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Pre-treatment neutrophil-to-lymphocyte ratio is associated with neutrophil and T-cell infiltration and predicts clinical outcome in patients with glioblastoma

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Abstract

Background: Markers of systemic inflammation are correlated with patient survival in various cancers. The prognostic value of neutrophil-to-lymphocyte ratio (NLR) was compared with that of platelet-to-lymphocyte ratio (PLR) in patients with glioblastoma. The association of NLR with neutrophil and T- cell infiltration was also explored.

Methods: A total of 152 patients with glioblastoma were retrospectively analyzed. Clinical information was obtained from electronic medical records. Kaplan-Meier analysis and the Cox proportional hazards models were used to examine the survival function of pre-treatment NLR and PLR in these glioblastoma patients. Neutrophil and $CD3^+$ T-cell infiltration was assessed by immunohistochemical staining of tissue microarray cores from glioblastomas.

Results: Pre-treatment NLR levels were significantly correlated with overall survival (OS) in glioblastoma patients (multivariate hazard ratio =1.050; 95 % confidence interval, 1.003–1.100; $P = 0.037$). Despite the correlation between NLR and PLR ($R = 0.509$, $P < 0.001$), NLR was superior to PLR as a prognostic factor. High pre-treatment NLR (≥4 versus < 4) was significantly associated with high neutrophil infiltration and low $CD3^+$ T-cell infiltration into tumors, and predicted poor OS (mean, 10.6 *vs.* 17.9 months, $P < 0.001$).

Conclusions: Pre-treatment NLR is of prognostic significance independent of MGMT status and is superior to PLR as a prognostic factor. Our results demonstrate a correlation between elevated peripheral blood NLR levels and increased tumor neutrophil infiltration/decreased $CD3^+$ T-cell infiltration.

Background

Glioblastoma is the most common malignant primary brain tumor in adults [1]. Currently, the standard treatment for glioblastoma patients is debulking surgery combined with radiotherapy and temozolomide (TMZ) chemotherapy [2]. Despite receiving the same treatment, glioblastoma patients show significant variation in their clinical outcomes because of the heterogeneity of the tumors and multiple systemic factors [3]. Therefore, prognostic markers that can guide individual adjuvant therapy and follow-up schedule are urgently needed. Prognostic markers in the peripheral blood are of considerable clinical value because of their accessibility [4].

Tumor-infiltrating immune cells, including neutrophils and lymphocytes, play an important role in glioblastoma progression and prognosis [5–10], reflecting the significance of local inflammatory factors. However, the heterogeneity in the amounts and the spatial localization of tumor-infiltrating immune cells, both between and within the patients' tumors, limits their clinical use as prognostic markers [7, 11]. Recently, the prognostic value of neutrophil-to-lymphocyte ratio (NLR) in peripheral blood, a marker of systemic inflammatory responses, was identified in glioblastoma [12, 13] and in many other types of cancer [14–16]. In patients with glioblastoma, NLR >4 is an independent prognostic indicator of poor outcome [12, 13]. These results raise the question

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of whether another inflammatory marker, the platelet-to-lymphocyte ratio (PLR) [15, 17], also has prognostic value in glioblastomas. Therefore, the influence of dynamic changes on the prognostic significance of NLR should be further examined, and the correlation between local and systemic inflammatory markers should be explored. In addition, the potential effects of the molecular marker *O*-methylguanine-DNA methyltransferase (MGMT) on the prognostic role of NLR remain unclear. In the present study, we retrospectively analyzed 152 patients with glioblastoma treated in our neurosurgical center to evaluate the prognostic value of NLR and PLR. The association of NLR with neutrophil and T-cell infiltration was also assessed.

Methods

Study population

Clinical samples and patient records corresponding to 217 consecutive patients diagnosed with glioblastoma at the Neurosurgery Department of the First Hospital of China Medical University between January 2010 and October 2014 were examined. Patients with other diseases, including diabetes mellitus, metabolic syndrome [18, 19], heart disease (acute coronary syndromes, rheumatic or congenital heart disease and cardiomyopathy), hypertension [20–22], severe renal or hepatic dysfunction, other cancers, inflammatory diseases, previous history of infection within 3 months and any medication usage related to inflammatory conditions that could significantly influence NLR or survival or those lacking complete data were excluded. Finally, 152 newly diagnosed patients were included in the analysis. Patients underwent surgical resection by neurosurgeons, who used similar operational techniques and principles. Tumor samples were immediately snap-frozen in liquid nitrogen after resection. One part of each sample was fixed with formalin, embedded with paraffin wax and, kept at room temperature. Glioblastomas were diagnosed by two neuropathologists according to the World Health Organization 2007 criteria. All patients received postoperative radio-chemotherapy according to the Stupp protocol [2].

Overall survival (OS) was defined as the interval between surgery and death from glioblastoma. The median follow-up period was 13 months (range, 1–53 months), during which 123 (80.9 %) patients died from glioblastoma. Data were censored at the last follow-up for patients who were alive at the time of the analysis. The present study was approved by the institutional review board of The First Hospital of China Medical University, and written informed consent was obtained from all glioma tissue donors who consented to the use of the tumor tissue and clinical data for future research. The research was in compliance with the Helsinki Declaration.

