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Original Article

Expression and prognostic value of ratios of platelet lymphocyte, neutrophil lymphocyte and lymphocyte monocyte in breast cancer patients

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Abstract: Background: The ratios of systemic inflammatory cells, neutrophil lymphocyte (NLR), platelet lymphocyte (PLR) and lymphocyte monocyte (LMR) can be used as prognostic indicators of breast cancer (BC). The purpose of this study was to explore the value of inflammatory markers in predicting the pathological reaction and prognosis of patients with BC after surgical treatment. Methods: A total of 144 BC patients who received standard neoadjuvant therapy in Shangqiu First People's Hospital from January 2016 to January 2018 were analyzed retrospectively. The clinical data of patients were collected and the effects of NLR, PLR and LMR on disease-free survival were evaluated by chi-square test and COX regression. The diagnostic value of NLR, PLR and LMR in BC recurrence was analyzed by receiver operating characteristic (ROC) curve. Results: Of the 144 patients, 20 (13.89%) had local or distant metastasis. The areas under the ROC curve of NLR, PLR and LMR in peripheral blood for the diagnosis of BC recurrence were 0.713, 0.683 and 0.765, respectively. Multivariate Cox regression analysis showed that T stage, lymph node metastasis, PLR, LMR and HER2 were independent risk factors for prognosis. Conclusion: Inflammatory markers based on NLR, PLR and LMR may become biological indicators to predict the pathological features and prognosis of invasive BC in the future.

Keywords: Breast cancer, platelet/lymphocyte ratio, neutrophil/lymphocyte ratio, lymphocyte/monocyte ratio

Introduction

Breast cancer (BC) is the most familiar malignancy among females at present [1]. In China, its morbidity is high, and its mortality ranks the third among tumor-related diseases [2]. The former is still on the rise, but the latter is on the decline. A recent epidemiological survey shows that there were 2 million new BC patients and 600,000 dead cases in 2018 [3]. Traditionally, BC has been classified as carcinoma in situ (ductal carcinoma and lobular carcinoma) or invasive disease according to morphological markers [4]. The diagnosis time is relevant to the prognosis. Clinical studies have found that the 5-year survival rate of early stage BC patients after treatment is as high as 70-90%; while for those with advanced ones, the rate can be as low as below 15% due to cancer cell proliferation or metastasis [5]. Thus, it is particularly important to explore the pathogenesis of BC and find potential targets for diagnosis, treatment and prognosis.

Early studies have shown that the occurrence of tumors is accompanied by inflammation, with different stages [6]. Inflammation promotes tumor occurrence and development. Recent study has found that tumors advance the persistence of inflammation, and they complement each other [7]. Platelet lymphocyte ratio (PLR), neutrophil lymphocyte ratio (NLR) and lymphocyte monocyte ratio (LMR) are the most common inflammatory indicators in blood routine [8]. Recent studies have found that PLR, NLR and LMR have high clinical value in the prognosis of various tumors, such as lung cancer, gastric cancer, colorectal cancer and BC [9-11]. For example, Spanish scholars confirmed that LMR strongly reflected the potential of DFS and OS in BC [12]. Another research found that PLR and NLR could be used as potential diagnostic indicators of BC and were relevant to the prognosis of patients [13]. However, there are still some controversies about the prognostic values of PLR, NLR and LMR in BC.

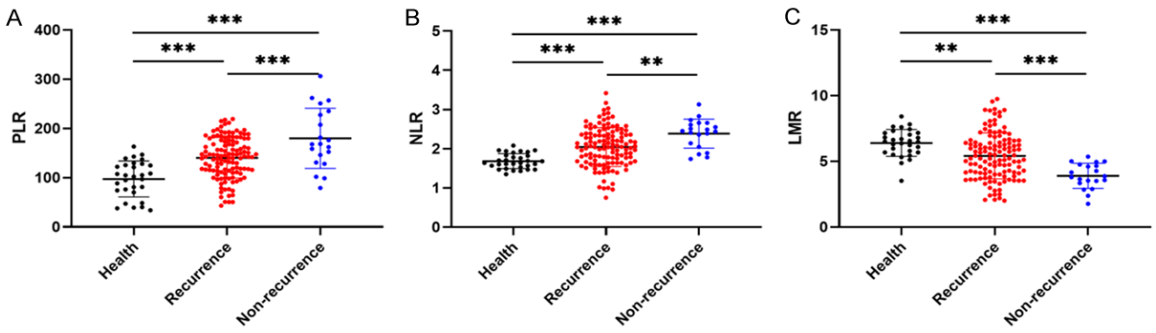

Figure 1. PLR, NLR and LMR in BC patients. A. The PLR of patients is detected by automatic hematology analyzer. B. The NLR of patients is detected by automatic hematology analyzer. C. The LMR of patients is detected by automatic hematology analyzer. $^{**}P < 0.01$, $^{***}P < 0.001$.

