostic role of peripheral inflammation markers in IBC, made a comparison with nIBC, and explored the relationship between these systemic markers and the local immune response.

2. Methodology

2.1. Patient selection

The medical records of all consecutively diagnosed IBC patients, between January 1, 1997 and December 31, 2017, at the GZA Hospital Sint-Augustinus and, between January 1, 2006 and December 31, 2017, at the Antwerp University Hospital were retrospectively reviewed after receiving ethical approval from the ethics committee (File number: 16/33/338). All cases ($n = 127$) had complete hospital records, were pathologically confirmed as invasive carcinoma and diagnosed as IBC using the clinical definition agreed on by international experts [23]. Patients with inflammatory skin changes in a breast that already had cancer or on the chest wall following mastectomy were excluded. Estrogen and progesterone receptor status were assessed using validated immunohistochemical tests and defined as positive if Allred score $\geq 3/8$. Tumor samples with documented amplification on a fluorescence in-situ hybridization (FISH) test were considered to be HER2-positive. Considering that systemic therapies have changed over the years, not all HER2+ patients received targeted therapy ($n = 28/58$). NACT consisted of an anthracycline-based regime combined with a taxane and pCR after completion of NACT was defined as the absence of residual invasive carcinoma in both the mastectomy specimen and the sampled regional lymph nodes.

2.2. Blood-based biomarkers

Anticoagulated whole blood was processed for the determination of the peripheral blood cell count and CA15.3 at the moment of diagnosis. These blood tests were performed as part of the routine management of the patients, before any therapeutic intervention. Absolute neutrophil count was divided by the absolute lymphocyte count to compute the NLR. PLR was calculated by dividing the absolute number of platelets by the absolute number of lymphocytes. Finally, the LMR was defined as the absolute lymphocyte count divided by the absolute monocyte count.

2.3. Stromal tumor infiltrating lymphocytes (sTIL) and PD-L1 scoring

PD-L1 expression and infiltration with sTIL were evaluated on pre-treatment tumor tissue samples by two researchers as previously reported [8]. In brief, TIL scoring was done according to the recommendations by the International TILs Working Group [24]. PD-L1 expression was assessed using the SP142 antibody and a score was assigned based on the percentage of the tumor area occupied by PD-L1+ immune cells. A consensus score was determined in case of discrepant results between the researchers.

2.4. Control group

To evaluate whether the peripheral immune markers were associated with the IBC phenotype or with a more advanced stage at diagnosis in IBC, we included a cohort of early stage breast cancer patients (eBC) ($n = 108$) and a cohort of locally advanced breast cancer patients (LABC) ($n = 74$). Furthermore, we also compared metastatic IBC disease ($n = 34$) with a control group of metastatic nIBC patients (mBC) ($n = 41$). This comparison between IBC cases and the nIBC control group was done using the pre-therapeutic blood-based biomarker values, and thus unaffected by later (neo) adjuvant therapy. Using the cancer registry, we retrospectively identified control patients who were consecutively diagnosed and treated between January 1, 2006 and December 31, 2017 at the Antwerp University Hospital. This cohort was randomly sampled in this timeframe to match the same period in which most IBC cases were diagnosed. All patients received adequate local and systemic treatment after a pathologically confirmed diagnosis. Exclusion criteria included IBC disease, previous breast cancer treatment, the diagnosis of ductal carcinoma in situ or the loss of follow-up.

2.5. Statistical analysis

Data were analyzed using R studio (Version 1.1.463 using the following packages: dplyr, tidyr, survival, survminer and ggplot2) [25] and cases with missing data were maintained in the database but excluded from the statistical analyses on a per test basis. To assess the relationship between the different cohorts, clinicopathological parameters and peripheral biomarkers a Pearson Chi2 test (categorical variables) and Kruskal-Wallis (continuous variables) with a post-hoc Dunn test were used. A multivariate logistic regression model included all significant parameters. We evaluated three survival endpoints: recurrence-free survival (RFS) defined as from the date of diagnosis to the date of cancer recurrence, distant metastasis-free survival (DMFS) that is defined as the interval between the date of diagnosis and distant relapse and overall survival (OS) defined as the interval between pathological diagnosis and death. Survival data were last updated by December 31st, 2018 and patients that were not relapsed or death at the time of analysis were censored at the date of their last follow-up visit. Survival curves were calculated with Kaplan-Meier estimates and compared

using the log-rank test. A multivariate cox proportional hazard model was used to evaluate the effects of all significant clinicopathological variables factors on survival. *P*-values were calculated two-sided and considered statistically significant when <0.05 .

3. Results

3.1. Study population

The baseline patient characteristics are summarized in [Table 1](#). Most IBC patients presented with nodal disease ($n = 121/127$) and more than a quarter of the patients ($n = 34/127$) had metastatic disease at the moment of diagnosis. Most patients without metastatic disease underwent a mastectomy after antracyclin-taxane based NACT and pCR was achieved by 25.6% ($n = 21/82$) of patients. Compared to the cohort of LABC patients, non-metastatic IBC patients where more often hormone receptor (HR) negative ($P = .02$), HER2-positive ($P = .005$) and less differentiated ($P < .001$) ([Table S1](#)).