Blood examination

Complete blood count was obtained preoperatively before any treatment (e.g., steroids) and repeated the first day after surgery as previously described [23]. Blood samples were tested by the staff at the Department of Clinical Laboratory within 2 h of collection using a Sysmex XE-2100 complete blood count analyzer (Sysmex, Kobe, Japan). The analysis included blood neutrophil, lymphocyte and platelet counts. The normal reference range is $1.8–6.3 \times 10^9$/L for neutrophils, $1.1–3.2 \times 10^9$/L for lymphocytes, and $125–350 \times 10^9$/L for platelets. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count, and the PLR was defined as the absolute platelet count divided by the absolute lymphocyte count.

Tissue microarrays and immunohistochemistry

Tissue microarrays were constructed using 152 glioblastoma clinical samples and analyzed by immunohistochemical staining as previously described [5, 24]. Samples were collected from the most phenotypically representative tumor regions. Diluted primary antibodies against human myeloperoxidase (MPO; ab134132, 1:250; Abcam, Cambridge, UK), CD15 (ab754, 1:50; Abcam) or CD3 (ab16669, 1:100; Abcam) were used and incubated overnight at 4 °C. Samples were then incubated with the horseradish peroxidase labeled secondary antibody in the immunohistochemical kit (KIT-5930, MaxVision, Fu Zhou, China) for 30 min at room temperature. Diaminobenzidine was used for color development and hematoxylin as counterstain. Results were visualized and photographed under a light microscope (Olympus BX-51; Olympus Optical Co., Ltd., Tokyo, Japan).

Semi-quantitative evaluation was performed by examining each section using at least ten randomly selected different high-power fields (HPF). Neutrophils and T-cells were identified as cells staining positive for anti-MPO and anti-CD3 antibodies, respectively, together with their characteristic morphology. Neutrophils were also verified by anti-CD15 staining. The number of infiltrating neutrophils and T-cells was manually counted independently by two investigators (YL and ZL) and an experienced neuropathologist (QL) blinded to the clinical background of the patients. The mean number of neutrophils and T-cells per field was calculated. The following scoring system was used: 0, <10 cells per field; 1, 10–20 cells per field; 2, 20–50 cells per field; 3, 50–100 cells per field; 4, 100 or more cells per field [8]. When strong differences in scoring between observers occurred, the core was re-evaluated to reach a concordant scoring [5].

MGMT promoter methylation status

Methylation-specific PCR (MSP) was performed as previously described [5, 25] to detect MGMT promoter

methylation. Briefly, tissue samples were lysed with 490 μl lysis buffer containing 20 mM Tris-Cl (pH 8.0), 5 mM EDTA (pH 8.0), 400 mM NaCl and 1 % (w/v) SDS, and digested with 10 μl proteinase K at 10 mg/ml at 37 °C for 12 h. Genomic DNA was purified from the lysate by phenol/chloroform extraction. One microgram of DNA was denatured by NaOH and modified by sodium bisulfite. MSP was performed using primer sequences for MGMT as follows: 5′-TTT GTG TTT TGA TGT TTG TAG GTT TTT GT-3′ (forward) and 5′-AAC TCC ACA CTC TTC CAA AAA CAA AAC A-3′ (reverse) for the unmethylated reaction; and 5′-TTT CGA CGT TCG TAG GTT TTC GC-3′ (forward) and 5′-GCA CTC TTC CGA AAA CGA AAC G-3′ (reverse) for the methylated reaction. Each PCR reaction (10 μl) was loaded onto nondenaturing 6 % polyacrylamide gels, stained with ethidium bromide, and visualized under UV illumination. The PCR reaction was repeated at least three times.

Statistical analysis

Univariate and multivariate Cox proportional hazards models were constructed. Sex, age, tumor size, pre-treatment Karnofsky performance status (KPS), degree of resection, body mass index (BMI), MGMT promoter methylation, NLR, PLR, neutrophil count, platelet count and lymphocyte count were included in the analysis. To adjust for potential confounders, age, tumor size, KPS, BMI, NLR, PLR, neutrophil count, platelet count and lymphocyte count were used as continuous variables and all of the other covariates were used as categorical variables. Tumor size was calculated based on preoperative MRI scans as follows: longest diameter × widest diameter × thickness (section thickness × the number of layers) × 1/2. MGMT promoter methylation status was dichotomized (methylation vs. unmethylation). According to previous reports [5, 26], tumor resection was defined as follows: (0) biopsy or residual tumor >30 %, (1) subtotal resection with residual tumor <30 %, and (2) gross total resection. The NLR was also analyzed as a dichotomous variable, according to previous data where a NLR ≥4 (versus NLR <4) conferred a worse prognosis [12, 13]. Kaplan-Meier survival analysis was used to determine the distribution of OS time, and the results were analyzed with the log-rank test.

