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

Sheng Han¹, Yang Liu¹, Qingchang Li², Zhonghua Li¹, Haipei Hou¹ and Anhua Wu¹*

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 (\( \geq 4 \) 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 dy-
namic 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 mo-
olecular marker O-methylguanine-DNA methyltransfer-
ase (MGMT) on the prognostic role of NLR remain
unclear. In the present study, we retrospectively ana-
lyzed 152 patients with glioblastoma treated in our
neurosurgical center to evaluate the prognostic value
of NLR and PLR. The association of NLR with neutro-
phil 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, in-
cluding diabetes mellitus, metabolic syndrome [18, 19], heart
disease (acute coronary syndromes, rheumatic or con-
genital heart disease and cardiomyopathy), hypertension
[20–22], severe renal or hepatic dysfunction, other can-
cers, inflammatory diseases, previous history of infection
within 3 months and any medication usage related to in-
flammatory conditions that could significantly influence
NLR or survival or those lacking complete data were
excluded. Finally, 152 newly diagnosed patients were in-
cluded in the analysis. Patients underwent surgical re-
section by neurosurgeons, who used similar operational
techniques and principles. Tumor samples were im-
mediately snap-frozen in liquid nitrogen after resection.
One part of each sample was fixed with formalin, embed-
ded with paraffin wax and, kept at room temperature.
Glioblastomas were diagnosed by two neuropathologists
independently by two investigators (YL and ZL) and an
experienced neuropathologist (QL) blinded to the clinical
background of the patients. The mean number of neutro-
phils 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 differ-
ences in scoring between observers occurred, the core was
re-evaluated to reach a concordant scoring [5].

Blood examination
Complete blood count was obtained preoperatively be-
fore 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, lym-
phocyte and platelet counts. The normal reference range is
1.8–6.3 × 10^9/L for neutrophils, 1.1–3.2 × 10^9/L for lym-
phocytes, and 125–350 × 10^9/L for platelets. The NLR
was defined as the absolute neutrophil count divided by
the absolute lymphocyte count, and the PLR was de-
 fined as the absolute platelet count divided by the abso-
lute lymphocyte count.

Tissue microarrays and immunohistochemistry
Tissue microarrays were constructed using 152 gli-
oblastoma clinical samples and analyzed by immunohis-
tochemical staining as previously described [5, 24].
Samples were collected from the most phenotypically
representative tumor regions. Diluted primary anti-
bodies 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 anti-ody in the immunohistochemical kit (KIT-5930, MaxVi-
sion, 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 exam-
ing 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 infil-
trating neutrophils and T-cells was manually counted in-
dependently by two investigators (YL and ZL) and an
experienced neuropathologist (QL) blinded to the clinical
background of the patients. The mean number of neutro-
phils 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 differ-
ences 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 pre-
viously 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 CAG TGG TTT TGA TGT TTG TAG GTT TTT GT-3' (forward) and 5'-AAC TCC ACA CTC TTC CAA AAA CA CA A-3' (reverse) for the unmethylated reaction; and 5'-TTT CGA CGT TCG TCT GTC TCG TTG TAG GTT TTC GC-3' (forward) and 5'-GCA CTC TTC CGG 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 ± 3.6 × 10⁹/L (range, 1.2–18.6 × 10⁹/L), 222.7 ± 61.3 × 10⁹/L (range, 113–413 × 10⁹/L) and 1.8 ± 0.7 × 10⁹/L (range, 0.7–4.6 × 10⁹/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-
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 \( \geq \) 4; \( P < 0.001 \)), whereas CD3\(^+\) T-cell

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

| Clinical or Molecular Feature | All Cases | Pre-treatment NLR | \( P \) |
|------------------------------|-----------|------------------|------|
|                              | No. | % | \(<4\) No. | % | \(\geq4\) No. | % |
| Total No. of patients         | 152 | 100 | 103 | 67.8 | 49 | 32.2 |
| Sex                          |      |     |     |     |     |     |
| Male                         | 95  | 62.5 | 65 | 68.4 | 30 | 31.6 |
| Female                       | 57  | 37.5 | 38 | 66.7 | 19 | 33.3 |
| Age, years                   |      |     |     |     |     |     |
| Mean ± SD                    | 50.4 ± 15.4 | 49.2 ± 16.2 | 53.1 ± 13.1 | 0.823 |
| ≤ 60                         | 112 | 73.7 | 80 | 71.4 | 32 | 28.6 |
| > 60                         | 40  | 26.3 | 23 | 57.5 | 17 | 42.5 |
| Tumor size, cm\(^3\)         |      |     |     |     |     |     |
| Mean ± SD                    | 60.6 ± 26.6 | 60.4 ± 26.1 | 61.0 ± 28.0 | 0.920 |
| KPS                          |      |     |     |     |     |     |
| Mean ± SD                    | 71.4 ± 14.2 | 70.4 ± 15.2 | 73.5 ± 11.6 | 0.212 |
| ≤ 70                         | 78  | 51.3 | 54 | 69.2 | 24 | 30.8 |
| 80–100                       | 74  | 48.7 | 49 | 66.2 | 25 | 33.8 |
| Resection                    |      |     |     |     |     |     |
| 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\(^2\)              |      |     |     |     |     |     |
| Mean ± SD                    | 24.2 ± 3.2 | 23.9 ± 3.3 | 24.8 ± 3.1 | 0.150 |
| MGMT promoter                |      |     |     |     |     |     |
| Methylated                   | 53  | 34.9 | 36 | 67.9 | 17 | 32.1 |
| Unmethylated                 | 99  | 65.1 | 67 | 67.7 | 32 | 32.3 |
| Pre-treatment PLR            |      |     |     |     |     |     |
| Mean ± SD                    | 135.0 ± 57.1 | 111.4 ± 34.7 | 184.8 ± 62.9 | <0.001 |
| OS, months                   |      |     |     |     |     |     |
| Mean ± SD                    | 15.6 ± 11.2 | 17.9 ± 11.0 | 10.6 ± 9.8 | <0.001 |

The differences between patients with pre-treatment NLR <4 and \( \geq4 \) 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
infiltration level was negatively correlated with pre-treatment NLR level \( (P = 0.006; \text{Table 4}) \). According to previous data and our results, a number of neutrophils \( \geq 10/200 \times \text{HPF} \) [8, 9] and a number of CD3\(^+\) T-cells \( \geq 20/400 \times \text{HPF} \) [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 \( \geq 4 \) 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 NLR 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.

**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
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
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
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 glioblastoma.
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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