3.1.1. Blood-based biomarkers in the different cohorts

The median NLR, PLR, LMR, CA15.3 and peripheral blood cell counts of the five cohorts are described in [Table 2](#) (and [Table S2](#)). NLR was significantly higher in IBC (Median NLR: 2.70, $P = .006$) compared to early stage breast cancer (eBC) (Median NLR: 2.14), while there was no significant difference between the IBC and the non-inflammatory LABC cohort (Median NLR: 2.44, $P = 0.428$, [Fig. 1A](#)). Patients with metastatic disease, both in patients with IBC (Median NLR: 2.89, $P = .003$) and nIBC disease (Median NLR: 3.49,

$P < .001$), had an elevated NLR compared to early stage BC, but the NLR was not significantly higher in mIBC patients compared to metastatic nIBC patients ($P = .75$, [Fig. 1A](#)). The PLR was higher in patients with metastatic disease (mIBC: Median PLR = 183, $P = .001$; mBC: Median PLR = 160, $P = .044$) compared to eBC, but again there was no difference between mIBC and mBC ($P = .86$, [Fig. 1B](#)). Interestingly, the number of lymphocytes between the three cohorts was comparable. The LMR was higher in the nIBC LABC stage (Median LMR: 4.56) in contrast to IBC (Median LMR: 3.29, $P < .001$), eBC (Median LMR: 3.67, $P = .010$) and mIBC (Median LMR: 3.41, $P = .002$, [Fig. 1C](#)). The number of monocytes in nIBC LABC was also significantly lower compared to IBC ($P = .001$) and eBC disease ($P = .002$). Finally, the CA15.3 was especially high in patients with metastatic disease compared to all other stages ([Fig. 1D](#)).

3.1.2. Clinicopathological parameters and blood-based biomarkers

After pooling all cohorts, we performed a univariate analysis of all clinicopathological parameters to investigate the impact on the blood-based biomarkers ([Table 3](#) & [Table S3](#)) and peripheral blood cell count ([Table S4](#)). All significant parameters were then fitted in a logistic regression model, after which only metastatic disease was associated with a high NLR (OR: 1.95, 1.09–3.55; $P = .03$) and PLR (OR: 2.0, 1.12–3.63; $P = .02$). Interestingly, LMR correlated with younger age in both univariate and multivariate analysis (OR: 0.98, 0.96–1; $P = .01$), unlike IBC disease or cM-stage.

We also looked at the association of the clinicopathological parameters with blood-based biomarkers in the group of IBC patients only ([Table S5](#)) and assessed the relationship between the peripheral immune cells and the tumor micro-environment by

Table 1
Baseline categorical parameters of patients in the five patient cohorts: IBC (inflammatory breast cancer), mIBC (metastatic inflammatory breast cancer) LABC (locally advanced non-inflammatory breast cancer), mBC (metastatic non-inflammatory breast cancer) and eBC (early stage non-inflammatory breast cancer). ER: Estrogen receptor, PR: Progesterone receptor, HR: hormone receptor.

		Non-metastatic disease			Metastatic disease	
		IBC (n = 93)	LABC (n = 74)	eBC (n = 108)	mIBC (n = 34)	mBC (n = 41)
cT-stage	1	0	4	62	0	4
	2	0	20	46	0	6
	3	0	32	0	0	10
	4	93	18	0	34	21
cN-stage	0	5	2	88	1	3
	1	33	25	20	13	15
	2	35	34	0	12	8
	3	20	12	0	8	15
cM-stage	0	93	74	108	0	0
	1	0	0	0	34	41
Pathological type	Ductal	89	61	95	34	33
	Lobular	2	8	11	0	4
	Mixed/other	2	5	2	0	4
Differentiation	Grade 1	2	12	24	0	5
	Grade 2	24	26	41	8	8
	Grade 3	63	24	40	21	10
ER status	Negative	49	22	21	11	10
	Positive	44	52	87	23	31
PR status	Negative	62	30	32	17	18
	Positive	31	44	76	17	23
HER2 status	Negative	50	56	83	19	32
	Positive	43	18	25	15	9
Molecular subtype	HR+HER2-	24	41	71	17	24
	HR+HER2+	24	11	16	6	7
	HR-HER2+	19	7	9	9	2
	HR-HER2-	26	15	12	2	8
Stage	I	0	0	51	0	0
	Ila	0	0	47	0	0
	Ilb	0	0	10	0	0
	IIla	0	45	0	0	0
	IIlb	73	17	0	0	0
	IIlc	20	12	0	0	0
	IV	0	0	0	34	41

Table 2

Baseline continuous parameters in the five patient cohorts: IBC (inflammatory breast cancer), LABC (locally advanced non-inflammatory breast cancer), eBC (early stage non-inflammatory breast cancer), mlBC (metastatic inflammatory breast cancer) and mBC (metastatic non-inflammatory breast cancer). Values are presented as a median (and range). NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, LMR: Lymphocyte-monocyte ratio. A filled column indicates the group with IBC disease. *: IBC = 92 & mlBC = 33; **: IBC = 92; ***: IBC = 92, mlBC = 33 & eBC = 96.

	Non-metastatic disease			Metastatic disease	
	IBC (n = 93)	LABC (n = 74)	eBC (n = 108)	mlBC (n = 34)	mBC (n = 41)
NLR	2.7 (0.852–9.56)	2.44 (0.795–20.1)	2.14 (0.573–29.9)	2.89 (1.38–8.21)	3.49 (1.07–9.25)
PLR*	150 (72.7–497)	145 (74.6–570)	136 (59.7–704)	183 (75.2–371)	160 (13.9–484)
LMR**	3.29 (0.935–9.55)	4.56 (1–10.6)	3.67 (1.28–11.9)	3.41 (1.37–6.08)	3.61 (1.73–21.4)
CA15.3***	22.2 (7.7–467)	18.6 (6.8–133)	19.7 (7.2–51)	63 (10.1–1850)	43.8 (4.6–3000)
Neutrophils	4.86 (2.42–13.2)	4.32 (1.67–16.1)	4.28 (1.47–13.4)	4.76 (2.45–7.72)	5.76 (2.45–18)
Monocytes**	0.49 (0.2–1.21)	0.39 (0.09–0.87)	0.5 (0.13–1.02)	0.465 (0.31–1.02)	0.47 (0.26–1.12)
Lymphocytes	1.72 (0.58–3.46)	1.92 (0.64–3.73)	1.82 (0.45–4.51)	1.58 (0.84–3.8)	1.57 (0.95–16.9)
Platelets*	275 (141–483)	271 (149–415)	248 (149–389)	294 (129–563)	281 (196–723)

