Original Article
dNLR and TILS can be used as indicators for prognosis and efficacy evaluation of neo-adjuvant chemotherapy in breast cancer

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Abstract: Background: To explore the clinical value of dNLR and TILS in the prognostic diagnosis of breast cancer (BC) and the efficacy evaluation of neo-adjuvant chemotherapy. Methods: From January 2012 to January 2014, 72 patients with BC who received neo-adjuvant chemotherapy in our hospital were selected for this study and analyzed retrospectively. The clinical value of dNLR and TILS in the prognosis of BC was observed, and the clinical data of the patients were collected for Cox regression analysis to observe their independent prognostic indicators. According to the clinical efficacy of neo-adjuvant chemotherapy, patients were divided into effective group (EG) (CR+PR) and invalid group (IG) (SD+PD), and the value of dNLR and TILS in the therapeutic effect of patients was observed. Results: After therapy, there were 10 cases of CR, 44 cases of PR, 13 cases of SD and 5 cases of PD in 72 patients. With the improvement of curative effect, the expression of dNLR was gradually declined (P < 0.05). The less effective the patients were, the less TILS positive rate was (P < 0.05). However, the expression of dNLR in the EG was obviously lower than that in the IG (P < 0.05). The area under dNLR curve was 0.844, while the area under TILS curve was 0.618. Multivariate Cox regression analysis revealed that TNM staging, dNLR and TILS were independent prognostic factors that affected the patients. Conclusion: Patients with high expression of dNLR and TILS-negative BC have poor efficacy of neoadjuvant chemotherapy, and dNLR and TILS can be used as prognostic observation indexes for BC.

Keywords: dNLR, TILS, breast cancer, prognosis, neo-adjuvant chemotherapy

Introduction

With the continuous improvement of living standard and the change of living environment, the incidence of basic diseases and tumors has been increasing [1]. A recent global estimation of morbidity and mortality showed that there were more than 18 million new tumor patients and 9.6 million deaths in 2018, among which the morbidity and mortality of BC accounted for 11.6% and 6.6% respectively [2]. BC is the most frequently diagnosed malignant tumor among women all over the world, and it is also one of the main causes of cancer death among women [3, 4]. With the updating of detection methods, the detection rate of BC is increasing in recent years, but the incidence rate is increasing year by year [5, 6]. With the deepening of scientific research, it is found that there are more and more pathogenic factors of BC [7]. Therefore, it is particularly important to seek the pathogenesis of BC and find potential targets for treatment, diagnosis and prognosis.

For now, the treatment methods of BC are progressing continuously, and the main clinical treatment methods are surgery, radiotherapy, chemotherapy, anti-hormone receptor therapy, molecular targeted therapy and biological immunotherapy, among which surgery combined with chemotherapy is commonly used in

Table 1. Clinical baseline data

Factors		N=72
Age	≥ 55 years	39
	< 55 years	33
Tumor size	≥ 5 cm	40
	< 5 cm	32
Lymph node metastasis	Metastasis	12
	No metastasis	60
TNM staging	I	35
	II	28
	III	9
TILS	Positive	25
	Negative	47
dNLR		2.40±1.17

clinical treatment [8, 9]. Chemotherapy before operation is called neo-adjuvant chemotherapy. Studies have revealed that neo-adjuvant chemotherapy can effectively improve the overall survival rate and disease-free survival time of patients with BC [10]. Although the continuous update of treatment plans is conducive to the selection of clinicians, there is a lack of prognostic indicators after BC treatment in clinical practice, which makes it impossible to effectively evaluate the prognosis of patients [11]. Neutrophil/(leukocytes-neutrophil) ratio (dNLR) is a newly discovered index in recent years, which can be calculated from complete blood cell parameters [12]. In many reports, it is mentioned that [12, 13] dNLR has certain clinical value in the diagnosis and prognosis of tumors. Tumor infiltrating lymphocytes (TILS) refer to lymphocyte groups that are recruited into the focus areas of tumor, which play an immune response and regulation role in tumor immune mechanism [14]. Previous studies have revealed that [15] TILS positive patients are positively correlated with the survival of tumor patients.

Therefore, this research was mainly designed to seek the clinical value of dNLR and TILS in BC, so as to provide potential reference indicators for clinical diagnosis and prognosis.

