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Original Article

Pre-treatment neutrophils count as a prognostic marker to predict chemotherapeutic response and survival outcomes in glioma: a single-center analysis of 288 cases

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Abstract: Background: Glioma is the most common and deadliest malignant primary intracranial brain tumor in adults. It remains unclear whether the pre-treatment peripheral blood test parameters might serve as biomarkers for treatment outcome. The purpose of the current study was to investigate the predictive and prognostic value of pre-treatment peripheral blood test parameters in glioma. Methods: In total, 288 glioma patients with complete results of pre-operation peripheral blood test, clinical information and tumor transcriptome data from Chinese Glioma Genome Atlas (CGGA project) were enrolled in our study. Receiver operating characteristic (ROC) curve, Kaplan-Meier analysis and Cox proportional hazards models were performed to evaluate the diagnostic and prognostic value of pre-treatment peripheral blood test parameters in glioma patients. Results: The white blood cells (WBC) and neutrophils (NEU) counts and neutrophil to lymphocyte ratio (NLR) were positively correlated with tumor grade. IDH mutation and 1p/19q codeletion occurred frequently in patients with higher NEU counts and NLR. We also found that glioma patients with higher NEU or NLR were more likely to have a significantly decreased overall survival. Meanwhile, NEU count was a prognostic marker for TMZ standard treatment GBM patients or IDH wild-type GBM patients. Further biological and functional analysis revealed that NEU count was positively associated with cell cycle and DNA duplication. Conclusion: Our study was first to highlight the clinical significance of NEU count in GBM clinical treatment, which should be fully valued for clinical prediction and precise management.

Keywords: Glioma, GBM, neutrophils, diagnostic marker, prognostic marker

Introduction

Glioma accounts for the most common malignant brain tumor in adults with an increased prevalence and incidence in the world [1]. Recent researches of genome-wide molecular profiles revealed the clinical and genetic landscape of gliomas comprehensively [2]. And the 2016 CNS World Healthy Organizations (WHO) classification system was updated by incorporating IDH 1/2 mutation, 1p/19q codeletion and histone H3-K27M mutation. Patients were benefited from the objective and precisely CNS classification system [3]. However, Glioblastoma (GBM) remains a highly lethal and aggressive tumor with a median survival of only 14.6 months [4]. Effective treatment and improvement of quality of life in glioma patients remains one of the biggest challenges in the world [5].

For decades, scientists found that various component of immune system played crucial roles in blocking cancer cells progression [6]. With numerous painstaking efforts and clinical failures, cancer immunotherapy, has recently received a significant breakthrough in treatment of solid tumors, such as melanoma and lung cancer [7, 8]. However, several clinical trials of immune checkpoint inhibitors that looked highly promising approved by FDA for glioblastoma were failed [9]. Researchers considered

that the "cold" immune property of GBM was one of the main reasons that limited the effectiveness of immunotherapies. "Cold" tumor means very few T-cell infiltrated in the tumor micro-environment and very low tumor mutation burden (TMB) in tumor cell [10]. Meanwhile, the physical blood-brain barrier (BBB) jointly, isolating CNS from immune system, was another reason for immune therapy failure [11].

Accumulated evidence indicated that the immune microenvironment of CNS was also interactive with peripheral immune system by overexpression of some chemokines, such as TGFβ and IL-10 [12]. Peripheral blood index, a straightforward reflection of human immune system, which always including white blood cells (WBC), neutrophils (NEU), lymphocyte (LYM), platelets (PLT) and red blood cells (RBC), played a crucial role in the diagnosis and management of patients in hospital [13]. Previous studies have shown that neutrophil-to-lymphocyte ratio (NLR) was positively correlated with the grade of glioma and associated with shorter overall survival of glioma patients [14-17]. However, the relationships between other peripheral immune cells and the clinicopathological characteristics of glioma are important clinical issues that to be elucidated.

In the present study, we investigated the diagnostic and prognostic value of pre-operation peripheral blood test parameters in a large cohort of glioma patients. Our results indicated that absolute neutrophil count was upregulated in GBM, IDH wild-type and 1p/19q intact glioma patients. Pre-operation neutrophil count was an unfavorable biomarker for standard TMZ treated or IDH wild-type GBM patients. Pre-treatment neutrophil counts were significantly positively correlated with cell-cycle proliferation features of gliomas. Meanwhile, we generated an effective prognostic nomogram module which integrated clinicopathological factors and neutrophil count. This integrated investigation into neutrophil may provide novel insights in robust glioma classification and prediction systems.

Materials and methods

Patients

We retrospectively reviewed patients diagnosed as cerebral diffuse glioma and reclassified those samples according to the 2016 WHO CNS classification, between January 2006 to December 2009 from Department of Neurosurgery, Beijing Tiantan Hospital in China. All patients included in this study had to meet the following criteria: 1) Gross total glioma resection. 2) No inflammation-related conditions like bacterial or viral infections or receiving drug treatments. 3) Radiotherapy plus concomitant and adjuvant temozolomide followed the Stupp regimen [18] for GBM patients. This study was approved by the Beijing Tiantan Hospital, and written informed consent was obtained from all patients. Ultimately, RNA sequencing data of 288 samples with clinicopathologic characteristics were included in this study. Complete demographic, clinical and pathological information were available for those patients (**Table 1**). The latest pre-operation full peripheral blood count test results of patients were collected from consulting hospital case document. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) were calculated as neutrophil count and platelet count divided by lymphocyte count using standard unit. RNA sequencing data, molecular pathological and clinical information were downloaded from the Chinese Glioma Genome Atlas (CGGA) database (http://www.cgga.org.cn).

DNA sequencing for IDH mutation

IDH1/2 mutations, valuable diagnostic and prognostic biomarkers, are the most prevalent mutation gene in gliomas. Genomic DNA was isolated from frozen tumor tissues using the QIAamp DNA Mini Kit (QIAGEN). IDH1/2 mutations were detected by pyrosequencing which were described in our previous study [19].

The performance of the nomogram in the CGGA cohort

Then the nomogram was used by totalling the points identified on the top scale for each independent covariate. The total points were vertically aligned to 1-yr, 2-yr, 3-yr and 5-yr overall survival to estimate median survival. The neutrophils nomogram performance was measured quantitatively using the concordance index. The C-index for this nomogram was applied to evaluate the discriminative ability of prognostic models in survival analysis and was 1000 times bootstrapping resampling cross validation. The value of the C-index can range from 0.5, which indicates no discriminative ability, to 1.0, which indicates perfect ability to dis-

Table 1. Clinical and molecular characteristics of 288 glioma patients

Characteristics	Value
Age in yrs	42 (12-70)
Sex	
Male	167
Female	121
WHO Grades	
II	127
III	61
IV	100
Histology	
A	58
AA	21
O	18
OA	52
AO	14
AOA	25
GBM	100
IDH Status	
Mutant	144
Wild-type	139
NA	5
Chromosome 1p/19q status	
Codel	51
Intact	218
NA	19
Standard TMZ Chemotherapy	
Yes	143
No	129
NA	16
White Blood Cells (WBC) in 10^9/L	6.87 (3.4-21.8)
Neutrophils (NEU) in 10^9/L	4.75 (1.5-20.1)
Lymphocytes (LYM) in 10^9/L	1.92 (0.4-3.9)
Neutrophil-to-Lymphocyte (NLR)	3.19 (0.68-30.75)
Platelet (PLT) in 10^9/L	229.99 (72-498)
Platelet-to-Lymphocyte (PLR)	131.77 (32.86-373.33)

tinguish between the patients who experience disease progression or death and those who do not. The nomogram calibration curves were assessed by plotting the observed survival fraction against the nomogram-assessed probabilities [20].