Pearson correlation analysis was used to examine the correlation between clinical variables. The chi-square test and ANOVA were used to determine statistical significance. The Student's *t*-test or Mann–Whitney *U*-test was used for variables with parametric distribution or non-parametric distribution, respectively. Statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA). A two-tailed *P*-value of <0.05 was regarded as significant.

Results

Clinicopathologic data of the 152 glioblastoma patients are summarized in Table 1; 106 (69.7 %) patients were under and 46 (30.3 %) were over 60 years of age. The KPS was 70–100 in 102 (67.1 %) patients and <70 in 50 (32.9 %) patients; 95 patients (62.5 %) were male. The mean OS was 15.6 ± 11.2 months. The corresponding 1- and 2-year survival rates were 56.6 and 22.4 %, respectively.

In the present study, the mean pre-treatment neutrophil, platelet and lymphocyte counts were $5.9 \pm 3.6 \times 10^9$/L (range, $1.2–18.6 \times 10^9$/L), $222.7 \pm 61.3 \times 10^9$/L (range, $113–413 \times 10^9$/L) and $1.8 \pm 0.7 \times 10^9$/L (range, $0.7–4.6 \times 10^9$/L), respectively. The mean pre-treatment NLR was 4.1 ± 3.8 (median, 2.54; range, 0.7–20.6), and the pre-treatment PLR was 135.0 ± 57.1 (median, 122.1; range, 46.6–311.5). As shown in Table 1, the pre-treatment NLR did not vary significantly with sex, age, tumor size, KPS, degree of resection, BMI and MGMT promoter methylation status.

Pre-treatment NLR and the survival of glioblastoma patients

Next, we examined the survival function of pre-treatment NLR in glioblastoma patients. Univariate and multivariate Cox regression analyses showed that pre-treatment NLR was an independent predictor of OS (multivariate hazard ratio = 1.050, 95 % confidence interval 1.003–1.100, $P = 0.037$, Table 2). As shown in Fig. 1a and b, Patients with high NLR (≥4) had lower 1-year and 2-year survival rates than those with low NLR (<4). The OS of patients with high NLR (≥4) was also shorter than that of patients with low NLR (<4; mean 10.6 *vs.* 17.9 months, $P < 0.001$; Fig. 1c). When the median pre-treatment NLR (2.54) was used as the cutoff point, similar results were obtained (Fig. 1d).

Multivariate analysis showed that KPS and MGMT promoter methylation were also independently associated with OS in glioblastoma (Table 2). In keeping with prior literature and prognostic nomograms [27], variables including age (≤60 vs. >60) and KPS (≤70 vs. 80–100) were also analyzed as categorical variables. As shown in Tables 1 and 3, similar results were obtained. We further examined the influence of pre-treatment NLR on OS across strata of other potential predictors, including age, KPS, degree of resection, and MGMT promoter methylation status. Pre-treatment NLR was an independent prognostic factor in all the subgroups (Fig. 2a).

However, postoperative NLR was not associated with patient outcome in glioblastoma (Table 2). After surgery, NLR increased significantly (mean ± SD: pre-treatment 4.1 ± 3.8 *vs.* postoperative 7.0 ± 6.7, $P < 0.001$; Fig. 2b) because of the effect of treatment, and so did PLR (pre-

Table 1 Clinical and molecular characteristics according to pre-treatment NLR in 152 glioblastoma cases

Clinical or Molecular Feature	All Cases		Pre-treatment NLR				*P*
			<4		≥4		
	No.	%	No.	%	No.	%	
Total No. of patients	152	100	103	67.8	49	32.2	
Sex							0.823
Male	95	62.5	65	68.4	30	31.6	
Female	57	37.5	38	66.7	19	33.3	
Age, years							0.136
Mean ± SD	50.4 ± 15.4		49.2 ± 16.2		53.1 ± 13.1		
≤ 60	112	73.7	80	71.4	32	28.6	0.118
> 60	40	26.3	23	57.5	17	42.5	
Tumor size, cm^3							0.920
Mean ± SD	60.6 ± 26.6		60.4 ± 26.1		61.0 ± 28.0		
KPS							0.212
Mean ± SD	71.4 ± 14.2		70.4 ± 15.2		73.5 ± 11.6		
≤ 70	78	51.3	54	69.2	24	30.8	0.731
80–100	74	48.7	49	66.2	25	33.8	
Resection							0.659
Biopsy	38	25.0	28	73.7	10	26.3	
Subtotal	39	25.7	26	66.7	13	33.3	
Gross total	75	49.3	49	65.3	26	34.7	
BMI, kg/m^2							0.150
Mean ± SD	24.2 ± 3.2		23.9 ± 3.3		24.8 ± 3.1		
MGMT promoter							0.975
Methylated	53	34.9	36	67.9	17	32.1	
Unmethylated	99	65.1	67	67.7	32	32.3	
Pre-treatment PLR							*<0.001*
Mean ± SD	135.0 ± 57.1		111.4 ± 34.7		184.8 ± 62.9		
OS, months							*<0.001*
Mean ± SD	15.6 ± 11.2		17.9 ± 11.0		10.6 ± 9.8		