Hence, we hope to verify the prognostic values of the three in patients after BC resection by retrospective study, and to provide reference for clinical practice.

Methods and data

General data

A total of 144 BC patients treated in the Shangqiu First People's Hospital from January 2016 to January 2018 were collected as an experimental group. The patients were 50.4± 4.2 years old on average. They underwent surgery after receiving standard neoadjuvant treatment. Meanwhile, 30 normal people, with a mean age of 48.1±5.1 years, who were medically examined in our hospital, were collected as a control group. This study was approved by the medical ethics committee of our hospital (HN25SQ10201). All the patients were well informed, and the informed consent was obtained.

Inclusion and exclusion criteria

Inclusion criteria: Female patients; All patients were diagnosed with invasive BC by imaging and pathological examination; Their clinical data, including follow-up data were complete; The patients met TNM staging criteria [14], and the stages were mainly distributed in II-III.

Exclusion criteria: Patients had other tumors; The TNM stage of patients was IV; Patients were intolerant to the treatment; Patients had serious heart, lung and blood diseases.

Data collection

The routine blood test of patients was completed by the clinical laboratory of our hospital. The peripheral blood of the patients was collected and stored in anticoagulant tube, and the blood routine test was performed by automatic hematology analyzer (Sysmex, Japan, XE-2100L). Data analysis was performed and judged by professional clinicians in our department. Neutrophil/lymphocyte ratio (NLR) = neutrophil count (10^9/L)/lymphocyte count (10^9/L). Platelet count/lymphocyte ratio (PLR) = platelet count (10^9/L)/lymphocyte count (10^9/L). Lymphocyte/monocyte ratio (LMR) = lymphocyte count (10^9/L)/monocyte count (10^9/L). Data collection included age, menstrual status, treatment plan, tumor size, lymph node metastasis, molecular classification, radiotherapy or not, recurrence and preoperative blood routine results.

Outcome measures

Main outcome measures: The PLR, NLR and LMR differences between BC patients and healthy people were analyzed. Patients were followed up and divided into recurrence group and non-recurrence group based on their recurrence. The differences in the three indicators between both groups were observed. The predictive values of PLR, NLR and LMR on recurrence of BC patients were evaluated by the receiver operating curve (ROC). Patients were included into different groups in light of the best cut-off value, and the relationship between the three indicators and clinical data was observed.

Secondary outcome measures: After all patients were followed up, their prognosis after treatment was evaluated through Cox regression analysis.

Statistical analysis

All the data were statistically assessed by SPSS20.0, and the pictures were drawn via GraphPad Prism 8. The counting data were

Table 1. ROC data

Indexes	AUC	95% CI	Specificity	Sensibility	Youden index	Cut-Off value
PLR	0.683	0.545-0.819	66.93%	65.00%	31.93%	> 158.365
NLR	0.713	0.603-0.824	72.58%	70.00%	42.58%	> 2.355
LMR	0.765	0.680-0.851	56.45%	95.00%	51.45%	< 5.290

Note: AUC: area under curve, 95% CI: 95% confidence interval, Cut-Off: optimal cut-off value.