Weighted gene co-expression network analysis

We used weighted gene co-expression network analysis (WGCNA) to generate a co-expression network correlation with neutrophil counts using the log2 transformed RNA sequencing data. The R package WGCNA was applied to construction the co-expression network. Briefly, WGCNA used topological overlap measure (TOM) to construct network modules with adjacency matrix (0.5 + 0.5 × correlation matrix, to the power β = 12). The TOM is a highly robust measure of network interconnectedness and essentially provides a measure of the connection strength between two adjacent genes and all other genes in a network. In this study, the power of 10 and the threshold of -2.5 were chosen for network and module built. We summarized the gene profile for each module and merged the highly correlated modules (absolute value of correlation index more than 0.7) [21].

Gene ontology and KEGG pathway analysis

Gene Ontology and KEGG pathway enrichment analyses were performed using cluster profiler package [22] in R and the DAVID online tool [23] to obtain the biological processes (BPs). $P < 0.05$, the threshold level for all gene ontology, was considered statistically significant.

Statistical analysis

Statistical analysis and graph were performed using R software (version, 3.6.2). Measurement analysis of continuous variables between different groups were compared using Student's test. The one-way analysis of variance (ANOVA) was used to compare the statistically significant differences for more than two groups. Receiver operating characteristic (ROC) curves were created using the peripheral blood cells as continuous variables to illustrate the prediction performance. Survival analyses of peripheral blood test parameters were performed using Kaplan-Meier method and log-rank test. Univariate and multivariate analysis of survival of neutrophils and clinical features were calculated with the cox proportional regression model. Nomogram was established based on all independent prognostic factors on multivariate analysis. The bootstrap method (B = 1000) was performed to calculate concordance index (C-index). The overall survival (OS) was calculated from the time of diagnosis and the time of

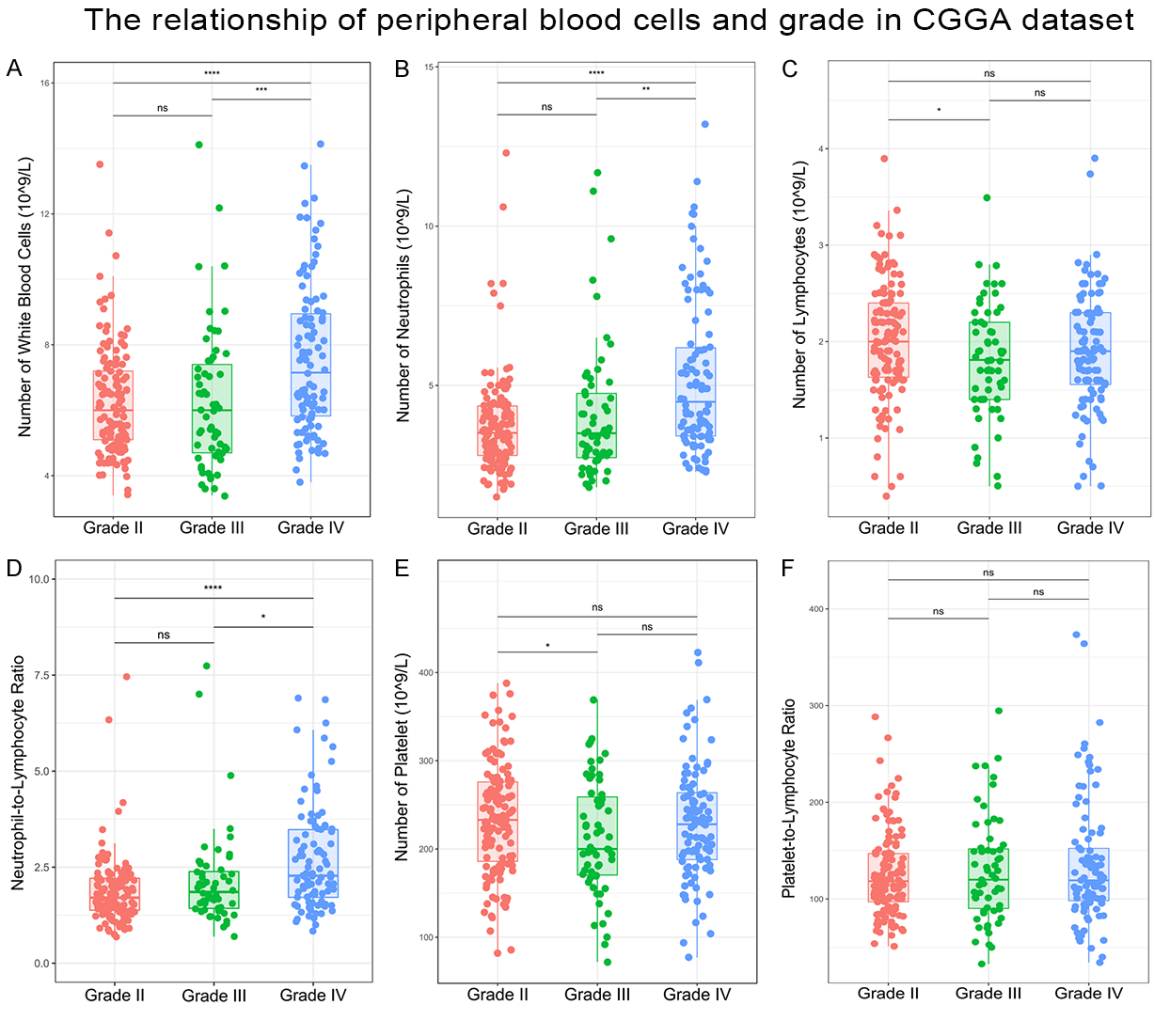

Figure 1. Comparison of six peripheral blood test parameters according to WHO grades. A. The count of WBC was significantly increased in WHO IV glioma (IV vs. III, P < 0.001; IV vs. II, P < 0.0001). B. The count of NEU was significantly increased in WHO IV glioma (IV vs. III, P < 0.01; IV vs. II, P < 0.0001). C. The count of LYM in WHO III glioma was increased than WHO II glioma (P < 0.05). D. The NLR was significantly increased in WHO IV glioma (IV vs. III, P < 0.05; IV vs. II, P < 0.0001). E. The count of PLT in WHO III glioma was increased than WHO II glioma (P < 0.05). F. The PLR was not statistical significantly difference among WHO grades. *, **, ***, and ****indicate P < 0.05, P < 0.01, P < 0.001 and P < 0.0001, respectively.

death. The progression-free survival (PFS) was defined as the period from the time of therapy initiation to the time of disease progression or death. A two-sided P < 0.05 was considered to indicate statistically difference.