The differences between patients with pre-treatment NLR <4 and ≥4 were compared. *P* value was calculated using chi-square test for categorical variables and using Student's *t*-test for continuous variables

BMI Body Mass Index, *KPS* Karnofsky Performance Scores, *MGMT* O(6)-methylguanine-DNA-methyltransferase, *NLR* Neutrophil to Lymphocyte Ratio, *OS* Overall Survival, *PLR* Platelet to Lymphocyte Ratio

treatment 135.0 ± 57.1 *vs.* postoperative 177.7 ± 123.9, $P < 0.001$; Fig. 2c), which may affect their prognostic value. Moreover, consistent with a previous study [12], pre-treatment neutrophil count, lymphocyte count, and platelet count were not independently correlated with patient survival (Table 2).

Pre-treatment NLR is superior to PLR as a prognostic factor in glioblastoma

In the present study, we observed a significant correlation between the two systemic inflammatory markers, pre-treatment NLR and PLR ($R = 0.509$, $P < 0.001$; Table 1 and Fig. 3). In univariate analysis, both NLR and PLR (univariate hazard ratio = 1.004, 95 % confidence interval 1.001–1.007, $P = 0.013$, Tables 2 and 3) were associated with patient survival. However, in multivariate analysis, the prognostic significance of PLR was markedly diminished (Tables 2 and 3).

Pre-treatment NLR and immune cell infiltration

Tissue microarrays were used to assess neutrophil and T-cell infiltration using immunohistochemical staining in 152 glioblastomas. To evaluate the association between pre-treatment NLR and immune cell infiltration, we semi-quantified the infiltrating neutrophils and $CD3^+$ T-cells. The level of neutrophil infiltration was significantly positively correlated with pre-treatment NLR level (NLR < 4 vs. NLR ≥ 4; $P < 0.001$), whereas $CD3^+$ T-cell

Table 2 Univariate and multivariate analyses of different prognostic parameters for overall survival of 152 glioblastoma patients

Variable	Univariate			Multivariate		
	P	HR	95 % CI	*P*	HR	95 % CI
Sex[a]	0.704	0.930	0.641–1.351	-	-	-
Age[b]	0.050	1.013	1.000–1.026	0.157	1.010	0.996–1.024
Tumor size[b]	0.559	0.998	0.991–1.005	-	-	-
KPS[b]	0.034	0.986	0.973–0.999	0.023	0.985	0.972–0.998
Resection[a]	0.047	0.800	0.641–0.997	0.183	0.860	0.688–1.074
BMI[b]	0.206	1.033	0.982–1.086	-	-	-
MGMT promoter[a]	0.011	0.605	0.411–0.890	0.041	0.659	0.442–0.984
Pretreatment PLR[b]	0.013	1.004	1.001–1.007	0.152	1.003	0.999–1.007
Postoperative NLR[b]	0.285	1.015	0.988–1.043	-	-	-
Pretreatment neutrophils[b]	0.620	1.010	0.971–1.051	-	-	-
Pretreatment lymphocytes[b]	0.531	0.999	0.996–1.002	-	-	-
Pretreatment platelets[b]	0.637	0.999	0.997–1.002	-	-	-
Pretreatment NLR[b]	<0.001	1.078	1.038–1.119	0.037	1.050	1.003–1.100

BMI Body Mass Index, *KPS* Karnofsky Performance Scores, *MGMT* O(6)-methylguanine-DNA-methyltransferase, *NLR* Neutrophil to Lymphocyte Ratio, *PLR* Platelet to Lymphocyte Ratio
[a]categorical variable; [b]continuous variable

infiltration level was negatively correlated with pre-treatment NLR level ($P = 0.006$; Table 4). According to previous data and our results, a number of neutrophils ≥10/200 × HPF [8, 9] and a number of $CD3^+$ T-cells ≥20/400 × HPF [7, 11] were used as cutoff points to define a high infiltration group and a low infiltration group. High neutrophil infiltration and low $CD3^+$ T-cell infiltration were more frequent in patients with pre-treatment NLR ≥4 than in those with pre-treatment NLR < 4 (69.4 *vs.* 36.9 %, $P < 0.001$ and 59.2 *vs.* 38.8 %, $P = 0.019$, respectively (Fig. 4a-c).

Kaplan-Meier analysis showed that increased neutrophil infiltration was correlated with shorter survival (log-rank, $P = 0.016$) (Fig. 4d-e). However, no association between $CD3^+$ T-cell infiltration and OS was observed (log-rank $P = 0.304$) (Fig. 4f), which could be attributed to the complexity of its components.