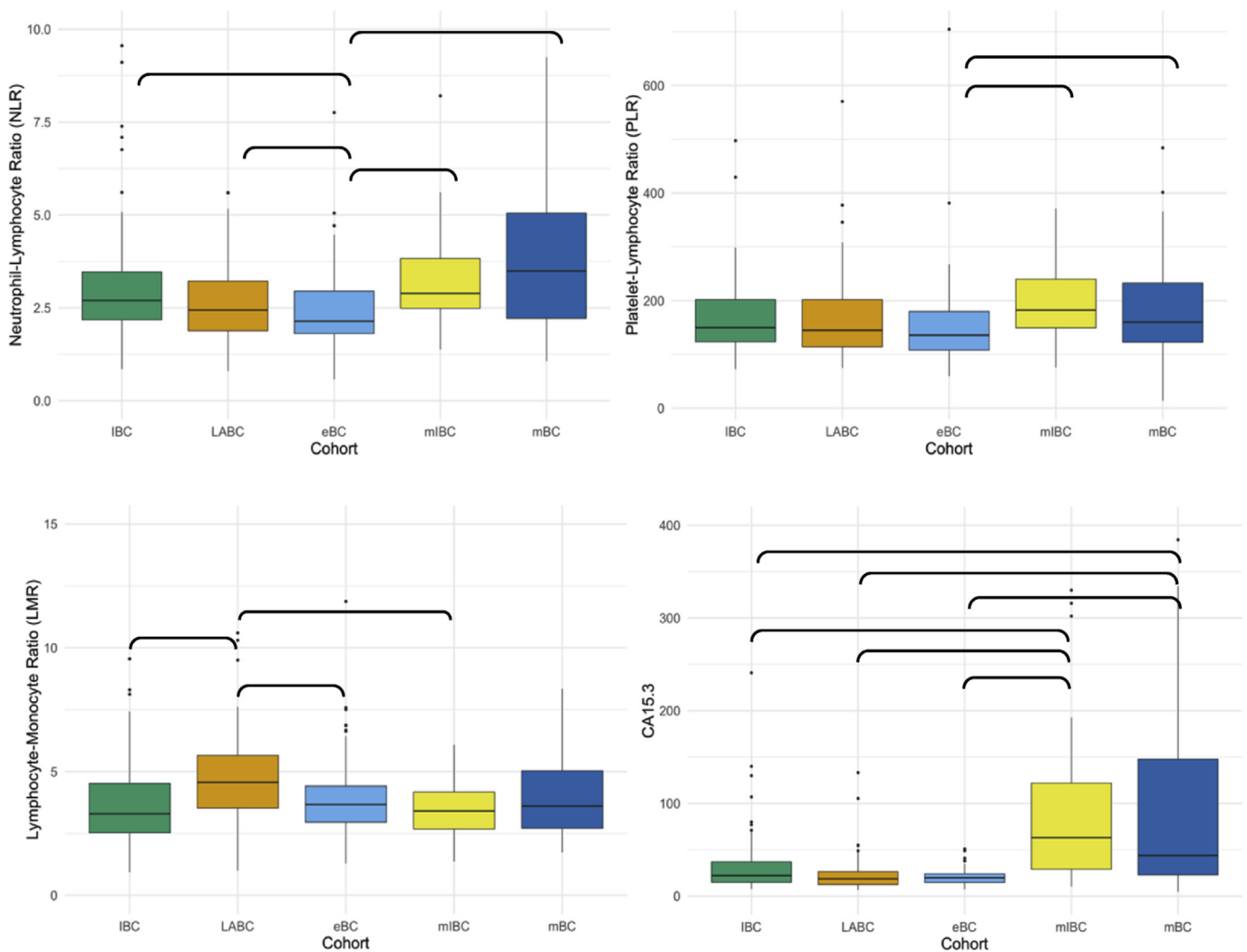


Fig. 1. Boxplot graphs of the median, interquartile range in the box and whiskers indicating > 1.5 and < 1.5 times the interquartile range above and below either end of the box per cohort. (A) NLR: Neutrophil-lymphocyte ratio, (B) PLR: Platelet-lymphocyte ratio (C) LMR: Lymphocyte-monocyte ratio and (D) CA15.3. Brackets indicate a statistically significant difference ($P < .05$).

examining the number of sTIL and PD-L1 expression on the infiltrating immune cells. However, no association was found with the biomarkers (Table S6) nor the peripheral blood cell counts in IBC. Finally, no marker could predict pCR after NACT (Table S6) in IBC.

3.1.3. Blood-based biomarkers and survival outcome

In the total IBC patient population, patients with a high NLR (>4.0) had a worse outcome compared to a lower NLR (≤ 4.0 ; HR: 0.51; 95% CI: 0.30–0.87; $P = .01$). Using this cut-off, based on the literature and representing the 8th decile, median survival was 5.54

Table 3
Association between clinicopathological parameters and NLR (A) or PLR (B) after pooling of all cohorts. Univariate analysis was done with Kruskal-Wallis test (Cont p-value) or after dichotomization with a chi-square test (Bin p-value). NLR high and low categories were based on the median value. Significant parameters were included in a logistic regression model (OR, 95% Confidence interval and P-value). ER: Estrogen receptor, PR: Progesteron receptor, HR: hormone receptor & OR: Odds Ratio, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio. Bold type indicates a statistically significant difference ($P < .05$).

