Original Article

The prognostic value of neutrophil-to-lymphocyte ratio in *de novo* stage IV breast cancer: a retrospective cohort study

Bin Shao, Huiping Li, Xiaoran Liu, Guohong Song, Hanfang Jiang, Ying Yan, Ruyan Zhang, Ran Ran, Jiayang Zhang, Yaxin Liu, Huan Wang, Jing Wang, Lijun Di

Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Breast Oncology, Peking University Cancer Hospital & Institute, Beijing, China

Contributions: (I) Conception and design: B Shao, H Li, L Di; (II) Administrative support: H Li; (III) Provision of study materials or patients: B Shao, H Li, L Di, X Liu, G Song, H Jiang, Y Yan, R Zhang, R Ran, J Zhang, Y Liu, H Wang, J Wang; (IV) Collection and assembly of data: B Shao, L Di; (V) Data analysis and interpretation: B Shao, L Di; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Huiping Li; Lijun Di. Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Breast Oncology, Peking University Cancer Hospital & Institute, 52 Fucheng Road, Haidian District, Beijing 100142, China.
Email: huipingli2012@hotmail.com; dilijun2012@163.com.

Background: The presence of a high neutrophil-to-lymphocyte ratio (NLR) has been associated with increased mortality in several malignancies. And the majority of studies on breast cancer (BC) analyzed patients with early-stage. Fewer studies focused on metastatic BC (MBC). *De novo* stage IV BC with no prior treatment is more suitable for analyzing prognostic factors. Herein, we examined the prognostic value of baseline NLR in *de novo* stage IV BC patients.

Methods: We retrospectively screened the medical records of female patients who were diagnosed with *de novo* stage IV BC at Peking University Cancer Hospital between January 2011 and December 2020. All patients were followed up by telephone every 6 months. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff value of NLR for progression-free survival (PFS). Peripheral blood lymphocyte subsets and tumor infiltrating lymphocytes (TILs) were analyzed by flow cytometry and immunohistochemistry, respectively. Correlations of PFS and overall survival (OS) with NLR and other clinicopathological factors were evaluated using Kaplan-Meier method and Cox regression analyses.

Results: A total of 128 patients between January 2011 and December 2020 were enrolled. 70 (54.7%) cases were hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative, 79 (61.7%) patients had visceral metastasis and 67 (52.3%) patients had more than 2 metastatic sites. The cutoff values of NLR were 2.9, optimized by ROC curve analysis. Totals of 77 and 51 patients were assigned to the NLR-low (≤2.9) and NLR-high (>2.9) groups, respectively. Compared with NLR-high patients, the NLR-low patients had significantly longer median PFS (14.8 *vs.* 7.2 months; hazard ratio =1.791; P=0.003). The OS showed no significant difference (64.1 *vs.* 56.0 months, P=0.980). The patients with NLR-low had a higher level of peripheral $CD3^+$ T cells (P=0.028) and a lower level of peripheral $CD4^+CD25^+$ regulatory T (Treg) cells (P=0.041). Patient samples with NLR-low also demonstrated higher levels of TILs than those with NLR-high (P=0.025).

Conclusions: The baseline NLR-high is associated with adverse PFS in patients with *de novo* stage IV BC. The NLR-high status may indicate immune suppression status, which can help identify patients with unfavorable prognosis and assist with physicians' treatment decision.

Keywords: Neutrophil-to-lymphocyte ratio (NLR); *de novo* stage IV breast cancer (*de novo* stage IV BC); peripheral blood lymphocyte subsets; tumor infiltrating lymphocytes (TILs); prognosis

Submitted Oct 18, 2022. Accepted for publication Dec 20, 2022. Published online Jan 09, 2023.
doi: 10.21037/atm-22-5612
View this article at: https://dx.doi.org/10.21037/atm-22-5612

Introduction

Breast cancer (BC) is the most common tumor in women and is a leading cause of cancer-related death worldwide (1). About 5–10% of patients with BC are diagnosed with *de novo* stage IV cancer (2). With advances in systemic therapy, the mortality rate for patients with stage IV BC is decreasing, but overall survival (OS) remains unsatisfactory (3). This is partly due to the fact that stage IV BC has a heterogeneous prognosis ranging from a few months to many years with variations in tumor biology and patient characteristics. These factors, such as age, race, performance status, molecular subtype, clinical stage, metastatic sites, number of metastatic sites, and previous medical treatments, affect the prognosis of patients (4-6). An accurate estimation of survival is critical to patient treatment decisions (7).

Neutrophils and lymphocytes are important components of the human immune system. Many studies have examined the prognostic role of neutrophils, particularly the neutrophil-to-lymphocyte ratio (NLR) and the association with clinical outcomes in many cancer types, including BC (8,9). Several studies on early-stage BC have shown that the baseline NLR predicts survival outcomes (9,10). In addition, a meta-analysis systematically analyzed the published data on NLR and treatment outcomes. NLR was found to be an independent prognostic factor for survival in most of studies on the early-stage BC. However, no significant correlation was found between survival and NLR for advanced BC patients (11). Analysis of prognostic factors in patients with metastatic BC (MBC) is confounded by changes that cancer cells develop at the time of distant relapse, as well as responses to treatment. Therefore, patients with *de novo* stage IV BC with no prior treatment are more suitable for studying prognostic factors.

The primary aim of this study was to analyze the influence of NLR's relationship with other biological and clinical factors on the survival of patients with *de novo* stage IV BC upon initial diagnosis. The secondary aim was to evaluate factors associated with baseline NLR. We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5612/rc).

Highlight box

Key findings

- The baseline NLR-high is associated with adverse PFS in patients with *de novo* stage IV BC.

What is known and what is new?

- The prognostic value of NLR MBC is not clear. To the best of our knowledge, the present study is the first one to evaluate the prognostic value of NLR in the *de novo* stage IV BC.
- In this study, NLR is independent prognostic factor for PFS, but not OS.

What is the implication, and what should change now?