Results

The distribution of peripheral immune cell counts in glioma patients

To investigate the regularity of peripheral blood test parameters in tumor progression, we analyzed the counts of peripheral blood tests parameters according to WHO tumor grades, IDH mutation status, chromosome 1p/19q status and histopathological types. As shown in **Figure 1**, the proportion of WBC, NEU and NLR were all significantly increased in grade IV patients compared with grade II (P < 0.0001; P < 0.0001; P < 0.0001) and grade III patients (P = 0.0005; P = 0.0022; P = 0.0402). However, the differences of LYM, PLT and PLR were not significant among WHO tumor grades. Furthermore, we observed that IDH wild-type glioma patients (Figure S1) significantly had a higher level of WBC, NEU and NLR than IDH mutant gliomas (P = 0.0041; P = 0.0006; P < 0.0001) while 1p/19q codeletion glioma (Figure

S2) harbored a higher level of NEU and NLR than 1p/19q intact gliomas (P = 0.0034; P < 0.0001). In addition, we also found that GBM patients had the highest level of WBC, NEU and NLR than patients in other histological subtypes (P < 0.0001) (Figure S3). These results indicated a potential link between peripheral immunity and glioma, even with the obstruction of blood-brain barrier. The variation of WBC and NEU were the main manifestation of glioma patients in the peripheral immune environment.

The efficacy of peripheral blood parameters in predicting clinicopathological marker in gliomas

In order to investigate the diagnostic value of peripheral blood test parameters in glioma patients, we used those parameters as continuous variable to predict GBM, IDH mutant and 1p/19q codeletion by generating ROC curves. The ROC curves of WBC (AUC = 0.676, 95% CI = 0.61-0.741, P < 0.0001), NEU (AUC = 0.726, 95% CI = 0.663-0.789, P < 0.0001) and NLR (AUC = 0.707, 95% CI = 0.641-0.772, P < 0.0001) exhibited a better accuracy for GBM prediction (**Figure 2A**). Meanwhile, the value of NEU (AUC = 0.626, 95% CI = 0.561-0.692, P = 0.0002) and NLR (AUC = 0.647, 95% CI = 0.583-0.712, P < 0.0001) could be used to estimate IDH mutant to some extent (**Figure 2B**). And the value of NEU (AUC = 0.626, 95% CI = 0.561-0.692, P = 0.0002) and NLR (AUC = 0.637, 95% CI = 0.558-0.717, P < 0.0001) could be used to predict 1p/19q codeletion preliminarily (**Figure 2C**). Those results indicated that WBC, NEU and NLR were closely related to GBM, IDH mutant and 1p/19q codeletion.

The prognostic value of peripheral blood parameters in glioma patients

To explore the influence of peripheral blood parameters on overall survival in glioma patients, we depicted the survival curves according to the optimal cutoff points (mean value) with dichotomized variables (**Figure 3**). The influence of WBC, NEU and NLR were similar to each other. Higher value of WBC (P = 0.00086), NEU (P < 0.0001) and NLR (P < 0.0001) were significantly associated with shorter overall survival in glioma patients. In contrast, patients with higher value of LYM (P = 0.16) and PLT (P = 0.23) had a longer survival, although not statistically significant. Meanwhile, PLR at diagnosis was not associated with OS. Then we investigated the prognostic value of WBC, NEU and NLR in GBM patients. As shown in Figure S4, the role of NEU (P = 0.0058) and NLR (P = 0.018) in GBM were similar to those in glioma. These results demonstrated that NEU and NLR were unfavorable prognosticator for GBM patients and there was probably a tight connection between peripheral NEU and NLR and the tumor microenvironment in central nervous system.

The development of the neutrophil prognostic nomogram model

Then we developed a nomogram model for predicting 1-yr, 2-yr, 3-yr and 5-yr overall survival of glioma patients. Seven clinicopathological variables, including gender, age, tumor grade, IDH mutation status, 1p/19q status, NEU and NLR, were included in our study. Firstly, the Cox regression assumption helped us to remove three characteristics (gender, IDH mutation status, NLR) before modules construction, and the nomogram model was built with four variables (age, NEU, grade, 1p/19q status) (**Figure 4A**). The concordance index for this nomogram was 0.792. And the calibrate plot of the proposed nomogram model showed that the predicted 1-yr, 2-yr, 3-yr and 5-yr OS corresponded closely to the actual survival time observed by Kaplan-Meier (**Figure 4B**). These results indicated that the predictions based on the neutrophils prognostic nomogram model showed a good agreement with the observed OS.

The association of neutrophil count with PFS and OS in GBM

Glioblastoma is the most prevalent malignant brain tumor in adults with the median survival of 14.6 months. It is difficult to predict the probability of tumor recurrence and overall survival time after resection of GBM patients [24]. The nomogram module proved that NEU played a critical role in predicting the clinical outcome for patients with glioma. Next, we investigated the prognostic role of NEU in GBM patients with different clinical stages and disease courses. The detailed information of GBM patients in our study was described in **Table 2**, and the median OS was 19.6 months and median PFS was 13.9 months. As shown in **Figure 5**, the NEU-high group (NEUHG) of GBM patients who received standard TMZ treatment showed a significant poor PFS (P = 0.0023) and OS (P = 0.0028)