Clinicopathological parameters		A. Neutrophil-lymphocyte ratio (NLR)						B. Platelet-lymphocyte ratio (PLR)					
				Univariate analysis		Multivariate analysis				Univariate analysis		Multivariate analysis	
		NLR high	NLR low	Binary p-value	Continuous p-value	OR (95% CI)	p-value	PLR low	PLR high	Binary p-value	Continuous p-value	OR (95% CI)	p-value
Age					.62						.88		
cT-stage	1	47	23	<.001	<.001			44	26	.05	.002		
	2	42	30			1.32 (0.65–2.69)	.44	37	35			1.56 (0.78–3.13)	.21
	3	21	21			1.47 (0.59–3.72)	.41	22	20			1.29 (0.51–3.24)	.59
	4	65	101			2.14 (0.84–5.61)	.11	71	93			1.58 (0.62–4.07)	.34
cN-stage	0	64	35	.003	0.002	0.73 (0.31–1.7)	.46	57	42	.11	.004	0.72 (0.31–1.69)	.46
	1	51	55			0.86 (0.43–1.7)	.66	53	53			0.68 (0.34–1.35)	.28
	2	37	52			1.12 (0.55–2.28)	.76	43	45			0.7 (0.34–1.43)	.33
	3	22	33					20	34				
cM-stage	0	151	124	<.001	<.001			149	125	.002	<.001		
	1	24	51			1.95 (1.09–3.55)	.03	25	49			2.0 (1.12–3.63)	.02
Pathological type	Ductal	155	157	.61	.35			154	156	.57	.13		
	Lobular	15	10					13	12				
	Mixed/other	5	8					7	6				
Differentiation	Grade 1	28	15	.10	.06			27	16	.12	0.27		
	Grade 2	57	50					56	50				
	Grade 3	74	84					72	85				
ER status	Neg	53	60	.49	.51			58	54	.73	.57		
	Pos	122	115					116	120				
PR status	Neg	77	82	.67	.76			77	81	.75	.57		
	Pos	98	93					97	93				
HER2 status	Neg	115	125	.30	.26			115	125	.30	.22		
	Pos	60	50					59	49				
Molecular subtype	HR + HER2-	90	87	.29	.40			84	93	.70	.55		
	HR + HER2+	34	30					34	29				
	HR-HER2+	26	20					25	20				
	HR-HER2-	25	38					31	32				
Group	IBC	50	77	.004	.005			54	71	.07	.01		
	nIBC	125	98			0.96 (0.44–2.13)	0.92	120	103			0.85 (0.39–1.84)	.67

years (95% CI: 3.71–14.93) in the low NLR group and 2.17 years (95% CI: 1.57 – NA) in the high NLR group (Fig. 2A). When using the median of 2.738 as a cut-off between high and low NLR, the survival difference reached only borderline significance (HR: 0.65; 95% CI: 0.41–1.03; $P = .07$) (Table S7). Furthermore, also PLR and CA15.3 were prognostic blood-based biomarkers in IBC, using the 8th decile as a cut-off (Fig. 2B and C). Subsequently, a cox proportional hazard analysis with all factors significantly associated with overall survival (Table S8) showed that a high NLR remained a significant predictor of worse overall outcome, besides the presence of metastatic disease in IBC patients (HR: 0.49; 95% CI: 0.24–1.00; $P = .05$, Table 4 and Table S8).

The association with RFS, DMFS and OS in the patients without metastatic disease at diagnosis was assessed for all clinicopathological parameters and blood-based biomarkers (Table S9). Both RFS and DMFS were only associated with the PLR (Fig. 3) and HR status. HR positive patients had a longer RFS (HR: 0.46; 95% CI: 0.26–0.82; $P = .009$) and DMFS (HR: 0.51; 95% CI: 0.28–0.92; $P = .024$). A lower PLR (≤ 210) was correlated with better RFS and

DMFS (HR: 0.51; 95% CI: 0.28–0.93; $P = .03$ and 0.47; 95% CI: 0.26–0.88; $P = .018$ respectively).

Finally, in IBC patients without distant disease a low NLR was also associated with a better OS (HR: 0.50, 95%CI: 0.25–1.01, $P = .049$). Median survival was 3.55 years (95% CI: 2.45 – NA) in the NLR high group and 13.56 years (95% CI: 5.54 – NA) in the NLR low group. In a multivariate model including cN-stage ($P = NS$) and HR status (HR: 0.42, 95%CI: 0.22–0.79, $P = .007$), the NLR remained borderline significant (HR: 0.51, 95%CI: 0.26–1.02, $P = .060$).

4. Discussion

In this study, we investigated commonly used peripheral inflammation markers (NLR, PLR and LMR) in a cohort of 127 IBC patients (of which 34 patients had metastatic disease). First, we demonstrated that the NLR is elevated in both IBC and metastatic nIBC compared to early stage nIBC, while there was no significant difference between the IBC and nIBC LABC, and the mIBC and mBC cohort. The PLR was higher in patients with distant disease, but