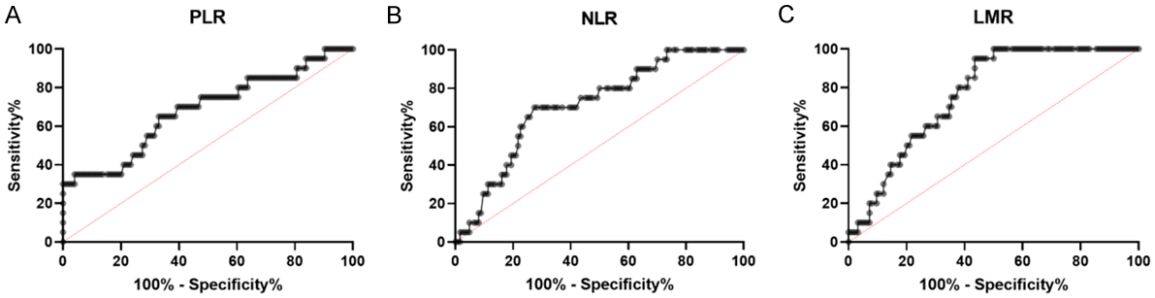

Figure 2. Area under PLR, NLR and LMR curves. A. The predictive value of PLR in the recurrence of BC is tested by ROC curve analysis. B. The predictive value of NLR in the recurrence of BC is tested by ROC curve analysis. C. The predictive value of LMR in the recurrence of BC is tested by ROC curve analysis.

Table 2. Relationship between PLR and clinical data of BC patients

Factor	PLR		*P* value
	> 158.365 (n=54)	≤ 158.365 (n=90)	
Age			0.298
≥ 50 years old (n=79)	27	52	
< 50 years old (n=65)	17	48	
Menopause			0.503
Premenopausal (n=53)	18	35	
Postmenopausal (n=91)	36	55	
T staging			0.005
T1-2 (n=100)	30	70	
T3-4 (n=44)	24	20	
Lymph node metastasis			0.016
N0-1 (n=95)	29	66	
N2-3 (n=49)	25	24	
ER			0.861
+ (n=60)	22	38	
- (n=84)	32	52	
PR			0.829
+ (n=65)	25	40	
- (n=79)	29	50	
HER2			0.240
+ (n=101)	41	60	
- (n=43)	13	30	

expressed as n (%) and assessed through Chi-square test. The differences in PLR, NLR and LMR among BC patients with recurrence, non-recurrence and healthy people were tested through univariate ANOVA and marked by F. Data between groups were compared by LSD-t test. The predictive values of PLR, NLR and LMR in BC recurrence were evaluated by ROC. The factors affecting their disease-free survival were analyzed through Cox regression. $P < 0.05$ was considered with statistical significance.

Results

Patient baseline data

A total of 144 BC patients were collected, with an average age of 50.4±4.2 years. There were 91 cases of menopause, 53 of premenopause; 100 of stage T1-2, 44 of stage T3-4; 95 of stage N0-1 and 49 of stage N2-3. The patients were staged according to the levels of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), including 67 cases of lumen type (44.44%), 46 (31.94%) of lumen type with $HER2^+$ and 31 (23.62%) with triple negative BC. 20 patients recurred after operation, and the median follow-up time was 32 months (range: 1-40 months). There were 30 patients included in the control group, with an average age of 48.1±5.1 years, and there was

Table 3. Relationship between NLR and clinical data of BC patients

Factor	NLR		*P* value
	> 2.355 (n=51)	≤ 2.355 (n=93)	
Age			0.721
≥ 50 years old (n=79)	29	50	
< 50 years old (n=65)	22	43	
Menopause			0.127
Premenopausal (n=53)	23	30	
Postmenopausal (n=91)	28	63	
T staging			0.015
T1-2 (n=100)	29	71	
T3-4 (n=44)	22	22	
Lymph node metastasis			0.330
N0-1 (n=95)	31	64	
N2-3 (n=49)	20	29	
ER			0.331
+ (n=60)	24	36	
- (n=84)	27	57	
PR			0.479
+ (n=65)	21	44	
- (n=79)	30	49	
HER2			0.291
+ (n=101)	33	68	
- (n=43)	18	25	

no obvious difference in age between the groups (P > 0.05).

PLR, NLR and LMR expression in BC patients

Totally 144 BC patients and 30 healthy people were compared. It was found that PLR and NLR of BC patients were higher than those of healthy people (**Figure 1A**, **1B**), while LMR was lower (**Figure 1C**). Besides, we also compared the three indices between patients with or without recurrence. The results manifested that patients with recurrence had higher PLR and NLR than those without recurrence. It is suggested that PLR, NLR and LMR have potential value in diagnosing BC.