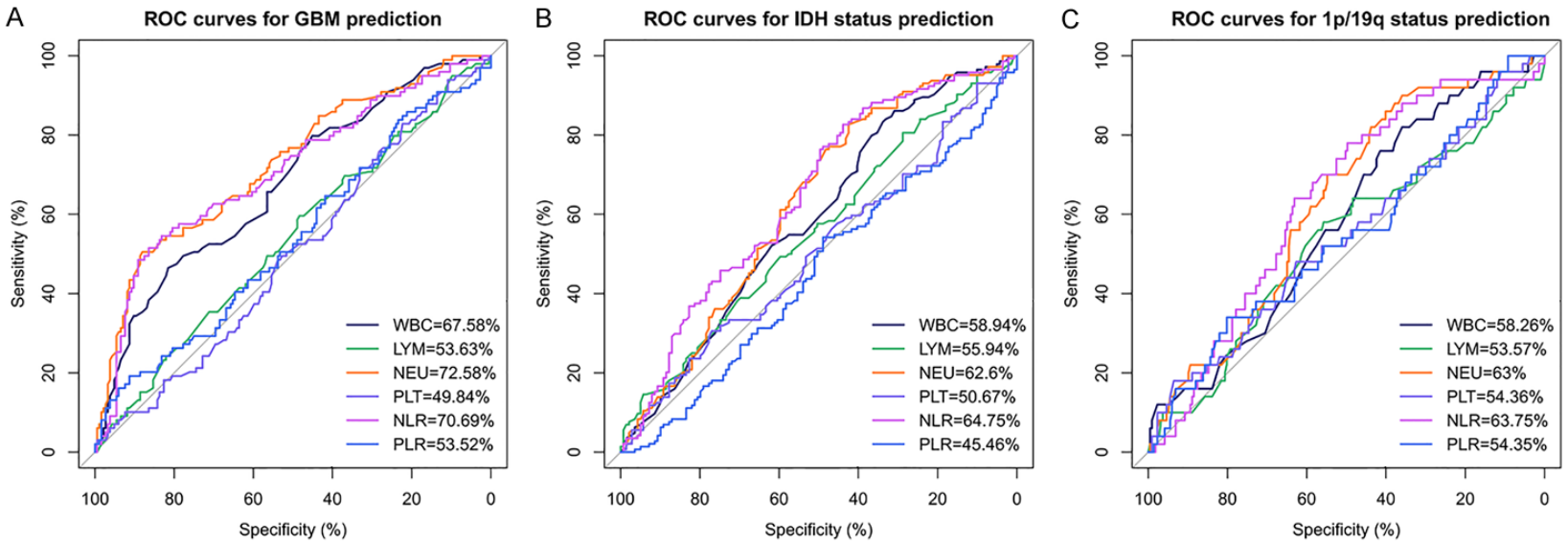

Figure 2. Receiver operating characteristic (ROC) curves exhibited the diagnostic efficacy of six peripheral blood test parameters in predicting GBM, IDH mutant and 1p/19q codeletion. A. WBC, NEU and NLR showed higher accuracy than LYM, PLT and PLR in predicting GBM. The area under the curve (AUC) was 0.676, 0.726 and 0.707, respectively. B. NEU and NLR showed higher accuracy in predicting IDH mutant. The AUC was 0.626 and 0.647, respectively. C. NEU and NLR showed higher accuracy in predicting 1p/19q codeletion. The AUC was 0.63 and 0.638, respectively.

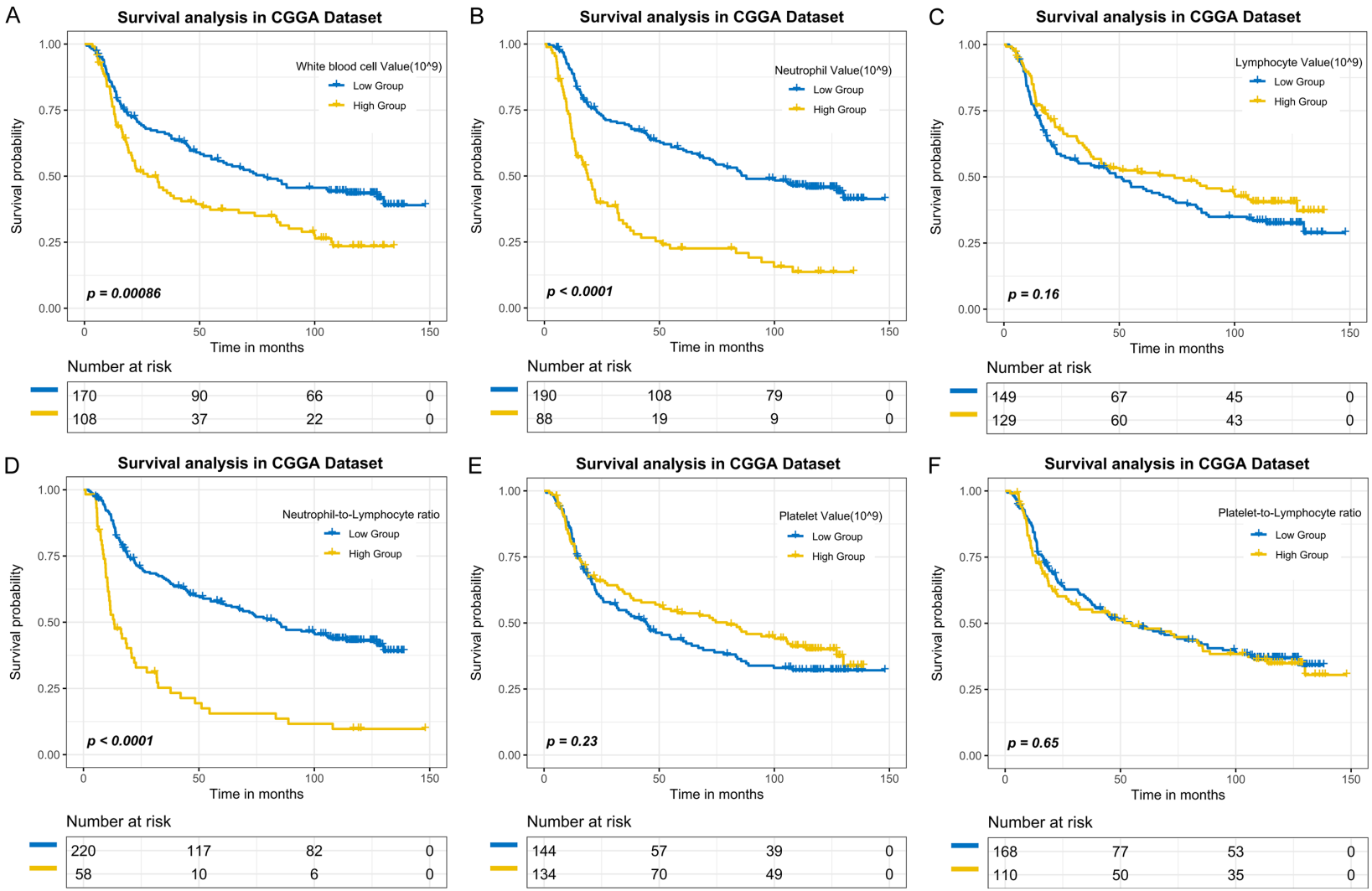

Figure 3. Survival analysis of six peripheral blood test parameters in glioma. A, B, D. Kaplan-Meier survival analysis revealed that WBC (P = 0.00086), NEU (P < 0.0001), NLR (P < 0.0001) were unfavorable prognosis factors in glioma. C, E, F. Meanwhile, LYM (P = 0.16), PLT (P = 0.23) and PLR (P = 0.25) were not prognostic indicators.