- The NLR-high status may indicate immune suppression status, which can help identify patients with unfavorable prognosis and assist with physicians' treatment decision.

Methods

We retrospectively identified 128 female patients who were diagnosed with *de novo* stage IV BC without prior treatment at Peking University Cancer Hospital between January 2011 and December 2020, without infectious diseases, auto immune diseases, concurrent hematological disorders, or other malignancies. All cases were histologically confirmed as invasive BC. Treatment efficacy was evaluated in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, version 1.1 (12). In this study, estrogen receptor (ER) and/or progesterone receptor (PR) positivity was defined as ≥1%. Human epidermal growth factor receptor 2 (HER2) was defined as negative with an immunohistochemical score of 0, 1+ or 2+ with fluorescence in situ hybridization (FISH) (–). All patients were followed up by telephone every 6 months. This study was approved by the Ethics Committee of the Peking University Cancer Hospital (No. 2017KT40) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Individual consent for this retrospective analysis was waived.

Overall response rate (ORR) was defined as the proportion of patients who achieved complete response (CR) or partial response (PR). Clinical benefit rate (CBR) was defined as the percentage of patients who achieved CR, PR, or stable disease (SD). Progression-free survival (PFS) was the time from the start of treatment to disease progression, death, or loss to follow-up. The OS was defined as the time from the beginning of treatment to death or loss to follow-up.

NLR

Peripheral blood was collected within 7 days prior to the first line therapy. The differential counts of the white blood cells were determined using a hemocytometer. The percentages of the differential counts were determined using

a BC-6900 Hematology Analyzer (Mindray, Shenzhen, China). The NLR calculations were made by dividing the serum neutrophil count by the lymphocyte count.

T-lymphocyte subtype detection in peripheral blood

Whole blood samples (200 µL) were incubated with conjugated antibodies as indicated in the dark for 10 minutes at room temperature before red blood cells were lysed. Samples pellets were re-suspended in 500 µL phosphate-buffered saline (PBS) after centrifugation for 5 minutes at 1,300 g at room temperature and analyzed by flow cytometry.

Conjugated antibodies used in this study were purchased from Beckman Coulter (Brea, CA, USA) and included CD3-PC5/CD4-FITC/CD8-PE (IM1650), CD3-FITC/(CD16$^+$/CD56)-PE (A07735), CD(14+16)-FITC/CD85k(ILT3)-PE/CD33-PC5 (A23413), CD4-FITC (A007750), CD8-FITC (A07756), CD19-PC5 (A07771), and CD25-PE (A07774).

Beckman-Coulter FC500 (Beckman Coulter, USA) and CXP analysis software (Beckman Coulter) was used for flow cytometry. There are 10,000 gated events in every analysis. Lymphocytes subtypes were selected according to physical characteristics including volume and transmissivity. The level of T lymphocyte subtype was expressed as percentage of the total number of lymphocytes.

Stromal tumor infiltrating lymphocytes (TILs) evaluation

Evaluation of TILs was performed by independent pathologists on a whole section of needle biopsy stained by hematoxylin and eosin (HE), based on guidelines of the International TILs Working Group (4). In short, TILs were expressed as the percentage of immune cells in the stroma within the tumor. The number of TILs was a continuous measurement and two categories were applied in the study: low TILs (≤10%) and high TILs (>10%).

Statistical analysis

The software SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The baseline characteristics of patients and treatment were described as mean ± standard deviation for continuous variables. Student's *t*-tests were used to compare normally distributed variables and Mann-Whitney U tests were used for non-normally distributed variables. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff for NLR. The chi-square test (or Fisher's exact test when necessary) was performed to compare clinicopathological characteristics between high NLR and low NLR groups. Survival was estimated using the Kaplan-Meier method. Log-rank test was used to compare PFS or OS between the different subgroups. NLR and other factors relevant to survival were tested by univariable and multivariable Cox proportional hazard models. All tests were two-tailed, and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

A total of 168 women were diagnosed with stage *de novo* stage IV BC between January 2011 and December 2020, of whom 128 patients were enrolled in our study based on the inclusion and exclusion criteria. The clinical characteristics of patients are presented in *Table 1*. The median age of cases was 53 (range, 29–76) years. At initial diagnosis, 70 (54.7%) cases were hormone receptor (HR)-positive/HER2-negative, 79 (61.7%) patients had visceral metastasis and 67 (52.3%) patients had more than 2 metastatic sites. A total of 116 (90.6%) cases received first line chemotherapy; 33 (73.3%) cases received anti-HER2 target therapy among the 45 patients with HER2 positive BC.

NLR and clinical characteristics in de novo stage IV BC patients

The optimal NLR cutoff value of 2.9 was determined by ROC curve analysis (*Figure 1*). The area under the ROC curve (AUC) was 0.684, with sensitivity at 0.59, and specificity at 0.75. Totals of 77 and 51 patients were classified into the NLR-low (≤2.9) group and NLR-high (>2.9) group, respectively. Cases in the NLR-high group had significantly higher proportion of T4 stage tumor than those in NLR-low group (P=0.002). The NLR did not show an association with other clinicopathological features, including molecular subgroups and treatment regimen (*Table 1*).

