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Clinical Significance of Routine Blood Test-Associated Inflammatory Index in Breast Cancer Patients

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: It is now widely acknowledged that chronic inflammation is closely associated with the process of cancer development. As a simple noninvasive blood-based test, hematological parameters in the routine blood test have been considered as inflammation markers. We aimed to evaluate platelet count (PC), red blood cell distribution width (RDW), white blood cell count (WBC), mean platelet volume (MPV), number of neutrophils/lymphocytes ratio (NLR), and platelet count/lymphocytes ratio (PLR) as surrogate inflammatory markers in breast cancer (BC) patients, and we compared these to those in healthy individuals.

Material/Methods: A retrospective study was conducted in Zhongnan Hospital of Wuhan University from July 2014 to April 2015, including 110 cases of pathologically diagnosed BC patients and 78 cases of healthy females. Retrospective analysis of selected hematological parameters was performed between the 2 groups, as well as assessment of the correlation between these indexes and clinicopathological characteristics of the 110 breast cancer patients.

Results: The mean values of RDW, MPV, NLR, and PLR were significantly higher in BC patients compared to the control group. The level of MPV exhibited positive correlations with lymph node metastasis and the Ki67 proliferation index in preoperative BC patients ($P<0.05$). Logistic regression analysis further showed that MPV was independently associated with the risk of BC lymph node metastasis ($P<0.05$).

Conclusions: Hematological parameters of RDW, MPV, NLR, and PLR can be used as an adjuvant tool for the diagnosis of BC. More importantly, the value of MPV can reflect the Ki67 proliferation index before surgery and identify patients with positive lymph node metastasis.

MeSH Keywords: Breast Neoplasms • Erythrocytes • Mean Platelet Volume

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Background

In 2016, with approximately 246 660 newly diagnosed cases and 40 450 deaths in the United States, breast cancer (BC) is still reported to be the most common cancer in women worldwide [1]. In spite of the remarkable progress made during recent decades, approximate 20% of patients with BC still suffer from recurrence or distant metastasis within 5 years due to diagnosis at advanced stage [2]. Therefore, represented by the liquid biopsy (such as circulating tumor cells, circulating DNA, circulating miRNA, circulating lncRNA, and exosome), some newly developed diagnostic biomarker have been used to screen cancer at an early stage; however, their clinical use is still limited due to their uncertain role and high costs [3,4]. Accumulating evidence demonstrates that cancer-related systemic inflammation plays a significant role in the development and progression of several neoplastic diseases, including BC [5]. Furthermore, along with the physical immune response, several inflammatory molecules in the peripheral blood have been verified to be overexpressed by tumor cells as a response to systemic inflammation, including C reactive protein, white cell counts, neutrophil, platelet count, and platelet-to-lymphocyte ratio [6,7].

Red blood cell distribution width (RDW), reflecting the size variability of circulating erythrocytes, has recently been shown to be associated with chronic inflammation, which is a key determinant of disease progression and survival in various cancers [8–10]. Mean platelet volume (MPV) is a simple measurement of the average size of platelets, and the results are often used to make inferences about platelet production in bone marrow or platelet destruction problems. Elevated MPV usually leads to thrombocytosis in the neoplasms [11]. Other inflammatory markers derived from the routine blood test are the neutrophils/lymphocytes ratio (NLR) and platelet count/lymphocytes ratio (PLR). Neutrophils are reported to have the potential to promote adhesion and seeding of circulating tumor cells to distant organ sites by secreting circulating growth factors such as vascular endothelial growth factor (VEGF) and proteases [12,13]. In contrast, lymphocytes demonstrate the host immune response to malignancy through inducing cancer cell death and inhibiting tumor cell proliferation and migration [14]. In this regard, hematological parameters in the routine blood test have the potential to serve as a marker for reflecting cancer-related inflammation during disease progression. Therefore, we performed this study and retrospectively analysis to assess whether hematological parameters can be used as an adjuvant tool to distinguish individuals with BC patients from healthy individuals. We further examined the correlation between levels of the selected parameters and the clinicopathological characteristics of the 110 BC patients.