Discussion

The identification of prognostic factors is clinically significant for glioblastoma patients and can guide clinical treatment and studies [28]. Previous studies show a strong linkage between inflammation and cancer [29, 30]. Meanwhile, inflammatory markers have been associated with patient prognosis in glioblastoma [5, 7]. As a marker of systemic inflammation, pre-treatment NLR has been recognized as a prognostic factor in glioblastomas [12, 13]. However, this prognostic effect should be re-evaluated in the era of standard therapy [2], when molecular markers such as MGMT promoter methylation are taken into consideration [28, 31]. In the present study, NLR levels did not correlate with MGMT promoter methylation status, and the prognostic role of NLR was not significantly modified by MGMT promoter methylation status, suggesting that these two prognostic factors may influence clinical outcome via different pathways and mechanisms (Tables 1, 2 and 3).

Although pre-treatment NLR was an independent predictor of clinical outcome in glioblastoma patients, postoperative NLR had no prognostic value. Postoperatively, the NRL as well as the PLR level increased significantly (Fig. 2b-c), indicating that the stress of surgery had an impact on systemic inflammation. Thus, postoperative NLR cannot reflect the baseline impact of systemic inflammation on clinical outcome in glioblastoma patients. Moreover, some diseases, such as cardiovascular diseases and infection, or drug treatments might affect neutrophil and lymphocyte counts; therefore, the ratio of these two parameters might be changed [32]. To minimize potential confounders, patients with such medical history were excluded from this study. In addition, the NLR was measured before any treatment, including steroids. Therefore, our results may reflect the influence of basic systemic inflammation, possibly induced by the tumor, on the prognosis. Consistent with a previous study, we established a positive correlation between the two markers of systemic inflammatory responses, NLR and PLR [15]. Nevertheless, the prognostic value of NLR and PLR may vary among different cancers. In esophageal cancer, PLR is a better prognostic factor than NLR [15], whereas in endometrial cancer, NLR is a better prognostic factor [33]. We showed that pre-treatment NLR was superior to PLR as a prognostic factor in patients with glioblastoma. However, the underlying mechanism needs further research.