NLR and clinical outcome in de novo stage IV BC patients following first line treatments

After a median follow-up of 32 (range, 2.6–169.2) months, 110 patients progressed, and 51 deaths were reported. The ORR was 43.8% (56/128), with CR 0.8% (1/128),

Table 1 Clinicopathologic characteristics of the *de novo* stage IV BC patients

Characteristics	All (n=128)	NLR ≤2.9 (n=77)	NLR >2.9 (n=51)	P value
Age (years), n (%)				0.859
≤50	54 (42.2)	32 (41.6)	22 (43.1)	
>50	74 (57.8)	45 (58.4)	29 (56.9)	
Histology, n (%)				0.269*
IDC	109 (85.2)	63 (81.8)	46 (90.2)	
ILC	4 (3.1)	4 (5.2)	–	
Others	15 (11.7)	10 (13.0)	5 (9.8)	
Grade, n (%)				0.690*
1	1 (0.8)	1 (1.3)	–	
2	69 (53.9)	42 (54.5)	27 (52.9)	
3	23 (18.0)	12 (15.6)	11 (21.6)	
Unknown	35 (27.3)	22 (28.6)	13 (25.5)	
T stage, n (%)				0.002*
T0	2 (1.6)	1 (1.3)	1 (2.0)	
T1	18 (14.1)	15 (19.5)	3 (5.9)	
T2	39 (30.5)	28 (36.4)	11 (21.6)	
T3	9 (7.0)	6 (7.8)	3 (5.9)	
T4	42 (32.8)	15 (19.5)	27 (52.9)	
Unknown	18 (14.1)	12 (15.6)	6 (11.8)	
N stage, n (%)				0.158*
N0	2 (1.6)	1 (1.3)	1 (2.0)	
N1	20 (15.6)	15 (19.5)	5 (9.8)	
N2	16 (12.5)	11 (14.3)	5 (9.8)	
N3	67 (52.3)	34 (44.2)	33 (64.7)	
Unknown	23 (18.0)	16 (20.8)	7 (13.7)	
Molecular subtype, n (%)				0.853
HR^+HER2^-	70 (54.7)	43 (55.8)	27 (52.9)	
$HER2^+$	45 (35.2)	27 (35.1)	18 (35.3)	
TNBC	13 (10.2)	7 (9.1)	6 (11.8)	
Ki-67, n (%)				0.540
≤20%	26 (20.3)	17 (22.1)	9 (17.6)	
>20%	97 (75.8)	57 (74.0)	40 (78.4)	
Unknown	5 (3.9)	3 (3.9)	2 (3.9)	

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	All (n=128)	NLR ≤2.9 (n=77)	NLR >2.9 (n=51)	P value
Site of metastasis, n (%)				
Liver metastasis	43 (33.6)	24 (31.2)	19 (37.3)	0.475
Lung metastasis	49 (38.3)	31 (40.3)	18 (35.3)	0.571
Brain metastasis	6 (4.7)	4 (5.2)	2 (3.9)	1.000*
Bone metastasis	82 (64.1)	52 (67.5)	30 (58.8)	0.315
Distant lymph nodes metastasis	72 (56.3)	43 (55.8)	29 (56.9)	0.909
Malignant pleural effusion	26 (20.3)	12 (15.6)	14 (27.5)	0.090
Visceral metastasis (liver, lung, brain), n (%)				0.846
No	49 (38.3)	30 (39.0)	19 (37.3)	
Yes	79 (61.7)	47 (61.0)	32 (62.7)	
Number of metastases, n (%)				0.182
≤2	61 (47.7)	33 (42.9)	28 (54.9)	
>3	67 (52.3)	44 (57.1)	23 (45.1)	
First-line treatment, n (%)				0.762*
Chemotherapy	116 (90.6)	69 (89.6)	47 (92.2)	
Endocrine therapy	12 (9.4)	8 (10.4)	4 (7.8)	
Anti-HER2 treatment, n (%)				0.470*
No	12 (26.7)	7 (25.9)	5 (27.8)	
Transtuzumab based regimen	28 (62.2)	18 (66.7)	10 (55.6)	
Transtuzumab + pertuzumab based regimen	4 (8.9)	1 (3.7)	3 (16.7)	
Tyrosine kinase based regimen	1 (2.2)	1 (3.7)	0 (0.0)	

*, P values determined by Fisher's exact test; all other P values determined by chi-squared test. BC, breast cancer; NLR, neutrophil-to-lymphocyte ratio; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

PR 43.0% (55/128), SD 46.1% (59/128), and progressive disease (PD) 10.2% (13/128). The NLR levels were not associated with ORR (45.5% in NLR-low *vs.* 41.2% in NLR-high, P=0.633) but were associated with CBR (94.8% in NLR-low *vs.* 82.4% in NLR-high, P=0.022) (*Table 2*).

To investigate whether an elevated NLR is associated with the clinical outcome of BC, univariate and multivariate analyses were performed. Kaplan-Meier analysis revealed that patients with NLR-high had significantly shorter PFS than those with NLR-low (7.2 *vs.* 14.8 months, P=0.004) (*Figure 2A*). Univariate analysis identified liver metastasis, visceral metastasis, and NLR as prognostic factors (*Table 3*). A multivariate Cox proportional hazard model including NLR, liver metastasis, or visceral metastasis was established and NLR was identified as an independent prognostic factor for PFS [hazard ratio =1.791; 95% confidence interval (CI): 1.213–2.644; P=0.003]. When stratifying different subgroups, NLR-high was only associated with PFS in the HER2-positive and triple negative BC (TNBC) subgroups, but not in the HR^+HER2^- subgroup (*Figure 2B-2D*).

The median OS of all patients was 56.0 (95% CI: 29.7–82.4) months. The OS had no significant difference between NLR-low (≤2.9) and NLR-high (>2.9) group in univariate survival analysis (64.1 *vs.* 56.0 months, P=0.980) (*Figure 3A*). The NLR also had no significant prognostic value in different molecular subtypes (*Figure 3B-3D*). In the multivariate analysis, we included all of the factors that might affect OS, including age, pathologic grade, molecular

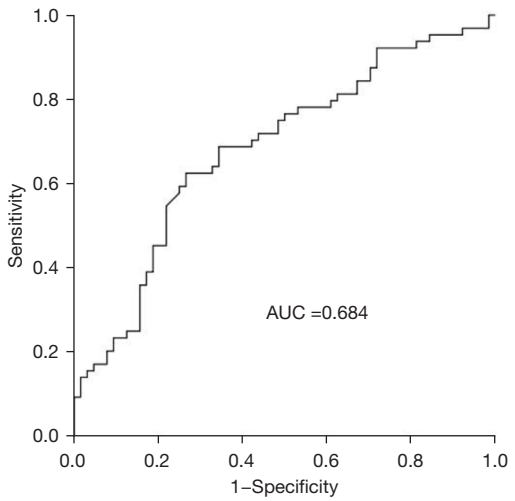

Figure 1 ROC curves assessing the cutoff point of NLR. AUC, area under the ROC curve; ROC, receiver operating characteristic; NLR, neutrophil-to-lymphocyte ratio.

subtype, Ki-67, visceral metastasis, number of metastasis sites, first line therapy, and NLR. Visceral metastasis was a significant prognostic factor for OS in the univariate analysis (P=0.006) and multivariate analysis (hazard ratio =2.425; 95% CI: 1.008–5.833; P=0.048) (*Table 4*).