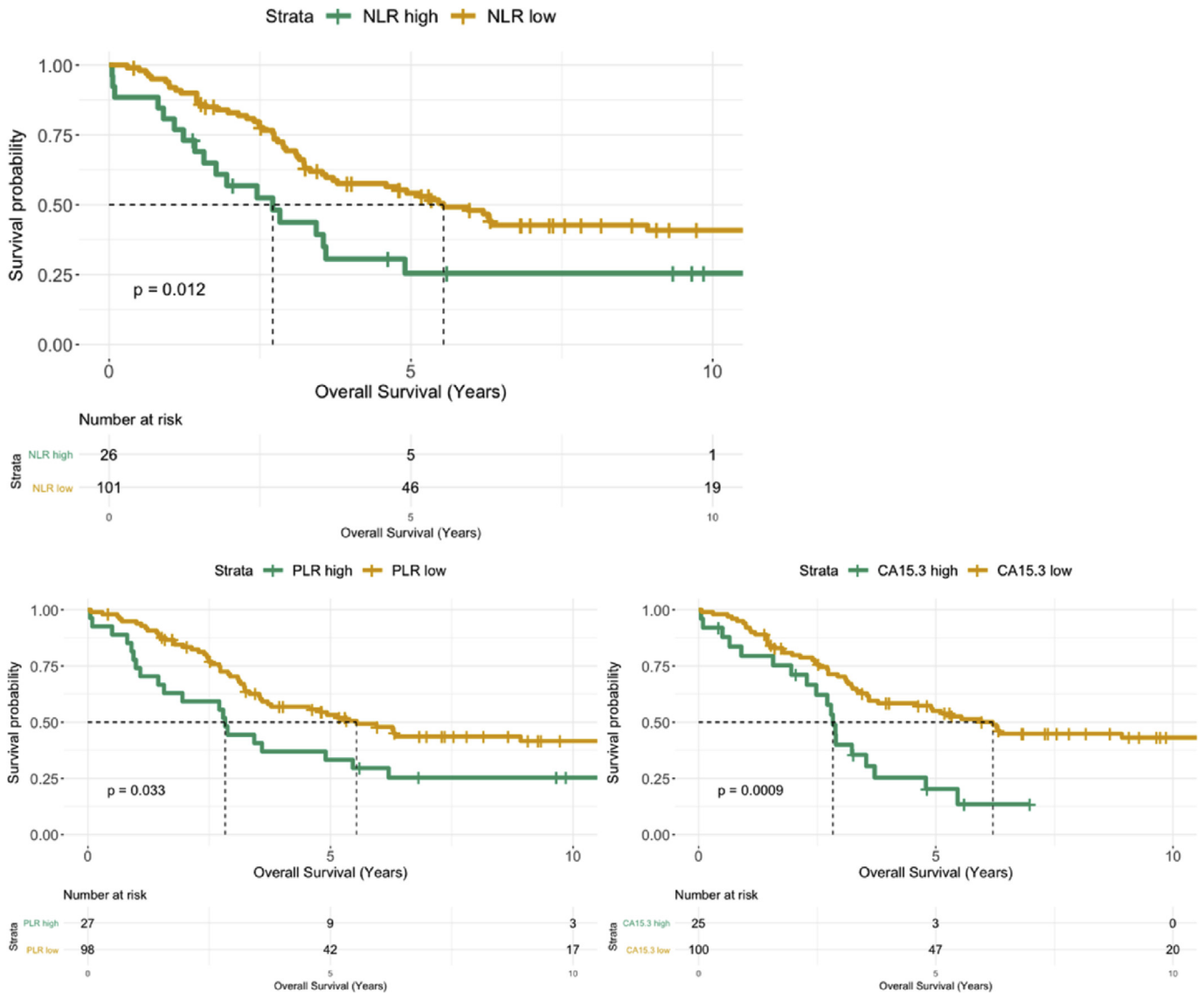


Fig. 2. A. Kaplan-Meier plot comparing patients with a low NLR (≤ 4.0 , $n = 101$) versus high NLR (>4.0 , $n = 26$). Median survival: 5.54 years (95% CI: 3.71 – 14.93) versus 2.17 (95% CI: 1.57 – NA). HR: 0.51; 95% CI: 0.30 – 0.87; $P = .01$. B. Kaplan-Meier plot comparing patients with a low PLR (≤ 220 , $n = 98$) versus high PLR (>220 , $n = 27$). Median survival: 5.54 years (95% CI: 3.59 – NA) versus 2.83 (95% CI: 1.57 – 6.20). HR: 0.57; 95% CI: 0.34 – 0.96; $P = .03$. C. Kaplan-Meier plot comparing patients with a low CA15.3 (≤ 66.0) versus high CA15.3 (>66.0). Median survival: 6.20 years (95% CI: 3.78 – NA) versus 2.83 (95% CI: 2.48 – 4.79). HR: 0.41; 95% CI: 0.24 – 0.71; $P < .001$. OS: overall survival; NLR: neutrophil-lymphocyte ratio, HR: Hazard ratio; PLR: platelet-lymphocyte ratio.

Table 4

Cox regression analysis for overall survival in IBC with all biomarkers: NLR = Neutrophil-Lymphocyte Ratio; PLR = Platelet-Lymphocyte ratio; HR = hazard ratio; CI = confidence interval. Bold type indicates a statistically significant difference ($P < .05$).

	HR	95%CI	p-value
NLR (low vs. high)	0.49	0.24–1.00	.05
PLR (low vs. high)	0.98	0.49–1.95	.95
CA15.3 (low vs. high)	0.73	0.38–1.38	.33
cN-stage (cN0/N1 vs. cN2/N3)	0.61	0.36–1.04	.07
cM-stage (cM1 vs. cM0)	3.31	1.96–5.58	<.001
Age	1.01	0.99–1.03	.17

again there was no difference between IBC and nIBC. Interestingly, the number of lymphocytes between all cohorts was comparable, indicating that the higher NLR and PLR can be originated by increased numbers of peripheral neutrophils and platelets.

Furthermore, we have built a logistic regression model (after pooling the three cohorts) showing that both NLR and PLR were associated with distant disease but not with the IBC phenotype. Therefore, the elicited peripheral immune response in IBC in both local and metastatic disease seems similar to the response in nIBC. However, even though the number of immune cells is comparable, the function of these leukocytes in IBC might be impaired. Mego et al. showed that IBC patients with detectable circulating tumor cells (CTCs) had lower percentages of T-helper cells as well as NK cells, accompanied by a higher percentage of T-reg cells in the peripheral blood [26] and a compromised function of dendritic cells [27]. By looking at the number of sTIL and the PD-L1 expression on the infiltrating immune cells, we aimed to assess the relationship between peripheral immune cells and the local tumor micro-environment. Some authors reported reduced numbers of neutrophils in patients with more sTIL, although no association between NLR and sTIL was demonstrated [28]. Similarly, an association

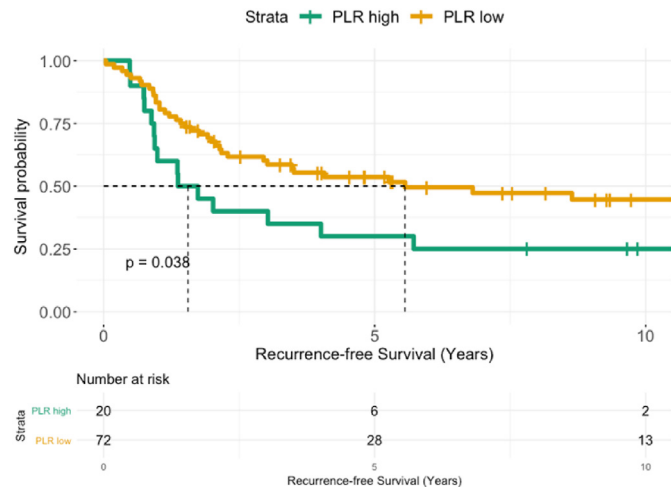


Fig. 3. Kaplan-Meier plot comparing recurrence free survival (RFS) between patients with a low PLR (≤ 210 , $n = 72$) versus high PLR (>210 , $n = 20$). Median survival: 5.56 years (95% CI: 2.95 – NA) versus 1.55 (95% CI: 0.94 – NA). HR: 0.51; 95% CI: 0.28 – 0.93; $P = .03$.