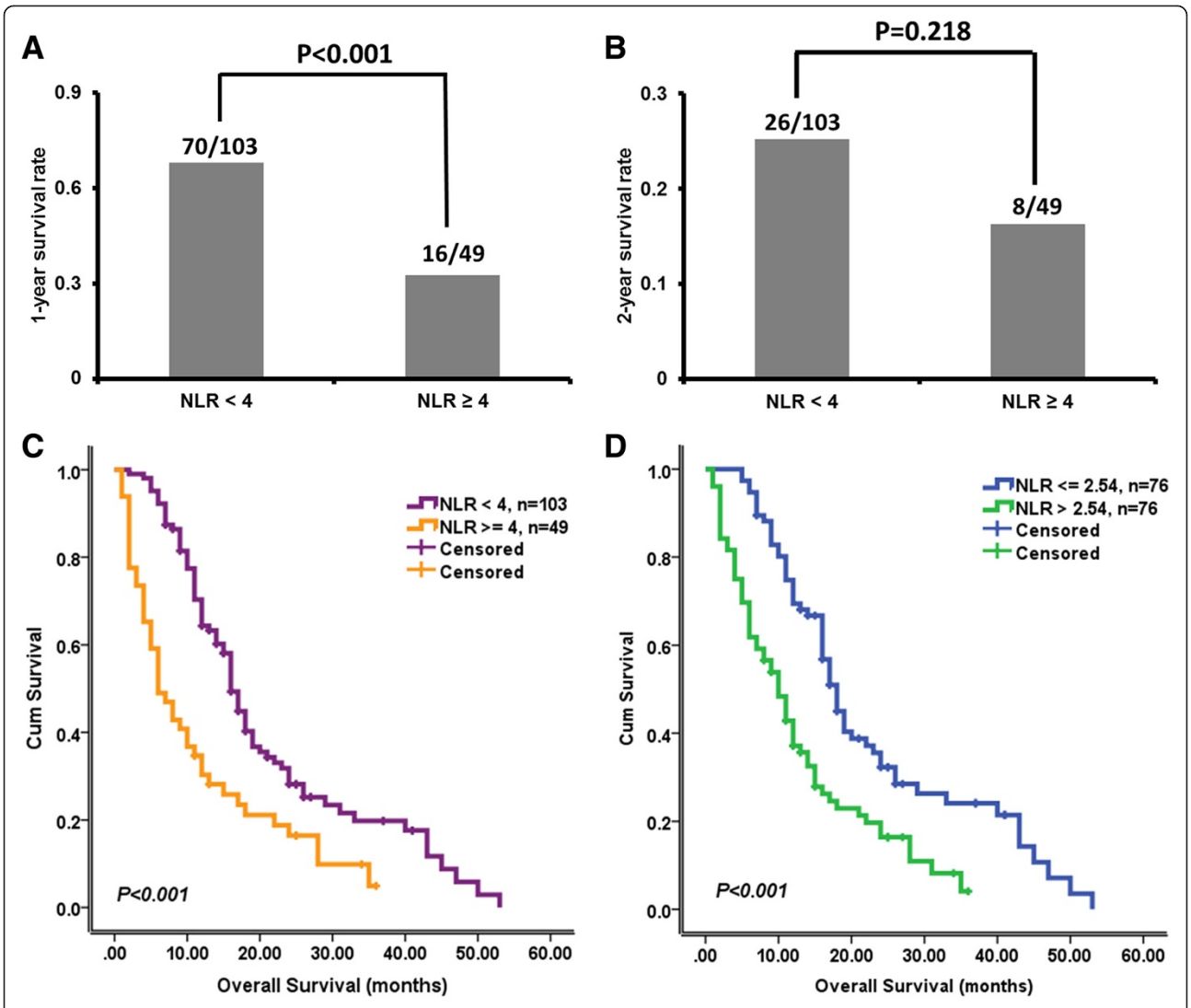

Fig. 1 Pre-treatment neutrophil-to-lymphocyte ratio (NLR) and prognosis. **a**, **b** Pre-treatment NLR levels and 1-year (**a**) and 2-year (**b**) survival rates. **c**, **d** Kaplan-Meier survival curves stratified by pre-treatment NLR levels. Survival time was significantly shorter among patients with NLR ≥4 (or >2.54) than among those with NLR <4 (or ≤2.54)

Table 3 Univariate and multivariate analyses of different prognostic parameters for overall survival of 152 glioblastoma patients

Variable	Univariate			Multivariate		
	P	HR	95 % CI	*P*	HR	95 % CI
Age (≤60 vs. >60)	0.025	1.603	1.060–2.424	0.478	1.168	0.755–1.806
KPS (≤70 vs. 80–100)	0.032	0.670	0.465–0.966	0.045	0.794	0.633–0.995
Resection (biopsy vs. STR vs. GTR)	0.047	0.800	0.641–0.997	0.110	0.738	0.509–1.071
MGMT promoter (unmethylated vs. methylated)	0.011	0.605	0.411–0.890	0.041	0.660	0.443–0.983
Pretreatment PLR (<135 vs. >135)	0.039	1.463	1.020–2.097	0.918	1.023	0.668–1.565
Pretreatment NLR (<4 vs. ≥4)	<0.001	2.139	1.464–3.125	0.002	2.068	1.304–3.277

GTR gross total resection, *KPS* Karnofsky Performance Scores, *MGMT* O(6)-methylguanine-DNA-methyltransferase, *NLR* Neutrophil to Lymphocyte Ratio, *PLR* Platelet to Lymphocyte Ratio, *STR* subtotal resection

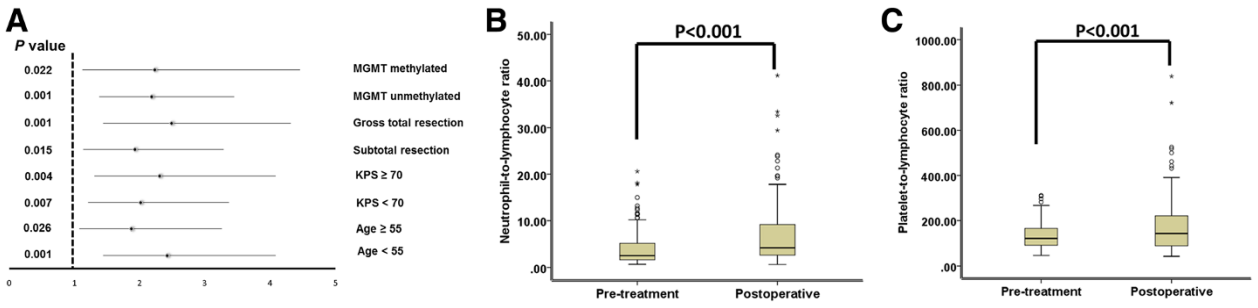

Fig. 2 Correlation between pre-treatment NLR and other clinical factors in glioblastoma patients. **a** Stratified analysis of pre-treatment NLR level and overall mortality. Hazard ratios and 95 % confidence intervals in various strata are shown. **b** Relationship between pre-treatment NLR and postoperative NLR. **c** Relationship between pre-treatment PLR and postoperative PLR. NLR and PLR were assessed as continuous variables

Definitive reasons for the association between elevated NLR and poor survival in cancer patients have not been identified to date. In the present study, we showed that high pre-treatment NLR (≥4 vs. < 4) was significantly associated with high neutrophil infiltration and low $CD3^+$ T-cell infiltration into glioblastomas. Previous data show that neutrophil infiltration plays an important role in stimulating tumor growth, angiogenesis and metastasis [34–36]. In gliomas, there is a positive correlation between tumor grade and the extent of neutrophil infiltration [9]. Liang et al. reported that increased recruitment of neutrophils promotes glioma progression and treatment resistance [8]. Blocking neutrophil infiltration has been suggested for the treatment of glioblastoma [37]. Consistently, we found a correlation of increased neutrophil infiltration with shorter survival in glioblastoma patients (Fig. 4d-e). Furthermore, neutrophils may suppress the immune function [38] by inhibiting the cytolytic activity of $CD8^+$ T-cells and natural killer cells [39, 40], and by enhancing the suppressive activities of $CD4^+$ suppressor T cells [41]. T-lymphocytes play an important role in host defenses against tumors, as they inhibit the proliferation and invasion of tumor cells via the induction of cytotoxic cell death and cytokine production [30, 42]. Moreover, Kmiecik et al. reported an association between elevated $CD3^+$ T-cell infiltration and prolonged survival in glioblastoma patients [11]. We found a negative correlation between pre-treatment NLR level and the number of infiltrating $CD3^+$ T-cells; however, $CD3^+$ T-cell infiltration was not associated with patient outcome. The complexity of $CD3^+$ T-cell subpopulations may affect its prognostic significance. While tumor-infiltrating effector T cells (cytotoxic and helper) may correlate with a better survival, the association between