NLR and peripheral blood T lymphocyte subtype

The distribution of lymphocyte subtype in the peripheral blood could partially represent the immune status of the patients. We compared the distribution pattern of different peripheral blood lymphocyte subtypes between the NLR-low (≤2.9; 52 cases) and NLR-high (>2.9; 35 cases) groups. The patients with NLR-low had higher levels of $CD3^+$ T cells (P=0.028) and lower levels of $CD4^+CD25^+$ regulatory T (Treg) cells (P=0.041) than those with NLR-high (*Figure 4*, *Table 5*).

NLR and TILs

We also explored the association of the TILs level with NLR level. The TILs in 30 cases were assessed. The median value of TILs was 7.5% (1–80%). A total of 12 (40%) cases had higher TILs levels (>10%) and 18 (60%) cases had low TILs levels (≤10%) With 15 cases with NLR-low and 15 cases with NLR-high, the NLR-low patients had higher levels of TILs (P=0.025; *Figure 5*).

Discussion

The predictive value of NLR has been shown in a variety of solid tumor types, including MBC (9,10,13-21). Although stage IV and recurrent MBC share molecular characteristics and biological behaviors, recurrent MBC patients tend to have a worse prognosis compared to stage IV MBC patients (22), which may be related to the fact that stage IV MBC, with its treatment-naïve status, is more sensitive to systemic treatments. Whether NLR has different roles between the two groups remains mostly unknown. In addition, metastatic patterns have also been associated with survival in MBC. Patients with visceral metastasis were shown to have shorter survival than those with non-visceral metastasis. The number of metastatic sites is also correlated with survival (23). Our study showed that NLR-high was associated with a shorter PFS in *de novo* MBC patients, without a significant role in OS. We found no association between NLR and metastatic patterns, except for T4 stage tumors, which demonstrated higher levels of NLR and were often associated with a large tumor mass or tumor rupture.

Previous results on the role of NLR in different MBC molecular subtypes have been contradictory (9,24-28). In our cohort, we found that NLR was associated with a poor prognosis in patients with HER2-positive or TNBC, but not in patients with HR^+HER2^-. TNBC and $HER2^+$ BCs with aggressive biological behavior demonstrate higher levels of both genomic instability and tumor mutation burden (TMB), which may promote the presentation of new antigens and result in a potential sensitization in response to immunotherapies in these patients (29).

We then investigated the relationship between NLR and immunological factors, including peripheral lymphocyte classification and the TILs. Our results demonstrated that NLR-low was associated with higher levels of $CD3^+$ T cells, lower levels of $CD4^+CD25^+$ Treg cells, and higher levels of TILs. Correlation of NLR with immunological factors is consistent with an immune active status in NLR-low patients. Neutrophils inhibit the immune response by inhibiting the activity of immune cells [lymphocytes, activated T cells, and natural killer (NK) cells]. Lymphocytes play a key role in tumor immune monitoring by inducing cytotoxicity and apoptosis in tumor cells (30,31). The $CD4^+$ T helper (Th) cells play multiple roles in the induction of immune responses against tumor cells. The effector $CD4^+$ T cells are classified into subsets including Th1, Th2, Th17,

Table 2 The correlation of NLR and treatment outcome

Treatment outcome	All (n=128)	NLR ≤2.9 (n=77)	NLR >2.9 (n=51)	P value
CR, n (%)	1 (0.8)	1 (1.3)	0 (0.0)	0.098
PR, n (%)	55 (43.0)	34 (44.2)	21 (41.2)	–
SD, n (%)	59 (46.1)	38 (49.4)	21 (41.2)	–
PD, n (%)	13 (10.2)	4 (5.2)	9 (17.6)	–
ORR (CR + PR), n (%)	56 (43.8)	35 (45.5)	21 (41.2)	0.633
CBR (CR + PR + SD), n (%)	115 (89.6)	73 (94.8)	42 (82.4)	0.022
PFS (95% CI) (months)	12.0 (9.8–14.1)	14.8 (11.9–17.8)	7.2 (3.5–10.9)	0.004
OS (95% CI) (months)	56.0 (29.7–82.4)	64.1 (32.3–95.9)	56.0 (33.2–78.8)	0.980

NLR, neutrophil-to-lymphocyte ratio; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; CBR, clinical benefit rate; PFS, progression-free survival; CI, confidence interval; OS, overall survival.