between peripheral immune cells or biomarkers and sTIL was not identified in our IBC cohort. Systemic immune cell counts might be affected by many systemic factors (e.g. infection, strenuous exercise, emotional stress, treatment side effects, etc.). Nevertheless, it seems that they are not reflected by local infiltration with sTIL or the expression of PDL1 on the infiltrating immune cells.

Although we could not establish a relationship between peripheral inflammatory indices and the local immune response, we showed that an elevated NLR (>4.0) had a negative effect on OS (HR: 0.49; 95% CI: 0.24–1.00; $P = .05$) and that a high PLR (>210) was associated with shorter RFS and DMFS (HR: 0.51; 95% CI: 0.28–0.93; $P = .03$ and 0.47; 95% CI: 0.26–0.88; $P = .018$ respectively). It is interesting that a high PLR, which is associated with metastatic disease, predicts relapse in a cohort without distant disease. In a meta-analysis, including 8563 breast cancer patients, a significant prognostic effect for NLR on both OS and RFS was found [12]. Other researchers also demonstrated a significant association between a low NLR and better outcome in breast cancer [13–16]. PLR [17] and LMR [18] are the other peripheral blood-derived prognostic inflammation markers that showed prognostic significance in breast cancer. However, it remains unclear which marker is superior [19,20]. Furthermore, different studies assessed the role of these markers in the context of breast cancer molecular subtype. Noh et al. showed that an increased NLR was an adverse prognostic marker in luminal A breast cancer [29], but most authors suggested that the prognostic effect of NLR and PLR is higher in the group of HR negative patients [30,31]. This might indicate the importance of a good lymphocytic response in the more aggressive, proliferative subtypes although the association between NLR, PLR and sTIL remains unclear. The negative prognostic effect of a high NLR and PLR could reflect a disbalance between the antitumor response of lymphocytes and increased peripheral neutrophils or platelets. Experimental data suggests that platelets may increase metastatic potential by the formation of platelet clumps around neoplastic cells or by the secretion of a significant number of growth factors, like platelet-derived growth factor (PDGF), that enhance cancer activities [32]. Neutrophils can promote tumor development by inducing a number of pro-cancerogenic factors such as vascular endothelial growth factor (VEGF), neutrophil elastase and matrix metalloprotein 9 (MMP9). Besides, neutrophils could suppress the cytotoxic activity of lymphocytes, natural killer cells, and activated T-cells thereby counteracting an anti-tumor immune response. Lastly, the metastatic process might also be enhanced by

neutrophil-derived leukotrienes that aid the colonization of distant tissues [33].

The LMR appeared to be increased in LABC compared to eBC and IBC, however multivariate analysis only showed an association with younger age and an increase of monocytes with ageing has been described [34]. Furthermore, we could not demonstrate any prognostic significance of the LMR in IBC. This finding is in line with the study of Husnzo et al. [30]. In the study of Peng et al. the LMR was similarly associated with age. However, they found that a low LMR was a favorable factor for response to NACT [35]. Moreover, other researchers reported that a lower pretreatment LMR was associated with more CTCs [36] and a poor prognostic factor for patients with LABC [18] or mBC [36], findings that we could not confirm. Beside the LMR, a low PLR [21], a low NLR [22], or a combination of both [37,38] were related to chemosensitivity in breast cancer in several studies. In our study the NLR, the PLR or a combination of markers were unable to predict pCR after NACT in IBC, possibly explained by the number of HR+ patients in our IBC cohort.

Rubio et al. could not demonstrate a significant association between NLR and survival in mBC, concluding that the prognostic effect of NLR is probably derived from the association with other clinicopathological factors [39]. However, our data are consistent with the growing body of evidence suggesting that the NLR is a good, independent inflammatory prognostic biomarker, especially for OS [40]. Nevertheless, it remains unclear how the NLR and the other markers that are indeed easy to measure and quickly available, can be used in the daily practice (for risk prediction). Among different breast cancer subtypes and stages, different cut-off values and different methods to calculate these values make it very difficult to compare studies and might partially explain some conflicting results. In this study we used a cut-off value of 4 (the 8th decile) for the NLR based on a number of large trials [11,15,16], but some researchers used a value of 2 based on a receiver operating characteristic (ROC) analysis to define the best cut-off for survival outcome [40,41]. Interestingly, in our IBC cohort both the NLR (for OS) and PLR (for DFS) remained significant when using different cut-offs determined by a ROC analysis (Table S7). Furthermore, an optimal cut-off value for OS might not be the best to predict chemosensitivity [42]. The parameters are also vulnerable to different systemic conditions (e.g. infection, exercise, stress, etc.) and therefore prone to changes in time. Interestingly, this evolution of the NLR also seems to be important. Patel et al. demonstrated that NLR elevation could persist until 1 year after the treatment completion and shortened survival in TNBC patients [43].

We acknowledge that this study has some limitations. It is a retrospective study with a double-center design and systemic treatment strategies have changed during this long study interval. Additionally, the sample size was not large enough to do molecular subtype specific analysis, although an IBC cohort of 127 patients should be representative for this rare form of breast cancer. However, we managed to explore some peripheral immune parameters in IBC over a long period of time (1997–2017) and showed that especially the NLR was a robust prognostic marker for survival.