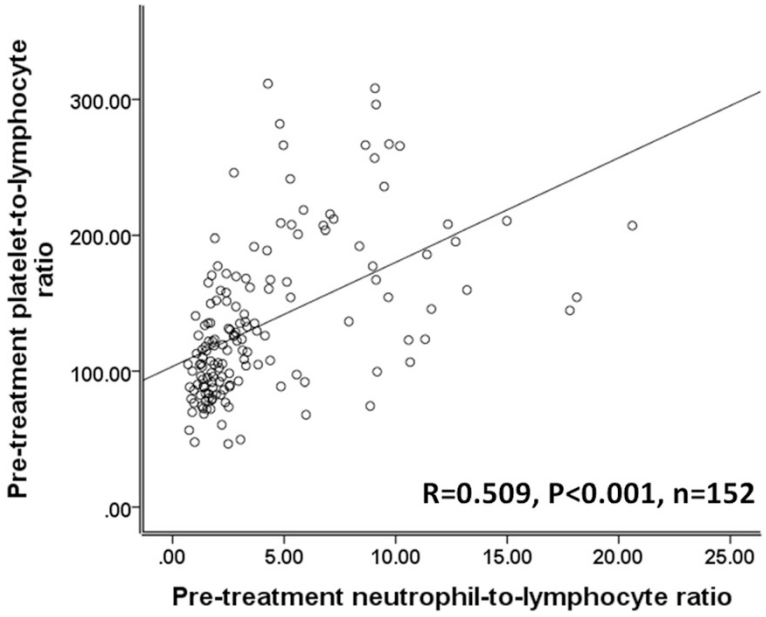

Fig. 3 Pre-treatment NLR levels were significantly correlated with pre-treatment PLR levels. NLR and PLR were assessed as continuous variables

Table 4 Correlation between neutrophils and $CD3^+$ T-cells infiltration and pre-treatment NLR in 152 Glioblastomas

	Neutrophils infiltration (200 × HPF)			$CD3^+$ T-cells infiltration (400 × HPF)		
	NLR ≥ 4 ($n = 49$)	NLR < 4 ($n = 103$)	*P*	NLR ≥ 4 ($n = 49$)	NLR < 4 ($n = 103$)	*P*
0 (<10)	15 (30.6 %)	65 (63.1 %)	<0.001	18 (36.7 %)	12 (11.7 %)	0.006
1 (10–20)	8 (16.3 %)	28 (27.2 %)		11 (22.5 %)	28 (27.2 %)	
2 (20–50)	17 (34.7 %)	7 (6.8 %)		11 (22.5 %)	37 (35.9 %)	
3 (50–100)	2 (4.1 %)	1 (1 %)		8 (16.3 %)	19 (18.4 %)	
4 (>100)	7 (14.3 %)	2 (1.9 %)		1 (2.0 %)	7 (6.8 %)	

tumor-infiltrating regulatory T cells and patient outcome remains unclear in glioblastoma [5, 7, 43, 44]. Our results indicate that, for glioblastoma patients, there could be a correlation and interaction between systemic and local inflammation, which may influence clinical outcome. In future studies, analysis of T-cell subsets would enable us to better understand this underlying relationship, and the molecular mechanism also needs to be explored.

The present study had several limitations. Firstly, the retrospective design of the study may lead to bias. Secondly, although a standard primary treatment regimen was applied, the post-progression salvage treatments were heterogeneous, which may have affected the survival analysis. Moreover, other unknown physiological and pathophysiological factors potentially affecting NLR may have influenced our analysis.

Conclusion

We showed that pre-treatment NLR was superior to PLR as a predictor of clinical outcome in patients with