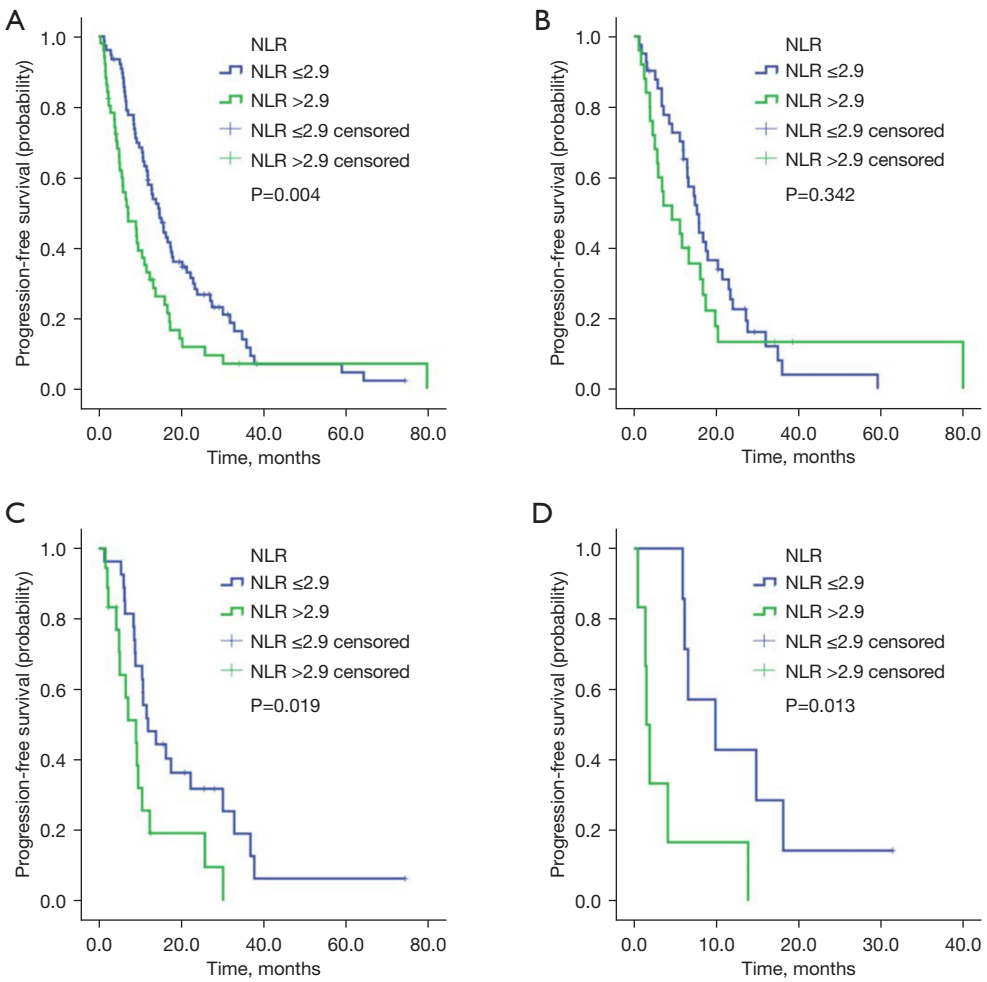

Figure 2 Kaplan-Meier plots of PFS in *de novo* stage IV BC patients according to baseline NLR. (A) PFS according to baseline NLR in the whole population. (B) PFS according to baseline NLR in HR-positive subgroup. (C) PFS according to baseline NLR in HER2-positive subgroup. (D) PFS according to baseline NLR in TNBC subgroup. NLR, neutrophil-to-lymphocyte ratio; PFS, progression-free survival; BC, breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

Table 3 Univariable analyses of PFS

Characteristics	N	Median PFS (months)	95% CI (months)	P value
Age (years)				0.687
≤50	54	12.5	7.4–17.4	
>50	74	11.7	9.4–14.2	
Pathological grade				0.384
Grade 1 and 2	70	13.3	9.8–16.8	
Grade 3	23	10.8	6.9–14.8	
HR				0.090
Positive	85	13.3	9.7–16.9	
Negative	43	8.5	5.7–11.3	
HER2				0.895
Positive	45	10.8	8.2–13.4	
Negative	83	13.0	9.7–16.2	
Ki-67				0.650
≤20%	26	12.0	8.9–15.1	
>20%	97	11.7	8.7–14.6	
Molecular subtype				0.112
HR^+HER2^-	70	13.3	9.7–16.9	
$HER2^+$	45	10.6	8.7–12.5	
TNBC	13	6.1	3.3–9.0	
Liver metastasis				0.002
Yes	43	9.3	6.6–11.9	
No	85	14.0	10.7–17.2	
Visceral metastasis				0.009
Yes	79	11.1	8.3–14.0	
No	49	14.8	9.9–19.7	
Number of metastatic sites				0.227
≤2	61	13.2	9.3–17.1	
>2	67	11.2	9.6–12.8	
Treatment				0.979
Chemotherapy	85	13.0	10.1–15.8	
Endocrine therapy	10	9.3	0.0–20.7	
NLR				0.004
≤2.9	77	14.8	11.9–17.8	
>2.9	51	7.2	3.5–10.9	

PFS, progression-free survival; CI, confidence interval; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; NLR, neutrophil-to-lymphocyte ratio.