5. Conclusion

There is no difference in peripheral immune cell counts and blood biomarkers between IBC and nIBC in both local and metastatic disease. It thus seems that both IBC and nIBC elicit the same peripheral immune response and the PLR and NLR should be seen as markers of extensive disease. However, patients with a high NLR (>4.0) have a worse outcome in IBC, and a high PLR is associated with an adverse RFS independent of disease stage or molecular subtype. Further research is necessary in order to better understand the peripheral immune response to (inflammatory) breast

cancer and its prognostic significance.

Funding

This work was supported by a PhD grant for CVB, from the Research Foundation - Flanders (FWO) [grant number 1189617N].

Declaration of competing interest

All authors have declared no conflicts of interest.

Ethical approval

This study is conducted in accordance with the ethical standards of the University of Antwerp and received ethical approval from the ethics committee (File number: 16/33/338).

Abbreviations

CTC	Circulating tumor cells
DMFS	distant metastasis-free survival
eBC	early stage breast cancer
ER	Estrogen receptor
FISH	fluorescence in-situ hybridization
HR	Hormone receptor
IBC	Inflammatory breast cancer
LABC	locally advanced breast cancer
LMR	lymphocyte-monocyte ratio
mBC	metastatic breast cancer
NACT	neoadjuvant chemotherapy
nIBC	non-inflammatory breast cancer
NLR	neutrophil-to-lymphocyte ratio
OS	overall survival
pCR	complete pathological response
PD-L1	Programmed Death Ligand 1
PLR	platelet-lymphocyte ratio
PR	Progesteron receptor
RFS	recurrence-free survival
ROC	receiver operating characteristic
STIL	stromal tumor infiltrating lymphocytes

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.breast.2020.08.006>.

References

- [1] Levine PH, Veneroso C. The epidemiology of inflammatory breast cancer. *Semin Oncol* 2008;35(1):11–6.
- [2] Fouad TM, Barrera AMG, Reuben JM, Lucci A, Woodward WA, Stauder MC, et al. Inflammatory breast cancer: a proposed conceptual shift in the UICC-AJCC TNM staging system. *Lancet Oncol* 2017;18(4):e228–32.
- [3] Woodward WA. Inflammatory breast cancer: unique biological and therapeutic considerations. *Lancet Oncol* 2015;16(15):e568–76.
- [4] Rypens C, Marsan M, Van Berckelaer C, Billiet C, Melis K, Lopez SP, et al. Inflammatory breast cancer cells are characterized by abrogated TGFbeta1-dependent cell motility and SMAD3 activity. *Breast Canc Res Treat* 2020.
- [5] Van Laere SJ, Ueno NT, Finetti P, Vermeulen P, Lucci A, Robertson FM, et al. Uncovering the molecular secrets of inflammatory breast cancer biology: an integrated analysis of three distinct affymetrix gene expression datasets. *Clin Canc Res : an official journal of the American Association for Cancer Research* 2013;19(17):4685–96.
- [6] Lim B, Woodward WA, Wang X, Reuben JM, Ueno NT. Inflammatory breast cancer biology: the tumour microenvironment is key. *Nat Rev Canc* 2018;18(8):485–99.
- [7] Bertucci F, Finetti P, Colpaert C, Mamessier E, Parizel M, Dirix L, et al. PDL1 expression in inflammatory breast cancer is frequent and predicts for the pathological response to chemotherapy. *Oncotarget* 2015;6(15):13506–19.
- [8] Van Berckelaer C, Rypens C, van Dam P, Pouillon L, Parizel M, Schats KA, et al. Infiltrating stromal immune cells in inflammatory breast cancer are associated with an improved outcome and increased PD-L1 expression. *Breast Cancer Res* 2019;21(1):28.
- [9] Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* 2018;19(1):40–50.
- [10] Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: big 02-98. *J Clin Oncol* 2013;31(7):860–7.
- [11] Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014;106(6):dju124.
- [12] Ethier JL, Desautels D, Templeton A, Shah PS, Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. *Breast Cancer Res* 2017;19(1):2.
- [13] Templeton AJ, Rodriguez-Lescure A, Ruiz A, Alba E, Calvo L, Ruiz-Borrego M, et al. Prognostic role for the derived neutrophil-to-lymphocyte ratio in early breast cancer: a GEICAM/9906 substudy. *Clin Transl Oncol* 2018;20(12):1548–56.
- [14] Pistelli M, De Lisa M, Ballatore Z, Caramanti M, Pagliacci A, Battelli N, et al. Pre-treatment neutrophil to lymphocyte ratio may be a useful tool in predicting survival in early triple negative breast cancer patients. *BMC Cancer* 2015;15:195.
- [15] Dirican A, Kucukzeybek BB, Alacacioglu A, Kucukzeybek Y, Erten C, Varol U, et al. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? *Int J Clin Oncol* 2015;20(1):70–81.
- [16] Koh CH, Bhoo-Pathy N, Ng KL, Jabir RS, Tan GH, See MH, et al. Utility of pre-treatment neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as prognostic factors in breast cancer. *Br J Canc* 2015;113(1):150–8.
- [17] Zhu Y, Si W, Sun Q, Qin B, Zhao W, Yang J. Platelet-lymphocyte ratio acts as an indicator of poor prognosis in patients with breast cancer. *Oncotarget* 2017;8(1):1023–30.
- [18] Ni XJ, Zhang XL, Ou-Yang QW, Qian GW, Wang L, Chen S, et al. An elevated peripheral blood lymphocyte-to-monocyte ratio predicts favorable response and prognosis in locally advanced breast cancer following neoadjuvant chemotherapy. *PLoS One* 2014;9(11):e111886.
- [19] Cho U, Park HS, Im SY, Yoo CY, Jung JH, Suh YJ, et al. Prognostic value of systemic inflammatory markers and development of a nomogram in breast cancer. *PLoS One* 2018;13(7):e0200936.
- [20] Azab B, Mohammad F, Shah N, Vonfrolio S, Lu W, Kedia S, et al. The value of the pretreatment neutrophil lymphocyte ratio vs. platelet lymphocyte ratio in predicting the long-term survival in colorectal cancer. *Canc Biomarkers* 2014;14(5):303–12.
- [21] Cuello-Lopez J, Fidalgo-Zapata A, Lopez-Agudelo L, Vasquez-Trespalacios E. Platelet-to-lymphocyte ratio as a predictive factor of complete pathologic response to neoadjuvant chemotherapy in breast cancer. *PLoS One* 2018;13(11):e0207224.
- [22] Asano Y, Kashiwagi S, Onoda N, Noda S, Kawajiri H, Takashima T, et al. Predictive value of neutrophil/lymphocyte ratio for efficacy of preoperative chemotherapy in triple-negative breast cancer. *Ann Surg Oncol* 2016;23(4):1104–10.
- [23] Dawood S, Merajver SD, Viens P, Vermeulen PB, Swain SM, Buchholz TA, et al. International expert panel on inflammatory breast cancer: consensus statement for standardized diagnosis and treatment. *Ann Oncol : official journal of the European Society for Medical Oncology/ESMO* 2011;22(3):515–23.
- [24] Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol : official journal of the European Society for Medical Oncology/ESMO* 2015;26(2):259–71.
- [25] RStudio Team. RStudio. Boston, MA: Integrated Development for R. RStudio, Inc.; 2016. URL, <http://www.rstudio.com/>.
- [26] Mego M, Gao H, Cohen EN, Anfossi S, Giordano A, Sanda T, et al. Circulating tumor cells (CTC) are associated with defects in adaptive immunity in patients with inflammatory breast cancer. *J Canc* 2016;7(9):1095–104.
- [27] Mego M, Gao H, Cohen EN, Anfossi S, Giordano A, Tin S, et al. Circulating tumor cells (CTCs) are associated with abnormalities in peripheral blood dendritic cells in patients with inflammatory breast cancer. *Oncotarget* 2017;8(22):35656–68.
- [28] Yoon CI, Park S, Cha YJ, Lee HS, Bae SJ, Cha C, et al. Associations between absolute neutrophil count and lymphocyte-predominant breast cancer. *Breast* 2019.
- [29] Noh H, Eomm M, Han A. Usefulness of pretreatment neutrophil to lymphocyte ratio in predicting disease-specific survival in breast cancer patients. *J Breast Cancer* 2013;16(1):55–9.
- [30] Huszno J, Kolosza Z. Prognostic value of the neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratio in breast cancer patients. *Oncol Lett* 2019;18(6):6275–83.
- [31] Guo W, Lu X, Liu Q, Zhang T, Li P, Qiao W, et al. Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio for breast cancer patients: an updated meta-analysis of 17079 individuals. *Cancer Med* 2019;8(9):