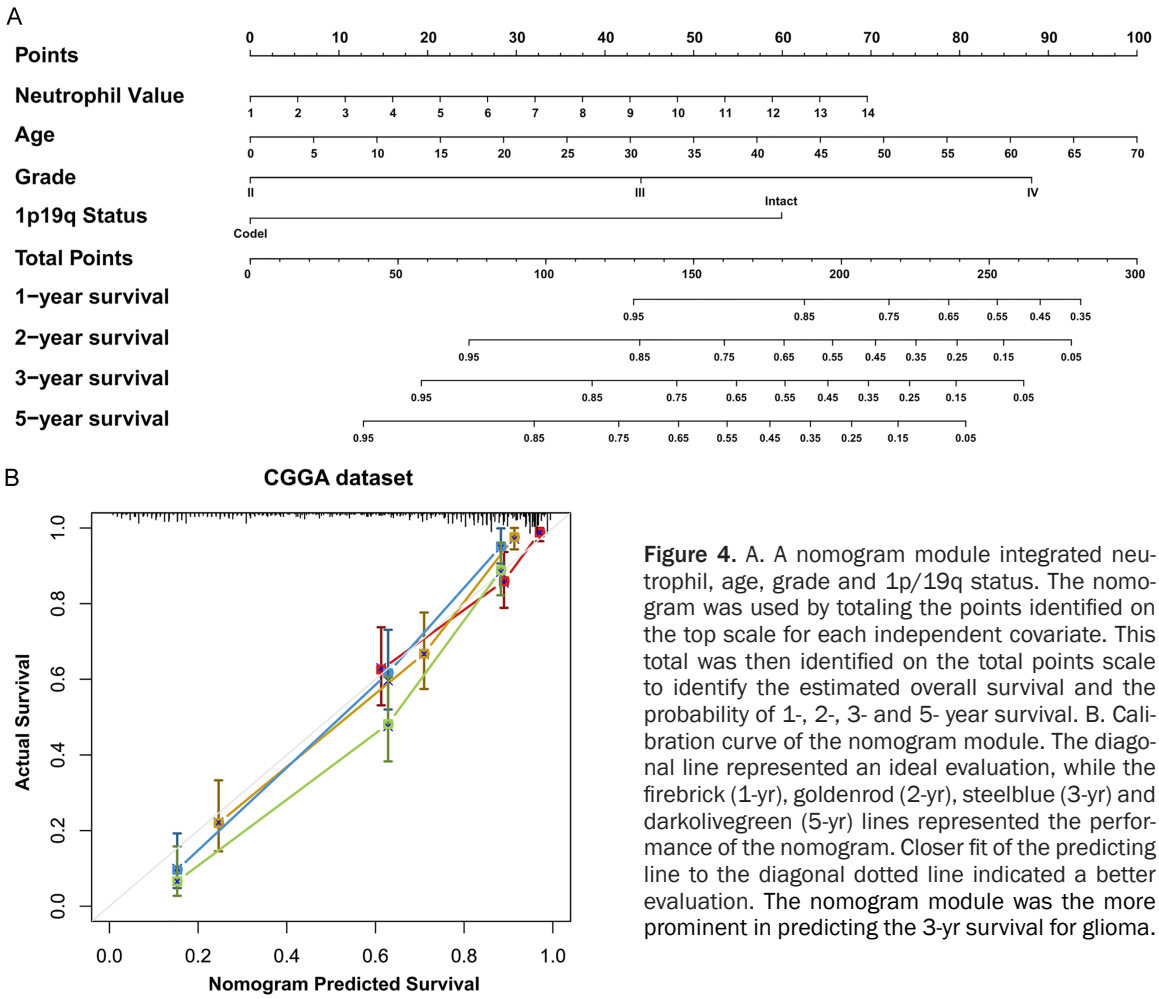

Figure 4. A. A nomogram module integrated neutrophil, age, grade and 1p/19q status. The nomogram was used by totaling the points identified on the top scale for each independent covariate. This total was then identified on the total points scale to identify the estimated overall survival and the probability of 1-, 2-, 3- and 5- year survival. B. Calibration curve of the nomogram module. The diagonal line represented an ideal evaluation, while the firebrick (1-yr), goldenrod (2-yr), steelblue (3-yr) and darkolivegreen (5-yr) lines represented the performance of the nomogram. Closer fit of the predicting line to the diagonal dotted line indicated a better evaluation. The nomogram module was the more prominent in predicting the 3-yr survival for glioma.

Table 2. Clinical and molecular characteristics of 100 GBM patients

Characteristics	Value
Median age (range, y)	46.6 (12-70)
Sex	
Male	48
Female	42
IDH Status	
Mutant	19
Wild-type	80
NA	1
Standard TMZ Chemotherapy	
Yes	60
No	34
NA	6

than those in NEU-low group patients (NEULG). However, the difference was not statistically significant for GBM patients in non-TMZ treatment group (PFS: P = 0.51; OS: P = 0.3). In addition, the PFS (P = 0.0014) and OS (P = 0.003) of IDH wild-type GBM patients were significant shorter than those in NEULG, while it was hard to predict the PFS (P = 0.103) and OS (P = 0.126) for IDH mutant patients using NEU count (**Figure 6**). These results implied that NEU may be a reflection of tumor status in peripheral blood and were helpful to assess the treatment response and monitor the recurrence or progression for GBM patients, especially for patients with the standard TMZ treatment or IDH wild-type tumors.

Network analysis for neutrophil-related biological functions

To identify the relationship between neutrophils and tumor progression, we performed weighted gene co-expression network analysis (WGCNA) by using RNA sequencing data to