Methods and data

Clinical data

From January 2012 to January 2014, 72 patients with BC who received neo-adjuvant chemotherapy in Tianjin Medical University Cancer Institute and Hospital were selected for this study and analyzed retrospectively. All the patients were female, ranging in age from 41 to 65 years with a median age of 55 years (**Table 1**). This study was approved by the medical ethics committee of our hospital.

Inclusion and exclusion criteria

Inclusion criteria: The patients were diagnosed as BC by pathological examination; All patients agreed to follow up after operation; They were informed of the research situation and signed the informed consent form. TNM staging is based on the seventh edition of TMN staging published by the International Cancer Control Alliance [16].

Exclusion criteria: All patients received tumor treatment before this study; The patients were complicated with other tumors; The clinical data of the patient was incomplete.

Therapeutic regimen

All patients recruited in this research were treated with TEC regimen, i.e. docetaxel (Jiangsu Hengrui Pharmaceutical Co., Ltd., China, H20163032) 75 mg/m^2, epirubicin (PFIZER Pharmaceutical (Wuxi) Co., Ltd., China, H200-0496) 75 mg/m^2 and cyclophos-phamide (Hanhui Pharmaceutical Co., Ltd., Zhejiang, China, H20093393) 500 mg/m^2, all of which were administered on the first day, with a cycle of 21 days. During the treatment period, the symptomatic and supportive treatments such as antiemetic and leukogenic drugs were supplemented. The tumor was measured by conventional CT scanning. The slice thickness of CT scanning was ≤ 5 mm, and the maximum diameter was recorded. According to the solid tumor formulated by the World Health Organization (WHO), the clinical efficacy was assessed [17]. The efficacy was assigned to four grades: complete remission (CR), partial remission (PR), stable disease (SD) and progression of disease (PD).

Follow-up

In this research, the survival of patients was followed up for 5 years, and the patients were followed up by telephone and clinical review of
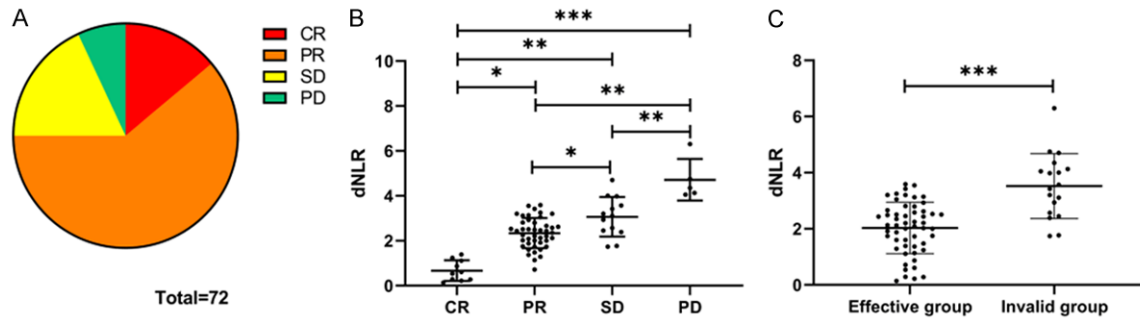
Figure 1. Relationship between dNLR, TILS and clinical efficacy of patients with BC. A. Clinical efficacy of patients after neo-adjuvant chemotherapy. B. Expression of dNLR in patients with different curative effects. C. T test was used to analyze the expression of dNLR in EG and IG. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 2. Efficacy analysis

Grouping	TILS (+)	TILS (-)	χ^2	P
EG	10	15	4.596	0.032
IG	8	39		

pathological data statistics at the 1st, 3rd, 6th, 9th and 12th month of each year.

Outcome measures

Main outcome measures: The clinical value of dNLR and TILS in the prognosis of BC was observed, and the clinical data of the patients were collected for Cox regression analysis to observe their independent prognostic indicators.

Secondary outcome measures: According to the clinical efficacy of neo-adjuvant chemotherapy, patients were divided into EG (CR+PR) and IG (SD+PD), and the value of dNLR and TILS in the therapeutic effect of patients was observed.