Predictive values of PLR, NLR and LMR in patients with recurrence

To further determine the relationship between PLR, NLR, LMR and BC recurrence, the value of the three was analyzed by ROC. Afterwards, we found that the area under ROC curve for PLR predicting recurrence was 0.683, and the Cut-Off was > 158.365. The area under ROC curve for NLR predicting recurrence was 0.713, and the Cut-Off was > 2.355. The area under ROC curve for LMR predicting recurrence was 0.765, and the Cut-Off was < 5.290. It is suggested that PLR, NLR and LMR have high clinical predictive value in BC recurrence (**Table 1**; **Figure 2**).

Relationship between PLR, NLR and LMR and clinical data of patients

In view of the Cut-Off values of PLR, NLR and LMR in ROC analysis, patients were randomized into high and low expression groups, and the relationship between the three indices and clinical data was observed. The results showed that PLR, NLR and LMR were correlated with T stage and lymph node metastasis (**Tables 2**-**4**).

Cox regression analysis

At the end of this research, we conducted Cox regression analysis on patients and analyzed the factors affecting their disease-free survival time. Univariate analysis manifested that clinical T stage, lymph node metastasis, HER-2, NLR, PLR and LMR were the factors affecting prognosis. Further multivariate Cox regression analysis documented that T stage, lymph node metastasis, PLR, LMR and HER2 were independent risk factors affecting prognosis (**Table 5**). Furthermore, the survival curves of PLR, NLR, LMR and disease-free survival were drawn (**Figure 3**).

Discussion

BC is a familiar female malignancy in clinical practice [15]. With the continuous progress of treatment methods, the mortality has obviously decreased in recent years [16]. Nevertheless, the number of patients has been increasing, and there is a lack of effective diagnostic and prognostic indicators [17]. Recent studies have found that some molecules have high clinical value in predicting BC diagnosis and prognosis [18]. But these molecular kits are expensive, and multiple tests will bring economic burden to patients. Thus, the key to improving this situation is to find simple, cheap and understandable clinical parameters.

Recent studies have shown that under inflammatory conditions, the occurrence and development of tumors can be effectively promoted

Table 4. Relationship between LMR and clinical data of BC patients

Factor	LMR		*P* value
	< 5.290 (n=63)	≥ 5.290 (n=81)	
Age			0.410
≥ 50 years old (n=79)	37	42	
< 50 years old (n=65)	26	39	
Menopause			0.446
Premenopausal (n=53)	21	32	
Postmenopausal (n=91)	42	49	
T staging			0.005
T1-2 (n=100)	64	36	
T3-4 (n=44)	17	27	
Lymph node metastasis			0.002
N0-1 (n=95)	33	62	
N2-3 (n=49)	30	19	
ER			0.201
+ (n=60)	30	30	
- (n=84)	33	51	
PR			0.628
+ (n=65)	27	38	
- (n=79)	36	43	
HER2			0.124
+ (n=101)	40	61	
- (n=43)	23	20	

[19]. Some studies have found that inflammatory cells play a vital role in tumor occurrence and prognosis [20]. With the progression of cancer, the necrosis and collapse of the adjacent tissues are gradually aggravated, which leads to more obvious non-specific inflammation [21]. Interestingly, inflammatory mediators accelerate tumor cell growth and metastasis by destroying the normal intracellular environment [22]. Neutrophils inhibit the cytolytic activity of lymphocytes, natural killer and activated T cells [23]. Monocytes can differentiate into tumor-related macrophages in tumor microenvironment [24]. Lymphocytes play crucial roles in cancer immune monitoring of proliferation and metastasis of target tumor cells [25]. All these indices can be detected by routine blood tests. Recent studies have found that NLR, PLR and LMR are tied to tumorigenesis and infection as inflammation-related markers [26]. These markers have been extensively considered as predictors of different diseases.

This research determined the clinical value of NLR, PLR and LMR in the prognosis of BC patients. First of all, we found that the expression of NLR and PLR increased, while that of LMR decreased, and the differences in the three indicators were more remarkable after recurrence; it indicated that the three indicators had potential value in BC diagnosis and recurrence prediction. To verify their predictive values in BC recurrence, we drew ROC curves, and the results supported our hypothesis. Patients were grouped according to the cut-off values of NLR, PLR and LMR. It was found that PLR, NLR and LMR were correlated with T stage and lymph node metastasis. It is suggested that the three can be expected to be markers of BC recurrence. Durhan et al. [27] found that the NLR and PLR values of postoperative BC patients decreased remarkably. While Moon et al. [28] discovered that the increase of NLR was an independent prognostic factor for late recurrence and could be used as a reliable, easily accessible and cost-effective test. These experiments reveal that NLR and PLR have a certain value in the prediction of BC recurrence, which is consistent with our results. There are few studies on LMR in BC recurrence. The diagnostic value of LMR in BC recurrence is first investigated in this study, but its mechanism is still vague and needs to be further explored.