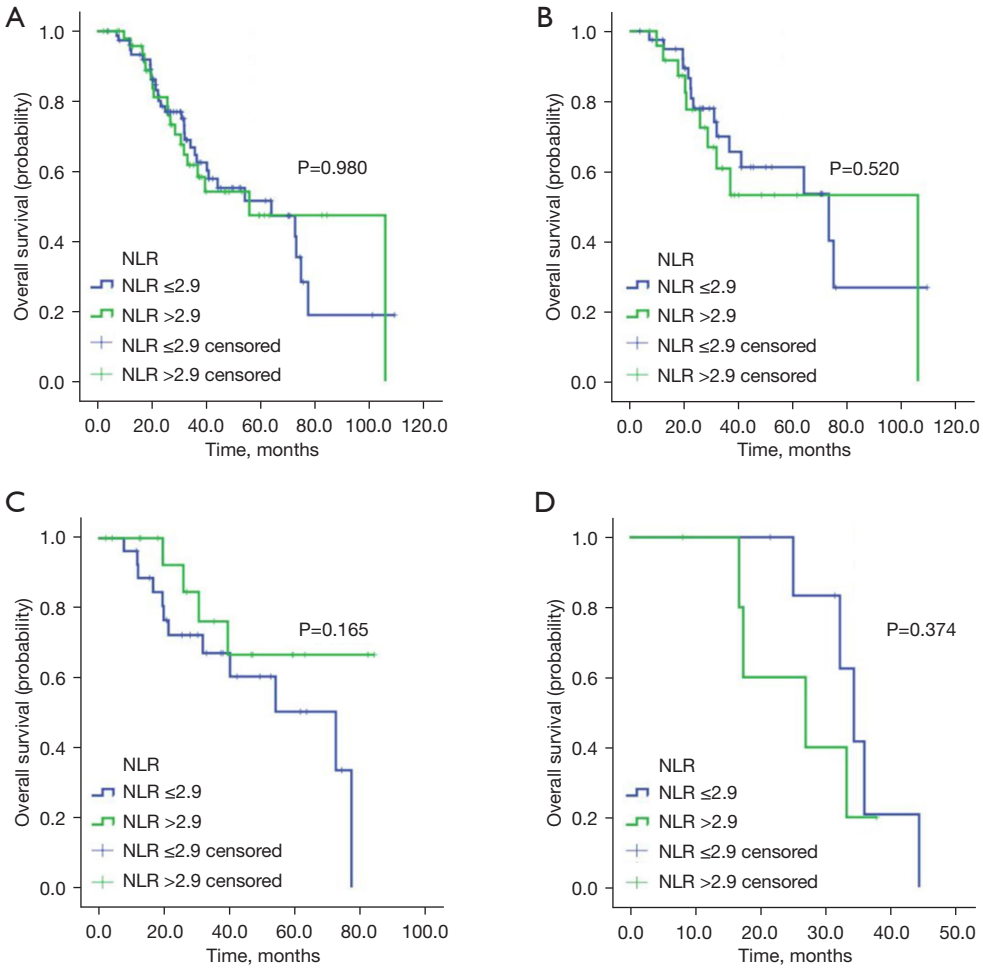

Figure 3 Kaplan-Meier plots of OS in *de novo* stage IV BC patients according to baseline NLR. (A) OS according to baseline NLR in the whole population. (B) O according to baseline NLR in HR-positive subgroup. (C) OS according to baseline NLR in HER2-positive subgroup. (D) OS according to baseline NLR in TNBC subgroup. NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; BC, breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer.

and Treg lymphocytes based on the synthesis of a specific cytokine profile (32). Tregs, which typically express CD25, are naturally present in the immune system, accounting for 5–10% of $CD4^+$ T cells; they are important in the control of immune responses by suppressing T cell proliferation and cytokine production and serve as regulatory factors in the tumor microenvironment (TME) by inhibiting $CD8^+$ T, NK, B, and antigen-presenting cells (APCs) (33-35). Tregs also secrete immunomodulatory cytokines [interleukin (IL)-10, transforming growth factor (TGF)-β, and IL-35], express granzyme/perforin, consume IL-2, and degrade adenosine triphosphate (ATP) (36,37).

With its endogenous antitumor response, higher levels of TILs were associated with a longer OS in early-stage BC independent of other prognostic clinicopathological parameters (38). The immune landscape of MBC has remained largely unexplored. As mentioned above, the TME in metastatic tumors appears to be in an inert status, compared to early-stage tumors, by showing lower TILs levels and depleted immune functions (downregulation of immune-activating molecules and the upregulation of those with immunosuppressive properties) (39-41). In our study, we found significantly higher levels of TILs in the NLR-low group (P=0.025).

Conclusions

In conclusion, our results suggest that the baseline NLR

Table 4 Univariable analyses of OS

Characteristics	N	Median OS (months)	95% CI (months)	P value
Age (years)				0.332
≤50	54		–	
>50	74	56.0	33.0–79.0	
Pathological grade				0.240
Grade 1 and 2	70	64.1	30.6–97.6	
Grade 3	23	34.4	29.2–39.5	
HR				0.237
Positive	85	64.1	42.0–86.2	
Negative	43	40.4	27.4–53.5	
HER2				0.277
Positive	45	72.8	51.1–94.6	
Negative	83	44.4	21.0–67.7	
Ki-67				0.561
≤20%	26	106.0	–	
>20%	97	44.4	13.1–75.6	
Molecular subtype				0.065
HR^+HER2^-	70	73.3	29.1–117.4	
$HER2^+$	45	72.8	51.0–94.6	
TNBC	13	33.2	24.3–42.2	
Liver metastasis				0.090
Yes	43	40.4	7.9–72.9	
No	85	64.1	32.1–96.1	
Visceral metastasis				0.006
Yes	79	40.4	21.7–59.1	
No	49	106.0	–	
Number of metastatic sites				0.129
≤2	61	73.3	60.3–86.2	
>2	67	41.0	24.5–57.4	
Treatment				0.754
Chemotherapy	85	56.0	25.9–86.1	
Endocrine therapy	10		–	
NLR				0.980
≤2.9	77	64.1	32.3–95.9	
>2.9	51	56.0	33.2–78.8	

OS, overall survival; CI, confidence interval; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; NLR, neutrophil-to-lymphocyte ratio.

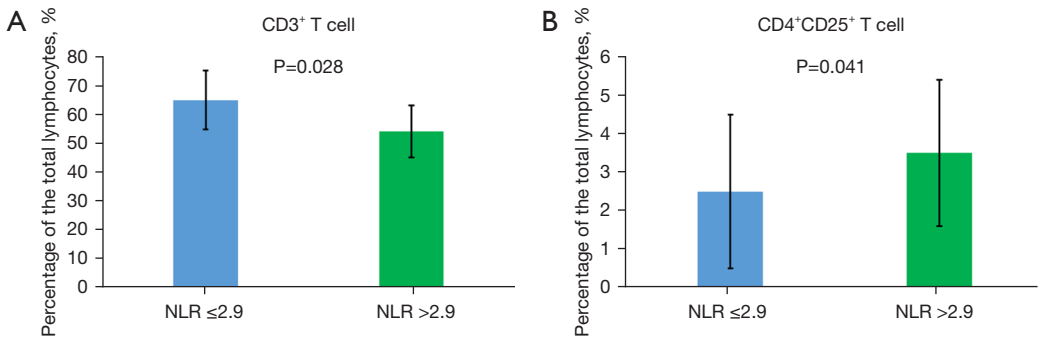

Figure 4 Peripheral blood total $CD3^+$ T cells (A) and $CD4^+CD25^+$ T cell (B) percentage in *de novo* stage IV BC patients according to baseline NLR. Data are presented as the percentage of cells mean of n=52 and n=35 patients for the NLR-high and NLR-low groups, respectively. NLR, neutrophil-to-lymphocyte ratio; BC, breast cancer.