- 4135–48.
- [32] Schlesinger M. Role of platelets and platelet receptors in cancer metastasis. *J Hematol Oncol* 2018;11(1):125.
- [33] Ocana A, Nieto-Jimenez C, Pandiella A, Templeton AJ. Neutrophils in cancer: prognostic role and therapeutic strategies. *Mol Canc* 2017;16(1):137.
- [34] Puissant-Lubrano B, Apoil PA, Guedj K, Congy-Jolivet N, Roubinet F, Guyonnet S, et al. Distinct effect of age, sex, and CMV seropositivity on dendritic cells and monocytes in human blood. *Immunol Cell Biol* 2018;96(1):114–20.
- [35] Peng Y, Chen R, Qu F, Ye Y, Fu Y, Tang Z, et al. Low pretreatment lymphocyte/monocyte ratio is associated with the better efficacy of neoadjuvant chemotherapy in breast cancer patients. *Canc Biol Ther* 2020;21(2):189–96.
- [36] De Giorgi U, Mego M, Scarpi E, Giordano A, Giuliano M, Valero V, et al. Association between circulating tumor cells and peripheral blood monocytes in metastatic breast cancer. *Ther Adv Med Oncol* 2019;11. 1758835919866065.
- [37] Kim HY, Kim TH, Yoon HK, Lee A. The role of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in predicting neoadjuvant chemotherapy response in breast cancer. *J Breast Cancer* 2019;22(3):425–38.
- [38] Graziano V, Grassadonia A, Iezzi L, Vici P, Pizzuti L, Barba M, et al. Combination of peripheral neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio is predictive of pathological complete response after neoadjuvant chemotherapy in breast cancer patients. *Breast* 2019;44:33–8.
- [39] Ivars Rubio A, Yufera JC, de la Morena P, Fernandez Sanchez A, Navarro Manzano E, Garcia Garre E, et al. Neutrophil-lymphocyte ratio in metastatic breast cancer is not an independent predictor of survival, but depends on other variables. *Sci Rep* 2019;9(1):16979.
- [40] Munoz-Montano W, Cabrera-Galeana P, Alvarado-Miranda A, Villarreal-Garza C, Mohar A, Olvera A, et al. Prognostic value of the pretreatment neutrophil-to-lymphocyte ratio in different phenotypes of locally advanced breast cancer during neoadjuvant systemic treatment. *Clin Breast Canc* 2020.
- [41] Gerratana L, Basile D, Toffoletto B, Bulfoni M, Zago S, Magini A, et al. Biologically driven cut-off definition of lymphocyte ratios in metastatic breast cancer and association with exosomal subpopulations and prognosis. *Sci Rep* 2020;10(1):7010.
- [42] Vano YA, Oudard S, By MA, Tetu P, Thibault C, Aboudagga H, et al. Optimal cut-off for neutrophil-to-lymphocyte ratio: fact or Fantasy? A prospective cohort study in metastatic cancer patients. *PloS One* 2018;13(4):e0195042.
- [43] Patel DA, Xi J, Luo J, Hassan B, Thomas S, Ma CX, et al. Neutrophil-to-lymphocyte ratio as a predictor of survival in patients with triple-negative breast cancer. *Breast Canc Res Treat* 2019;174(2):443–52.