Statistical analysis

The data were statistically analyzed by SPSS, and GraphPad Prism 8 was used to draw the pictures. The measurement data of normal distribution were represented by mean number ± standard deviation (Mean ± SD), and t test was applied. The counting data were represented by number of cases (N) and composition ratio (%), and chi-square test was applied. Kaplan-Meier was used to calculate survival time and plot survival curve. The receiver operating characteristic curve was used to analyze the evaluation value of dNLR and TILS in the efficacy of BC. Logistic regression analysis was used to analyze the independent risk factors affecting the curative effect of patients. Cox regression analysis was used to observe independent prognostic indicators of the patients. The difference was statistically significant with $P < 0.05$.

Results

Relationship between dNLR, TILS and clinical efficacy of patients with BC

First of all, the clinical efficacy of the patients was assessed after therapy. Through the evaluation, it was found that there were 10 cases of CR, 44 cases of PR, 13 cases of SD and 5 cases of PD in 72 patients after therapy (**Figure 1A**). By comparing the dNLR and TILS of patients with different curative effects, it was found that the expression of dNLR was gradually declined with the improvement of curative effect ($P < 0.05$) (**Figure 1B**). According to the clinical efficacy, the patients were divided into EG and IG. Chi-square test revealed that the patients with worse efficacy had less TILS positive rate ($P < 0.05$) (**Table 2**). However, the expression of dNLR in the EG was obviously lower than that in the IG ($P < 0.05$) (**Figure 1C**).

Evaluation value of dNLR and TILS in clinical efficacy of patients with BC

Then, the patients were divided into EG and IG according to their clinical efficacy. Further ROC curve analysis revealed that the area under dNLR curve was 0.844 (**Figure 2A**), while the area under the TILS curve was 0.618 (**Figure 2B**). This indicated that dNLR could be used as a potential index to evaluate the therapeutic
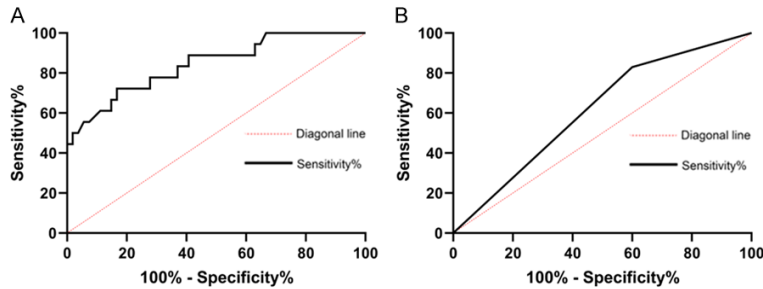
Figure 2. Evaluation value of dNLR and TILS in clinical efficacy of patients with BC. A. Evaluation value of dNLR in clinical efficacy of patients with BC. When Cut off was 55.56%, the best specificity was 83.33% and the sensitivity was 72.22%. B. Evaluation value of TILS in clinical efficacy of patients with BC. When Cut off was 23.64%, the best specificity was 65.66% and the sensitivity was 68.09%.

effect of neo-adjuvant chemotherapy, while it had a low predictive value for TILS.

dNLR and TILS were closely related to the prognosis of patients with BC

A total of 72 patients were followed up, 0 of whom were lost to follow-up, and the success rate of follow-up was 100%. The clinical data of patients were collected and analyzed by univariate analysis. The results revealed that TNM staging, dNLR, TILS and efficacy were independent factors affecting the patients. The indicators with differences were further analyzed by multivariate Cox regression analysis, and it was found that TNM staging, dNLR and TILS were independent prognostic factors affecting the patients (**Figure 3**; **Table 3**).

Discussion

As the most common female malignant tumor in clinical practice, BC has become the number one killer of women's health with the popularization of tumor screening and the increasing incidence of cancer year by year [18]. For now, chemotherapy combined with surgery is a common way to treat BC in clinic. Neo-adjuvant chemotherapy is a treatment method proposed in recent years. By reducing the tumor volume, it can achieve clinical decline, give patients the opportunity of surgical treatment, and improve the prognosis of patients [19, 20]. Although neo-adjuvant chemotherapy has improved the survival of patients to a certain extent, there is no better therapeutic effect and prognosis evaluation index at present.