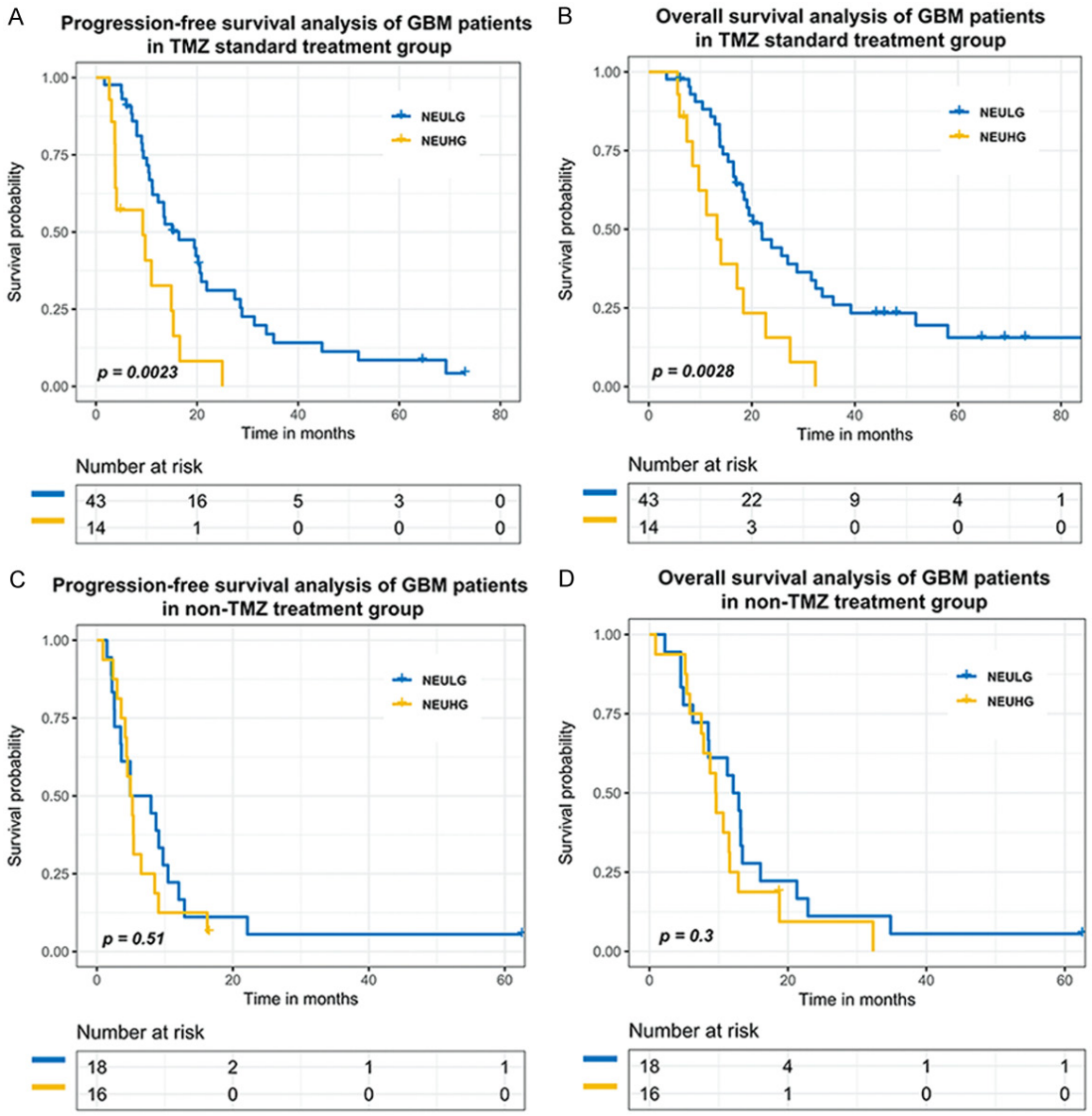

Figure 5. Survival analysis of NEU in GBM patients, with or without TMZ standard treatment. A, B. Kaplan-Meier survival analysis showed that TMZ standard treatment GBM patients in NEUHG had a significantly poor PFS (P = 0.0023) and OS (P = 0.0028). C, D. NEU was not a prognostic factor for non-TMZ treatment GBM patients.

explore the NEU-related biological functions. WGCNA, which was an unsupervised and unbiased analysis, has been widely applied to explore clusters of correlated transcripts. Pearson correlation test was performed to find genes significantly correlated with NEU. A total of 4125 genes co-expressed with NEU counts ($P < 0.05$) were selected from the RNA sequencing dataset. The WGCNA network modules were established with NEU co-expressed genes profile. As shown in **Figure 7A**, **7B**, four NEU-specified modules of which Pearson $|R| > 0.7$ and $P < 0.0001$ were selected in the following analysis. The blue module and turquoise module were positively correlated with NEU count, while the yellow module and grey module were negatively correlated with NEU count. The gene ontology analysis was performed to investigate the biological features of NEU-related genes in positive modules and negative modules. We found that genes in blue module and turquoise module were significantly involved in DNA replication, sister chromatid segregation, cell cycle G1/S phase transition and so on (**Figure 7C**). And genes in yellow and grey modules were mainly focused on normal biological progress-