Material and Methods

Sample collection and ethics statement

This retrospective study, conducted between July 2014 and April 2015, included 110 BC patients hospitalized at Zhongnan Hospital of Wuhan University. The study included newly diagnosed primary and metastatic BC cases that had not received any treatments likely to affect hematological parameters. The control group was 76 healthy controls randomly selected from the Medical Examination Center who were there for routine checkups during the same period. Data regarding patients' demographics and laboratory values were retrospectively reviewed through hospital medical database records. None of the subjects in the control group had any recorded history of malignancies and were matched to the cases in terms of age (P=0.1028) and sex (female). The clinical characteristics and laboratory data of the study population are summarized in Tables 1 and 2. Ethics

Table 1. Demographics and hematological parameters of the study populations

	Breast cancer patients n=110	Control group n=78	P-value
Age (year)	54.34±12.28	51.54±10.37	0.1028
PC (×10⁹/l)	206.20±52.93	218.50±43.67	0.0934
MPV (fl)	9.11±1.29	7.68±1.73	<0.0001
RDW (%)	13.45±1.14	11.39±0.67	<0.0001
WBC (×10⁹/l)	6.30±2.20	6.396±1.43	0.7251
NLR	2.60±2.47	1.86±0.51	0.0094
PLR	126.40±48.68	111.10±29.50	0.0142

PC – platelets count; MPV – mean platelet volume; RDW – red blood cell distribution width; WBC – white blood cell count; NLR – neutrophils/lymphocytes ratio; PLR – platelets count/lymphocytes ratio.

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Table 2. Relationship between selected hematological parameters and clinical characteristics of BC patients.

Clinical characteristic	No.of case	PC (×10⁹/l)		MPV (fl)		RDW (%)		WBC (×10⁹/l)		NLR		PLR	
		Mean ± SD	P-value	Mean ± SD	P-value	Mean ± SD	P-value	Mean ± SD	P-value	Mean ± SD	P-value	Mean± SD	P-value
Age (year)													
≤50	44	207.50± 54.24	0.82	9.12± 1.20	0.94	13.34± 1.26	0.41	5.98± 1.28	0.22	2.53± 1.41	0.80	131.30± 48.50	0.39
>50	66	205.20± 52.43		9.10± 1.35		13.52± 1.07		6.51± 2.62		2.65± 2.98		123.10± 48.90	
Tumor size (cm)													
≥2	47	207.50± 58.03	0.41	9.05± 1.36	0.90	13.46± 1.15	0.93	6.05± 1.2	0.93	2.12± 0.85	0.54	118.40± 42.90	0.79
<2	28	196.30± 55.42		9.09± 1.18		13.43± 1.22		6.02± 1.48,		2.28± 1.35		121.80± 65.55	
Intrinsic subtype													
Luminal A	48	207.70± 51.75	0.38	9.00± 1.25	0.43	13.51± 1.05	0.18	6.31± 2.93	0.95	2.80± 3.38	0.89	129.80± 41.09	0.27
Luminal B	13	197.80± 37.93		9.12± 1.37		13.08± 0.47		6.44± 1.72		2.50± 2.00		112.50± 41.80	
HER2 overexpressing	7	201.00± 70.70		9.84± 2.21		14.36± 1.99		5.76± 1.77		1.99± 0.74		118.00± 50.52	
Triple negative	17	215.8± 56.32		8.97± 0.74		13.46± 1.60		6.33± 1.44		2.81± 1.25		146.20± 68.44	
Estrogen receptor													
Positive	74	205.00± 51.24	0.79	9.01± 1.20	0.22	13.33± 0.87	0.12	6.37± 2.48	0.59	2.65± 2.89	0.79	123.50± 45.22	0.42
Negative	33	207.90± 53.62		9.43± 1.47		13.71± 1.62		6.12± 1.47		2.50± 1.27		131.80± 56.59	
Progesterone receptor													
Positive	65	197.50± 43.98	0.05	9.19± 1.32	0.52	13.39± 0.93	0.5	6.36± 2.58	0.68	2.73± 3.07	0.50	121.30± 42.92	0.24
Negative	43	217.3± 60.36		9.02± 1.26		13.54± 1.43		6.18± 1.50		2.34± 1.16		132.60± 56.3	
Her-2													
Positive	21	199.30± 48.66	0.42	9.40± 1.65	0.20	13.49± 1.32	0.98	6.22± 1.68	0.87	2.32± 1.62	0.48	114.30± 42.68	0.11
Negative	65	209.80± 52.65		8.99± 1.14		13.50± 1.202		6.32± 2.61		2.80± 2.96		134.10± 49.64	
Lymph node metastasis													
Yes	37	203.20± 62.00	0.85	9.52± 1.40	0.04*	13.38± 1.04	0.95	6.35± 3.17	0.73	2.78± 3.84	0.46	123.40± 48.79	0.98
No	49	205.40± 47.89		8.98± 1.23		13.37± 1.05		6.17± 1.59		2.33± 1.24		123.00± 49.05	
WHO stage													
I	4	235.80± 27.29	0.34	8.60± 0.85	0.50	13.68± 0.81	0.70	5.73± 0.85	0.72	2.35± 0.73	0.96	151.00± 28.14	0.43
II	61	204.40± 53.74		9.05± 1.39		13.26± 0.85		6.27± 2.63		2.72± 3.12		126.60± 46.70	
III	15	192.90± 46.26		9.41± 1.33		13.27± 1.32		6.72± 1.60		2.80± 1.74		117.00± 50.03	
Ki67 proliferation index													
≤15	31	219.20± 38.83	0.07	8.50± 0.91	<0.01*	13.29± 0.73	0.36	6.24± 1.49	0.90	2.27± 1.35	0.37	125.90± 43.40	0.99
>15	76	199.30± 55.61		9.37± 1.35		13.51± 1.29		6.30± 2.46		2.75± 2.84		126.10± 51.27	