Table 5 Peripheral lymphocyte subtypes distribution according to NLR

Peripheral lymphocyte subtypes (phenotype)	NLR		P value
	≤2.9 (n=52)	>2.9 (n=35)	
$CD3^+$ T cell	65.0±10.2	54.1±9.1	0.028
$CD3^+CD4^+$ T cell	33.1±6.2	28.4±7.2	0.068
$CD3^+CD8^+$ T cell	28.4±8.7	26.2±10.7	0.158
$CD4^+CD25^+$ T cell	2.5±2.0	3.5±1.9	0.041
$CD8^+CD28^+$ T cell	12.8±5.9	11.4±6.1	0.727
$CD8^+CD28^-$ T cell	19.1±8.9	20.2±9.2	0.587
$CD3^-CD16^+CD56^+$ NK cell	13.9±7.9	13.5±8.5	0.797
$CD19^+$ B cell	14.8±6.3	14.6±6.6	0.698

The data are presented as mean ± standard deviation. NLR, neutrophil-to-lymphocyte ratio; NK, natural killer.

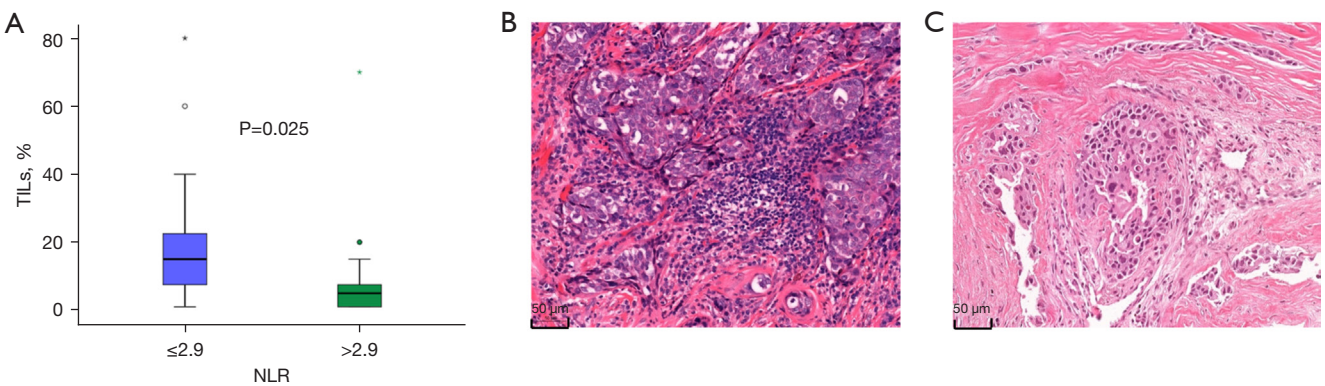

Figure 5 TILs in *de novo* stage IV BC patients according to baseline NLR. (A) The TILs ratio according to baseline NLR in 15 cases with NLR-low (≤2.9) and 15 cases with NLR-high (>2.9). (B) Immunohistochemistry stained by HE of high TILs with low NLR. (C) Immunohistochemistry stained by HE of low TILs with high NLR. Magnification times: ×400. "*", "°", special high value of TIL ratio. TILs, tumor infiltrating lymphocytes; NLR neutrophil-to-lymphocyte ratio; BC, breast cancer; HE, hematoxylin and eosin.

is a prognostic factor for PFS in *de novo* stage IV BC with first line treatment, especially in HER2 positive and TNBC subtypes. A NLR-low status may indicate immune activation at the baseline with activated immune profiles (high $CD3^+$ T cells, low $CD4^+CD25^+$ Tregs and high TILs). These results offer evidence for taking NLR into consideration when making treatment decisions in the first line setting in *de novo* stage IV BC patients. Future prospective and larger scale studies will help to validate these findings.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5612/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5612/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5612/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of the Peking University Cancer Hospital (No. 2017KT40) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Individual consent for this retrospective analysis was waived.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Heller DR, Chiu AS, Farrell K, et al. Why Has Breast Cancer Screening Failed to Decrease the Incidence of de Novo Stage IV Disease? Cancers (Basel) 2019;11:500.
3. Chia SK, Speers CH, D'yachkova Y, et al. The impact of new chemotherapeutic and hormone agents on survival in a population-based cohort of women with metastatic breast cancer. Cancer 2007;110:973-9.
4. Largillier R, Ferrero JM, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast cancer. Ann Oncol 2008;19:2012-9.
5. Bertaut A, Mounier M, Desmoulins I, et al. Stage IV breast cancer: a population-based study about prognostic factors according to HER2 and HR status. Eur J Cancer Care (Engl) 2015;24:920-8.
6. Leone BA, Vallejo CT, Romero AO, et al. Prognostic impact of metastatic pattern in stage IV breast cancer at initial diagnosis. Breast Cancer Res Treat 2017;161:537-48.
7. Balachandran VP, Gonen M, Smith JJ, et al. Nomograms in oncology: more than meets the eye. Lancet Oncol 2015;16:e173-80.
8. Forget P, Machiels JP, Coulie PG, et al. Neutrophil:lymphocyte ratio and intraoperative use of ketorolac or diclofenac are prognostic factors in different cohorts of patients undergoing breast, lung, and kidney cancer surgery. Ann Surg Oncol 2013;20 Suppl 3:S650-60.
9. 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;16:55-9.
10. Pistelli M, De Lisa M, Ballatore Z, 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.
11. Corbeau I, Jacot W, Guiu S. Neutrophil to Lymphocyte Ratio as Prognostic and Predictive Factor in Breast Cancer Patients: A Systematic Review. Cancers (Basel) 2020;12:958.
12. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
13. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic

role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 2014;106:dju124.
14. Azab B, Shah N, Radbel J, et al. Pretreatment neutrophil/lymphocyte ratio is superior to platelet/lymphocyte ratio as a predictor of long-term mortality in breast cancer patients. Med Oncol 2013;30:432.
15. Chen J, Deng Q, Pan Y, et al. Prognostic value of neutrophil-to-lymphocyte ratio in breast cancer. FEBS Open Bio 2015;5:502-7.
16. Iwase T, Sangai T, Sakakibara M, et al. An increased neutrophil-to-lymphocyte ratio predicts poorer survival following recurrence for patients with breast cancer. Mol Clin Oncol 2017;6:266-70.
17. Miyagawa Y, Araki K, Bun A, et al. Significant Association Between Low Baseline Neutrophil-to-Lymphocyte Ratio and Improved Progression-free Survival of Patients With Locally Advanced or Metastatic Breast Cancer Treated With Eribulin But Not With Nab-Paclitaxel. Clin Breast Cancer 2018;18:400-9.
18. Takuwa H, Tsuji W, Yamamoto Y, et al. Low neutrophil-lymphocyte ratio correlates with extended survival in patients with metastatic breast cancer who achieved clinically complete response following multidisciplinary therapy: A retrospective study. Oncol Lett 2018;15:6681-7.
19. Iimori N, Kashiwagi S, Asano Y, et al. Clinical Significance of the Neutrophil-to-Lymphocyte Ratio in Endocrine Therapy for Stage IV Breast Cancer. In Vivo 2018;32:669-75.
20. Vernieri C, Mennitto A, Prisciandaro M, et al. The neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios predict efficacy of platinum-based chemotherapy in patients with metastatic triple negative breast cancer. Sci Rep 2018;8:8703.
21. Imamura M, Morimoto T, Egawa C, et al. Significance of baseline neutrophil-to-lymphocyte ratio for progression-free survival of patients with HER2-positive breast cancer treated with trastuzumab emtansine. Sci Rep 2019;9:1811.
22. Yamamura J, Kamigaki S, Fujita J, et al. The Difference in Prognostic Outcomes Between De Novo Stage IV and Recurrent Metastatic Patients with Hormone Receptor-positive, HER2-negative Breast Cancer. In Vivo 2018;32:353-8.
23. Martin AM, Cagney DN, Catalano PJ, et al. Brain Metastases in Newly Diagnosed Breast Cancer: A Population-Based Study. JAMA Oncol 2017;3:1069-77.
24. Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014;25:1544-50.
25. Yao M, Liu Y, Jin H, et al. Prognostic value of preoperative inflammatory markers in Chinese patients with breast cancer. Onco Targets Ther 2014;7:1743-52.
26. Inoue Y, Fujishima M, Ono M, et al. Clinical significance of the neutrophil-to-lymphocyte ratio in oligometastatic breast cancer. Breast Cancer Res Treat 2022;196:341-8.
27. Liu Y, He M, Wang C, et al. Prognostic value of neutrophil-to-lymphocyte ratio for patients with triple-negative breast cancer: A meta-analysis. Medicine (Baltimore) 2022;101:e29887.
28. Takamizawa S, Shimoi T, Satomi-Tsushita N, et al. Neutrophil-to-lymphocyte ratio as a prognostic factor for patients with metastatic or recurrent breast cancer treated using capecitabine: a retrospective study. BMC Cancer 2022;22:64.
29. Gao C, Li H, Liu C, et al. Tumor Mutation Burden and Immune Invasion Characteristics in Triple Negative Breast Cancer: Genome High-Throughput Data Analysis. Front Immunol 2021;12:650491.
30. Seo AN, Lee HJ, Kim EJ, et al. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. Br J Cancer 2013;109:2705-13.
31. Hiraoka K, Miyamoto M, Cho Y, et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer 2006;94:275-80.
32. Golubovskaya V, Wu L. Different Subsets of T Cells, Memory, Effector Functions, and CAR-T Immunotherapy. Cancers (Basel) 2016;8:36.
33. Jafarzadeh A, Fooladseresht H, Minaee K, et al. Higher circulating levels of chemokine CCL22 in patients with breast cancer: evaluation of the influences of tumor stage and chemokine gene polymorphism. Tumour Biol 2015;36:1163-71.
34. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol 2002;2:389-400.
35. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature 2001;411:375-9.
36. Takeuchi Y, Nishikawa H. Roles of regulatory T cells in cancer immunity. Int Immunol 2016;28:401-9.
37. Jafarzadeh A, Jamali M, Mahdavi R, et al. Circulating levels of interleukin-35 in patients with multiple sclerosis: evaluation of the influences of FOXP3 gene polymorphism

and treatment program. J Mol Neurosci 2015;55:891-7.
38. Mahmoud SM, Paish EC, Powe DG, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011;29:1949-55.
39. Adams S, Schmid P, Rugo HS, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. Ann Oncol 2019;30:397-404.
40. Loi S, Giobbie-Hurder A, Gombos A, et al. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b-2 trial. Lancet Oncol 2019;20:371-82.
41. Szekely B, Bossuyt V, Li X, et al. Immunological differences between primary and metastatic breast cancer. Ann Oncol 2018;29:2232-9.

(English Language Editor: J. Jones)

Cite this article as: Shao B, Li H, Liu X, Song G, Jiang H, Yan Y, Zhang R, Ran R, Zhang J, Liu Y, Wang H, Wang J, Di L. The prognostic value of neutrophil-to-lymphocyte ratio in *de novo* stage IV breast cancer: a retrospective cohort study. Ann Transl Med 2023;11(2):45. doi: 10.21037/atm-22-5612