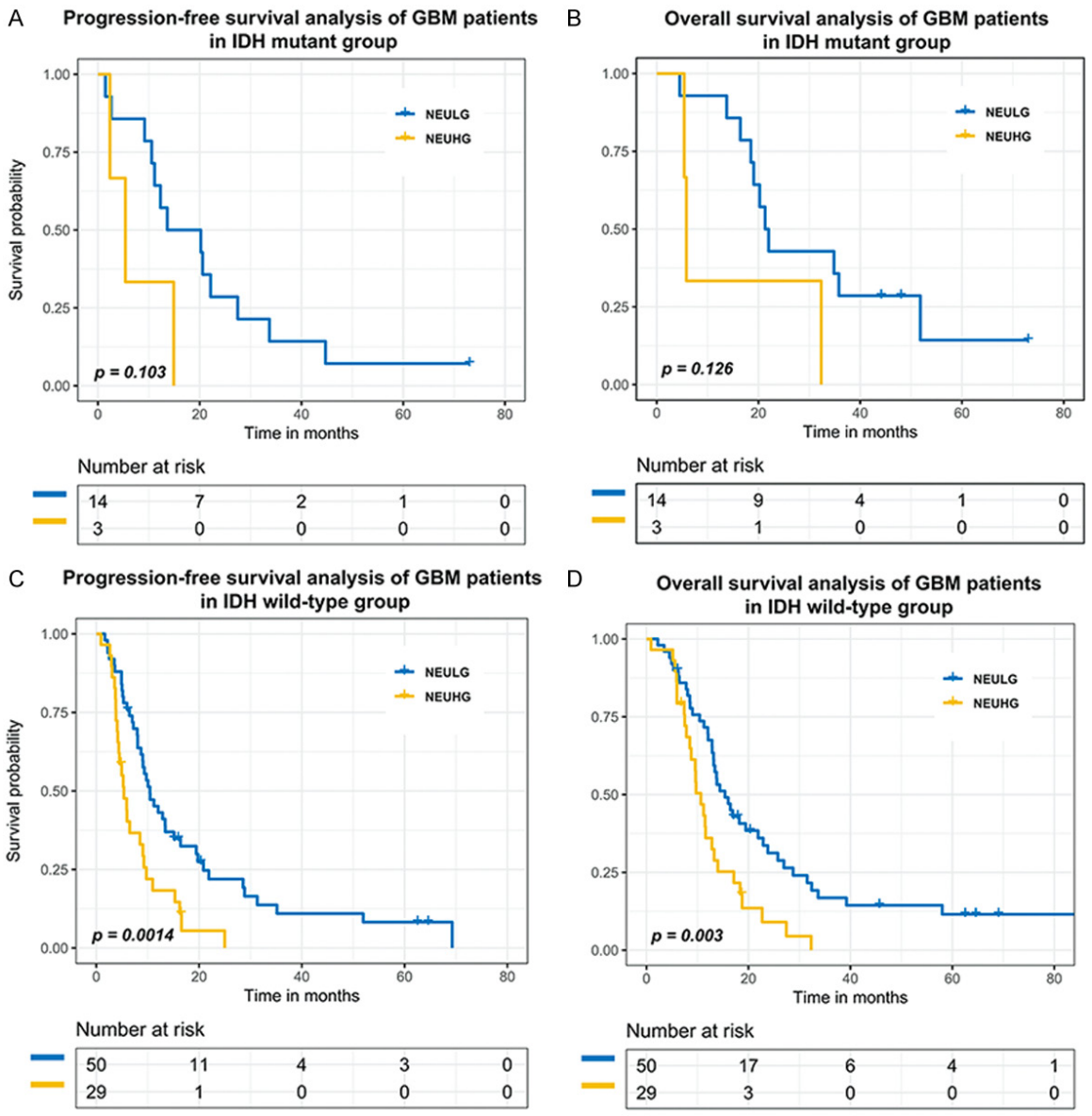

Figure 6. Survival analysis of NEU in IDH mutant GBM patients and IDH wild-type GBM patients. A, B. NEU was not a prognostic factor for IDH mutant GBM patients. C, D. Kaplan-Meier survival analysis revealed that IDH wild-type GBM patients in NEUHG had a significantly shorter PFS (P = 0.0014) and OS (P = 0.003) than patients in NEULG.

es, such as adult behavior and phospholipid transport (**Figure 7D**). These results indicated that NEU count might be a useful marker in reflecting cell-cycle regulation, cell proliferation and tumor progression functions of glioma patients and may partially interpret the reason that neutrophils were always accompanied by higher tumor grade in gliomas.

Discussion

The viewpoint that human brain was one of immune-privileged organs was revised in 2015 through observing immune responses in cervical lymph nodes by Aspelund et al. [25, 26]. Nonetheless, the trafficking and patrolling of the mediators and leukocytes were hard to observe between tumor microenvironment in CNS and peripheral immune system, especially in the early stages of tumor [27]. Even in non-small-cell lung cancer and liver cancer, without the influence of BBB, by single-cell sequencing analysis, the relationship of tumor-infiltrating lymphocytes and peripheral lymphocytes was not elucidated [28, 29].

Nowadays, several studies have reported that peripheral blood test parameters could serve

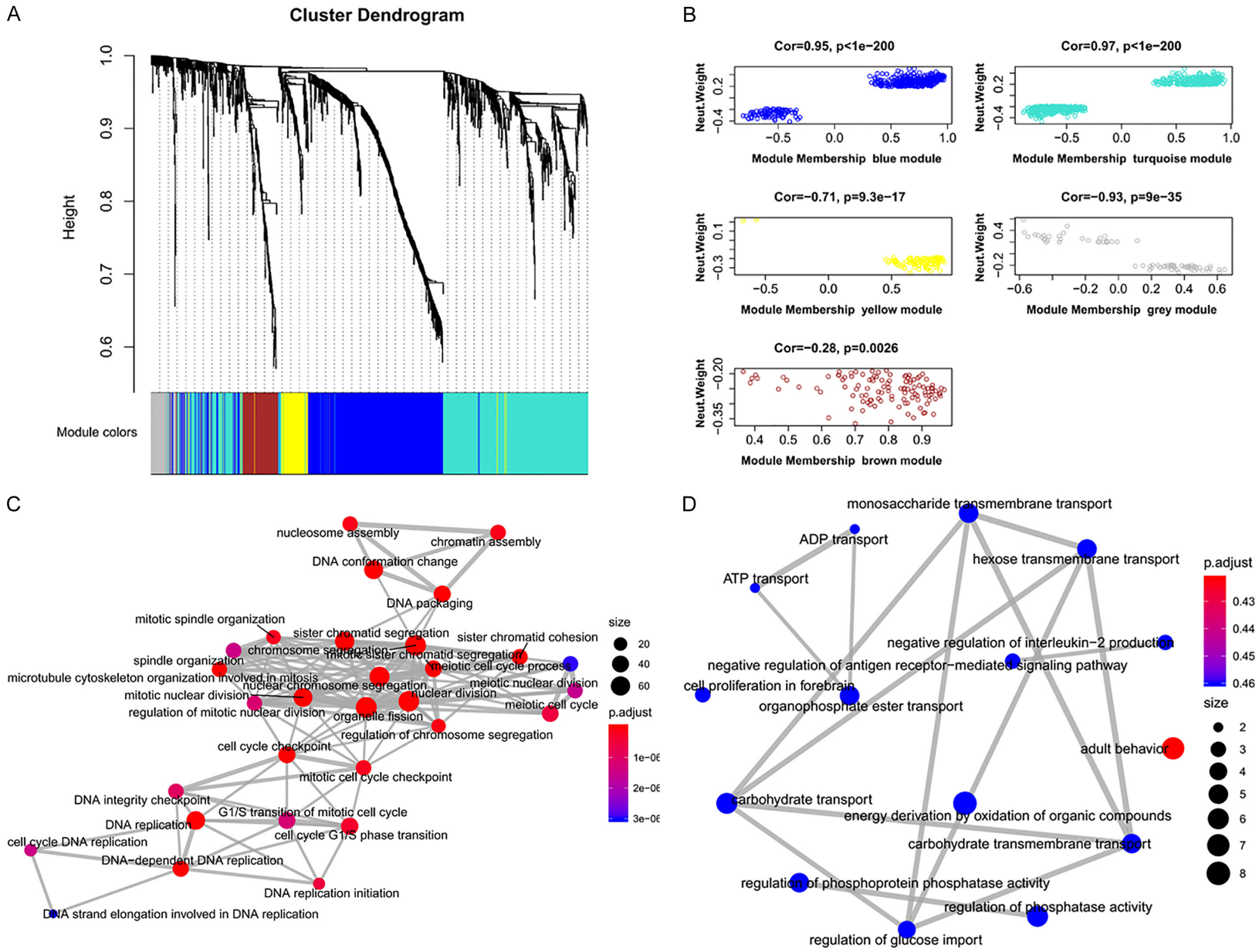
A
Cluster Dendrogram
Height
1.0
0.9
0.8
0.7
0.6
Module colors
B
Cor=0.95, p<1e−200
Neut.Weight
Module Membership blue module
Cor=0.97, p<1e−200
Module Membership turquoise module
Cor=−0.71, p=9.3e−17
Module Membership yellow module
Cor=−0.93, p=9e−35
Module Membership grey module
Cor=−0.28, p=0.0026
Module Membership brown module
C
nucleosome assembly
chromatin assembly
DNA conformation change
DNA packaging
mitotic spindle organization
sister chromatid segregation
sister chromatid cohesion
chromosome segregation
mitotic sister chromatid segregation
spindle organization
meiotic cell cycle process
microtubule cytoskeleton organization involved in mitosis
nuclear chromosome segregation
meiotic nuclear division
mitotic nuclear division
nuclear division
meiotic cell cycle
regulation of mitotic nuclear division
organelle fission
regulation of chromosome segregation
cell cycle checkpoint
mitotic cell cycle checkpoint
DNA integrity checkpoint
G1/S transition of mitotic cell cycle
DNA replication
cell cycle G1/S phase transition
cell cycle DNA replication
DNA−dependent DNA replication
DNA replication initiation
DNA strand elongation involved in DNA replication
size
20
40
60
p.adjust
1e−0
2e−0
3e−0
D
monosaccharide transmembrane transport
ADP transport
hexose transmembrane transport
ATP transport
negative regulation of interleukin−2 production
negative regulation of antigen receptor−mediated signaling pathway
cell proliferation in forebrain
organophosphate ester transport
adult behavior
carbohydrate transport
energy derivation by oxidation of organic compounds
carbohydrate transmembrane transport
regulation of phosphoprotein phosphatase activity
regulation of phosphatase activity
regulation of glucose import
p.adjust
0.43
0.44
0.45
0.46
size
2
3
4
5
6
7
8