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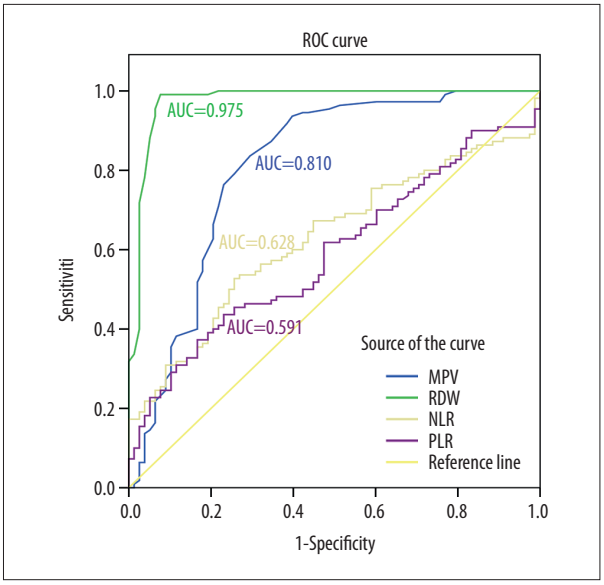

Figure 1. ROC curve analysis of RDW, MPV, NLR, and PLR. RDW – red blood cell distribution width; MPV – mean platelet volume; NLR – neutrophils/lymphocytes ratio; PLR – platelets count/lymphocytes ratio.

approval for the use of human subjects was obtained from the Ethics Committee of the Zhongnan Hospital of Wuhan University.

Blood assessment

Blood values had been taken into consideration at the time of diagnosis before administration of any treatment when patients were admitted to the hospital. Venous blood specimens were drawn into sterile standard tubes containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant and evaluated within 1 h after venipuncture using a Beckman Coulter UniCel DxH800 hematology analyzer. PC, RDW, WBC, MPV, NLR, and PLR values were then obtained directly from the routine blood tests through the medical database.

Statistical analysis

All data analyses were performed using SPSS version 17.0 (SPSS, Inc. Chicago, IL, USA) and data are presented as mean ± standard deviation (SD). For comparisons, the *t* test (2-tailed), one-way analyses of variance (ANOVA), and Mann-Whitney U test were performed based on the normality of the distribution as checked by the Shapiro-Wilk test. Receiver-operating characteristics (ROC) curve analysis was further performed to identify optimum cut-off values of selected hematological parameters. Logistic regression analysis was carried out to check the correlation between MPV value and the risk of BC lymph node metastasis. P value less than 0.05 was considered statistically significant.

Results

Comparisons between BC group and control group

A total of 110 BC patients with newly confirmed BC and 78 healthy individuals were enrolled into this retrospective study. The laboratory blood parameters of BC patients and control group are shown in Table 1. The levels of RDW, MPV, NLR, and PLR in the breast cancer group were significantly higher than in the control group ($P<0.05$), but age distribution, PC, and WBC were not significantly different ($P>0.05$) (Table 1). ROC curve analysis showed that the AUCs of RDW, MPV, NLR, and PLR were 0.975, 0.810, 0.628, and 0.591, respectively (Figure 1). Specifically, the sensitivity of RDW was 99.09% (CI: 95.04% to 99.98%) and its specificity was 92.31% (84.01% to 97.12%) when the cut-off value was 12.25%, which exhibited the best differential diagnosis potential and could be used as an adjuvant tool for BC screening.

Subgroups analysis of BC patients

Subgroup analyses based on age, tumor size, estrogen receptor, progesterone receptor, her-2, lymph node metastasis, WHO stage, and Ki-67 proliferation index were also conducted to analyze the correlation between selected hematological parameters and the clinical characteristics of BC patients. As shown in Table 2, the MPV value in the lymph node metastasis group was significantly higher than in the group without lymph node metastasis ($P=0.04$), and MPV in patients with Ki-67>15% is significantly higher than in those with Ki-67≤15 ($P=0.0015$), but there was no significant difference in PC, RDW, WBC, NLR, and PLR in each subgroup ($P>0.05$). Multivariate logistic regression analysis was further performed to verify whether the MPV value was independently correlated with breast cancer metastasis status. ER, PR, HER-2, RDW, MPV, NLR, and PLR were considered as independent variables. Measurement data were divided according to quartile level of the selected parameters (Table 3). The results demonstrated that MPV was an independent risk factor (OR=0.164, p=0.028) for lymph node metastasis in BC patients (Table 4).