Finally, we followed up patients and observed their DFS. During the follow-up period, 20 of 144 patients had recurrence with a recurrence rate of 13.88%, which was similar to other follow-up results [26]. Through regression analysis, we determined that the levels of NLR, PLR and LMR were correlated with DFS. Multivariate analysis documented that high PLR and low LMR were independent inflammatory markers related to poor DFS. What's more, Ma et al. [29] found that LMR could be used as a potential marker to predict treatment efficacy and prognosis of BC; while in this research, PLR was also a predictor of recurrence. Previously, it was reported that the increase of PLR suggested the poor prognosis of patients, which was consistent with our research. We think that the differences in the research of Tuma et al. may be caused by the small number of cases.

The above study confirmed the expression and prognostic value of PLR, NLR and LMR ratio in BC patients. Although there have been a large number of studies about the relationship between NLR, PLR, LMR and gastric cancer, larger sample size and prospective research are still necessary.

Table 5. Cox regression analysis

Factor	Univariate Cox			Multivariate Cox		
	95% CI	HR value	*P* value	95% CI	HR value	*P* value
Age (≥ 50 VS < 50 years old)	0.334-2.002	0.818	0.660	0.678-5.105	1.860	0.228
Menopause (Premenopausal VS Postmenopausal)	0.259-1.498	0.623	0.291			
T stage (T1-2 VS T3-4)	1.527-9.163	3.741	0.004	1.432-9.016	3.593	0.006
Lymph node metastasis (N0-1 VS N2-3)	2.072-13.175	5.225	< 0.001	1.681-11.519	4.401	0.003
ER (+ VS -)	0.322-1.866	0.775	0.570			
PR (+ VS -)	0.361-2.087	0.869	0.753			
HER2 (+ VS -)	0.028-0.440	0.110	0.002	0.016-0.793	0.113	0.028
PLR (> 158.365 VS ≤ 158.365)	0.085-0.579	0.222	0.002	0.087-0.629	0.234	0.004
NLR (> 2.355 VS ≤ 2.355)	0.143-0.852	0.350	0.021	0.566-4.862	1.659	0.356
LMR (< 5.290 VS ≥ 5.290)	0.04-0.495	0.141	0.002	0.069-0.920	0.252	0.037

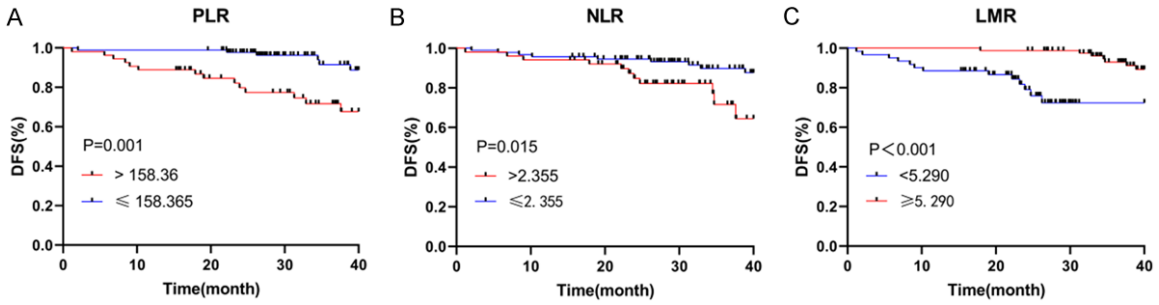

Figure 3. Relationship between PLR, NLR, LMR and disease-free survival of BC patients. A. Analysis of relationship between PLR and DFS in BC patients by Kmurm survival curve. B. Analysis of relationship between NLR and DFS in BC patients by Kmurm survival curve. C. Analysis of relationship between LMR and DFS in BC patients by Kmurm survival curve.

In conclusion, the inflammatory indicators based on NLR, PLR and LMR may become biological indicators to predict the pathological features and prognosis of invasive BC in the future.

Disclosure of conflict of interest

None.

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