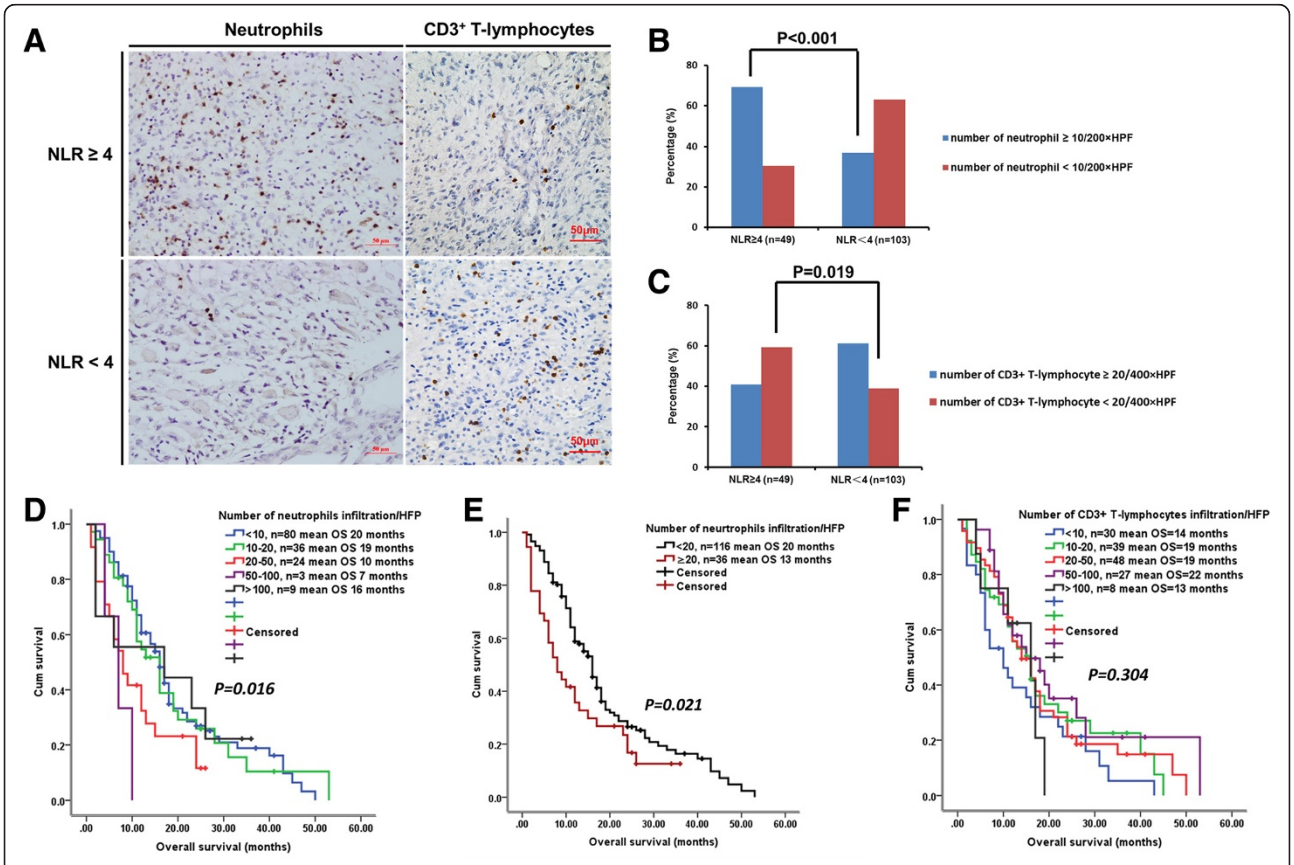

Fig. 4 Correlation between pre-treatment NLR and immune cell infiltration in glioblastomas. **a** Representative immunohistochemical images of neutrophil infiltration and $CD3^+$ T-cell infiltration in glioblastoma patients with pre-treatment NLR ≥4 and NLR <4. **b**, **c** Pre-treatment NLR ≥4 (versus NLR <4) significantly correlated with high neutrophil infiltration (**b**) and low $CD3^+$ T-cell infiltration (**c**). High neutrophil infiltration (number of neutrophils ≥10/200 × HPF) and low $CD3^+$ T-cell infiltration (number of $CD3^+$ T-cells <20/400 × HPF) were more frequent in patients with pre-treatment NLR ≥4 than in those with pre-treatment NLR < 4 (69.4 vs. 36.9 %, $P < 0.001$ and 59.2 vs. 38.8 %, $P = 0.019$, respectively). **d**-**f** Kaplan-Meier plots of overall survival, stratified by different levels of neutrophil infiltration (D and E) or $CD3^+$ T-cell infiltration (**f**). Scale bars, 50 μm

glioblastoma. NLR is of prognostic significance independent of MGMT status. Our results demonstrate a correlation between elevated peripheral blood NLR levels and increased tumor neutrophil infiltration/decreased $CD3^+$ T-cell infiltration. The association of NLR in the peripheral blood with immune cell infiltration in the tumor microenvironment provides insight into the mechanism by which NLR can predict prognosis.

Abbreviations

BMI: Body mass index; HPF: High-power fields; HR: Hazard ratio; KPS: Karnofsky performance status; MGMT: O-methylguanine-DNA methyltransferase; NLR: Neutrophil-to-lymphocyte ratio; OS: Overall survival; PLR: Platelet-to-lymphocyte ratio; TMZ: Temozolomide.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SH and AW conceived and designed the study. SH, YL, QL, ZL and HH performed the experiments and collected data. SH, YL and AW contributed to the statistical analysis and drafted the manuscript. SH and AW obtained funding. All authors read and approved the final manuscript.

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