Discussion

Breast cancer is still the most common cancer in women and ranks as the second cause of cancer-related deaths in the world [1]. Therefore, early detection and control of lymph node metastasis has become an important part of breast cancer prevention and treatment.

Studies have shown that long-standing inflammation is closely related to the occurrence and development of tumors [15,16]. Inflammatory breast cancer is a particular type of breast cancer

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Table 3. Value assignments of RDW, MPV, NLR and PLR.

	Value assignments			
	1	2	3	4
RDW	<12.70	12.70–	13.20–	>13.70
MPV	<8.30	8.30–	8.90–	>9.73
NLR	<1.65	1.65–	2.10–	>2.70
PLR	<91.36	91.36–	114.43–	>156.63

RDW – red blood cell distribution width; MPV – mean platelet volume; NLR – neutrophils/lymphocytes ratio; PLR – platelets count/lymphocytes ratio.

Table 4. Multivariate logistic regression analysis of risk factors for lymph node metastasis of BC patients.

Factor	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)
ER	–0.632	0.852	0.550	1	0.458	0.531	0.100~2.826
PR	0.811	0.817	0.984	1	0.321	2.249	0.453~11.157
HER-2	–0.781	0.699	1.247	1	0.264	0.458	0.116~1.803
RDW	0.377	0.776	0.236	1	0.627	1.458	0.318~6.681
MPV	–1.809	0.823	4.830	1	0.028*	0.164	0.033~0.822
NLR	1.310	0.929	1.990	1	0.158	3.706	0.600~22.880
PLR	–1.001	0.963	1.080	1	0.299	0.367	0.056~2.428
Constant	0.882	0.846	1.087	1	0.297	2.416	

* Indicates statistical significance. ER – estrogen receptor; PR – progesterone receptor; HER-2 – human epidermal growth factor receptor-2; RDW – red blood cell distribution width; MPV – mean platelet volume; NLR – neutrophils/lymphocytes ratio; PLR – platelets count/lymphocytes ratio.

which does not present as a lump, sometimes resulting in a delay in diagnosis [17]. Therefore, it should be a primary goal of modern medicine to overcome the diagnostic problems and limitations for cancer patients. Hematological parameters in the noninvasive routine blood test have long been considered as markers for systemic inflammatory response [18,19]. WBC and MPV are routinely measured hematological parameters and have been reported to be higher in patients with malignancies of the pancreas and liver in comparison with healthy controls [20]. Recently, more attention has been focused on RDW, NLR, and PLR. Seretis et al. [21] revealed that elevated RDW may indicate breast cancer with bone marrow infiltration, which may result in erythroid hematopoietic stem cell proliferation defect whereby a large volume of immature red blood cells is released into the peripheral blood in advance, which results in increased red blood cell size heterogeneity and RDW [8]. Some investigators also demonstrated that higher RDW levels might reflect underlying chronic inflammation, which can result in higher cardiovascular risk [22]. On the other hand, breast cancer-induced anemia can cause secondary elevated RDW [22]. The diagnostic value of MPV, NLR, and PLR in distinguishing gastric cancer patients and healthy people has been reported [23]. MPV is a platelet volume index directly reflecting platelet function state [24]. Our results indicate that, compared to healthy individuals, MPV values in breast cancer patients were also significantly increased, which is consistent with previous research conducted in gastric cancer [25]. Other inflammatory markers, such as PLR and NLR, has been proposed as reliable clinical diagnostic criteria in various types of cancer [26–29], and this was further verified in this retrospective study.

Conclusions

The routine blood test is the most accessible and fundamental examination, which has long been proposed as an essential assistant tool for disease diagnosis. Our results show that RDW, MPV, NLR, and PLR can be effective in distinguishing breast cancer patients from healthy individuals. Among them, RDW is the most effective indicator, thus making it possible to assist physicians in the early diagnosis of BC in combination with other tests. Furthermore, MPV is a convenient and inexpensive laboratory index that has value in evaluating axillary lymph node metastasis and Ki-67 proliferation index in breast cancer, and could serve as a new biomarker for prognosis evaluation of BC patients. However, our study has limitations, including

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its small sample size and single-center design, and our results need to be verified by large-scale clinical studies with follow-up study. However, to the best of our knowledge, this study is the first to provide comprehensive insights into hematological parameters of routine blood testing in BC patients.

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Conflicts of interest

None.

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