In recent years, the latest concept of DNLR has been put forward, in which white blood cell count minus neutrophil count can be regarded as equivalent to lymphocyte count, and neutrophilic leukocytosis or lymphopenia may lead to the change of DNLR [21]. According to the analysis in this study, the clinical efficacy of patients with high DNLR was significantly decreased before operation, while the efficacy of TILs positive patients was significantly increased after neo-adjuvant chemotherapy. It is suggested that DNLR and TILs may be potential observational indicators of curative effect after neo-adjuvant chemotherapy. More and more studies have shown that inflammation plays an important role in regulating the development and progression of tumors. Neutrophils, as an important factor of inflammatory immunity, promote the growth and metastasis of tumors by releasing a series of inflammatory and immune factors [22]. As an important member of immune monitoring, lymphocytes play an inhibitory role in tumor proliferation and metastasis [23]. Studies have shown that [24] tumor lymphocyte infiltration is an important marker to improve the prognosis of patients, and the increase of peripheral blood lymphocytes also indicates a better prognosis of patients. Neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR), as important indicators of inflammatory immune response, have been proved to be closely related to the prognosis of various tumors [25, 26]. The above research is similar to the results of our study, which reminds us that DNLR and TILs may be potential prognostic indicators of BC. To verify our conjecture, ROC curve was drawn and the efficacy prediction value of dNLR and TILS was further analyzed after neo-adjuvant chemotherapy. It was found that the area under dNLR curve was 0.844, which was a great observational indicator of curative effect, but the area under the TILS curve was only 0.618, which was worse than the prediction effect of dNLR. At the end of the research, the patients were also followed up for 5 years and the patients' clinical data were collected for Cox regression analysis. Through analysis, it was
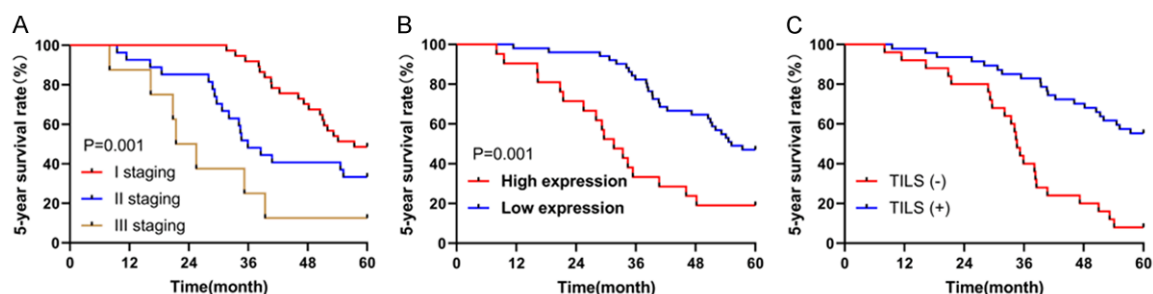
Figure 3. Relationship between Cox difference factors and survival. A. Difference between TNM staging and 5-year survival of patients. B. Difference between dNLR staging and 5-year survival of patients. C. Difference between TILS staging and 5-year survival of patients.

Table 3. Cox regression analysis

Factors	Univariate Cox			Multivariate Cox		
	P value	HR value	95% CI	P value	HR value	95% CI
Age (≥ 55 vs. < 55)	0.802	0.927	0.512-1.678			
Tumor size (≥ 5 cm vs. < 5 cm)	0.121	1.598	0.883-2.890			
Lymph node metastasis (metastasis vs. no metastasis)	0.128	0.565	0.271-1.179			
TNM staging (I vs. II vs. III)	< 0.001	2.144	1.396-3.294	0.001	2.277	1.429-3.627
dNLR (high expression vs. low expression)	< 0.001	0.331	0.179-0.612	0.001	0.341	0.179-0.649
TILS (positive vs. negative)	< 0.001	0.235	0.127-0.435	< 0.001	0.182	0.092-0.359
Efficacy (EG vs. IG)	0.010	0.438	0.233-0.824	0.110	0.523	0.237-1.157

found that dNLR and TILS were expected to be independent prognostic indicators of BC, suggesting that dNLR and TILS could be used as prognostic indicators of patients with BC.

However, there are still some limitations in this research. First, the samples collected in this research are relatively small. Second, this study, as it is retrospective, may have data bias. We hope to increase the number of our samples in future studies to improve our research conclusions.

To sum up, patients with high expression of dNLR and TILS-negative BC have poor efficacy after neoadjuvant chemotherapy, and dNLR and TILS can be used as prognostic indicators for BC.

Disclosure of conflict of interest

None.

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