Figure 7. WGCNA and GO analyses for NEU in glioma. A. Branches of the hierarchical cluster tree defined five co-expression modules which were assigned a special color as can be seen from the color band underneath the tree. B. The blue (Cor = 0.95, P < 0.0001) and turquoise modules (Cor = 0.97, P < 0.0001) contained genes that were positively correlated with the weight of NEU. And the genes in yellow (Cor = -0.71, P < 0.0001) and grey modules (Cor = -0.93, P < 0.0001) were negatively correlated with the weight of NEU. C. GO analysis revealed that the functions of genes in blue and turquoise modules were mainly associated with the process of DNA replication and cell cycle. D. And the functions of genes in yellow and grey modules were mainly associated with the process of material transport and cytokines regulation.

as the diagnostic and prognostic markers for human cancers. For instance, higher value of the pre-operation NLR was associated with poor PFS and OS in patients of different types of cancer, including gynecologic cancers [30], lung cancer [31], ovarian cancer [32], cervical cancer [33], endometrial cancer [34], melanoma [35], glioma [14] and bladder tumor [36]. PLR was another important marker of inflammation which reported in various cancers. Researches revealed that increased PLR was a negative prognostic marker in patients with gastric cancer [37], colorectal cancer [38], hepatocellular carcinoma [39], ovarian cancer [40], non-small cell lung cancer [41] and head and neck cancer [42]. Nonetheless, the problem of whether the peripheral blood test parameters could be used to detect the clinical characteristics, monitoring the treatment response, and predicting the survival prognosis of gliomas was still remained.

In our study, the pre-operation peripheral blood test information of 288 glioma patients accompanied with their tumor RNA sequencing data, the largest patient cohort to the best of our knowledge, were retrospectively analyzed to investigate the relationship between peripheral blood test parameters and clinicopathological characteristics in glioma. In line with other researches [17], we found that WBC count, NEU count and NLR were positively associated with the WHO grade of glioma. However, the LYM count, PLT count and PLR did not show any significant correlation with tumor malignancy. To date, few studies have investigated the relationship between peripheral blood test parameters and IDH status or 1p/19q status of glioma. We provided the first evidence that both of NEU count and NLR were significantly higher in patients with IDH wild-type or 1p/19q intact glioma. Meanwhile, the prognostic analyses of pre-operation peripheral blood test parameters revealed that the NEU could be used to predict GBM. However, the ROC curves confirmed that the sensitivity and specificity of peripheral blood test parameters were not strong enough for glioma prognostication. And those result implied that NEU count and NLR were more reliable and stable in reflecting the tumor progression. Interestingly, the positive correlation between NEU-related modules and cell-cycle and DNA replication from WCGNA and GO analyses could potentially explained that phenomenon. The inhibition of cell-cycle in combination with TMZ treatment might be a useful therapeutic strategy for suppressing GBM.

In the previous studies, pre-operation NLR was recognized as an adverse prognostic factor in glioma and GBM patients. Whether other peripheral blood test parameters could be used to predict the clinical prognosis of glioma patients was unknown. In our study, we demonstrated that glioma patients with the reduced number of WBC, NEU and NLR would have a favorable OS, and both NEU and NLR were proved to be negatively correlated with OS in patients with GBM. These results highlighted the pivotal role of NEU and NLR in determining prognosis and questioned the use of traditional risk factors for clinical prediction, although these markers needed to be validated in large, prospective studies. After comprehensively taking NEU, NLR and other robust indicators into consideration, the multiple cox regression analysis helped us develop a neutrophil prognostic nomogram module, which showed superior validity in predicting the survival time for gliomas. Therefore, integrating NEU into a traditional prognostic system may be potentially helpful for precision medicine.

TMZ, an oral alkylating agent, is commonly used as first-line chemotherapy for newly diagnosed GBM patients [43]. Currently, tumor associated neutrophils have been recognized to be associated with acquired chemoresistance and higher glioma grade at later stages [44, 45]. However, the role of peripheral neutrophils in tumor chemoresistance was unclear. We found that the NEUHG GBM patients received

TMZ standard treatment were more likely to recur and had a shorter OS. Simultaneously, the NEU was not a prognostic marker for GBM patients with non-TMZ treatment. Our study was first to demonstrate the association between NEU and the treatment response of TMZ in GBM.

Tumor associated neutrophils play important roles in tumorigenesis and cancer progression. The tight junctions of BBB would be impaired during GBM development and peripheral NEU was recruited at tumor site by CXCL8 to execute cell-mediated effects [12]. Our findings also enhanced the hypothesis that the peripheral NEU might be transported into tumor microenvironment which may induced by TGF-β and G-CSF stimulation [46]. Furthermore, patients with IDH wild-type glioma in NEUHG had a significantly reduced PFS and OS than those in NEULG. This suggested that IDH mutation status of glioma may affect the production of peripheral neutrophils.

Conclusions

In summary, the main advantage of our research was using large peripheral blood test and RNA sequencing data matched glioma samples to comprehensively analyze the prognostic value and potential biological functions of peripheral blood parameters. We demonstrated that pre-operation NEU and NLR were associated with GBM, IDH mutation and 1p/19q codeletion. Moreover, NEU count, which was significantly associated with cell cycle related functions, could predict worse survival for GBM patients. Our findings highlighted the important role of pre-operation peripheral neutrophils in GBM clinical treatment and management. Neutrophils may become a critical target in devising the treatment strategies for GBM patients in future.

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Disclosure of conflict of interest

None.

Abbreviations

CGGA, Chinese Glioma Genome Atlas; ROC, receiver operating characteristic; CNS, central nervous system; WHO, World Healthy Organizations; WBC, white blood cells; NEU, neutrophils; LYM, lymphocyte; PLT, platelets; RBC, red blood cells; NLR, neutrophil to lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; BBB, blood-brain barrier; TMB, low tumor mutation burden; AUC, area under the ROC curve; ANOVA, one-way analysis of variance; WGCNA, weighted gene co-expression network analysis; OS, overall survival; PFS, progression free survival; NEUHG, NEU-high group; NEULG, NEU-low group.

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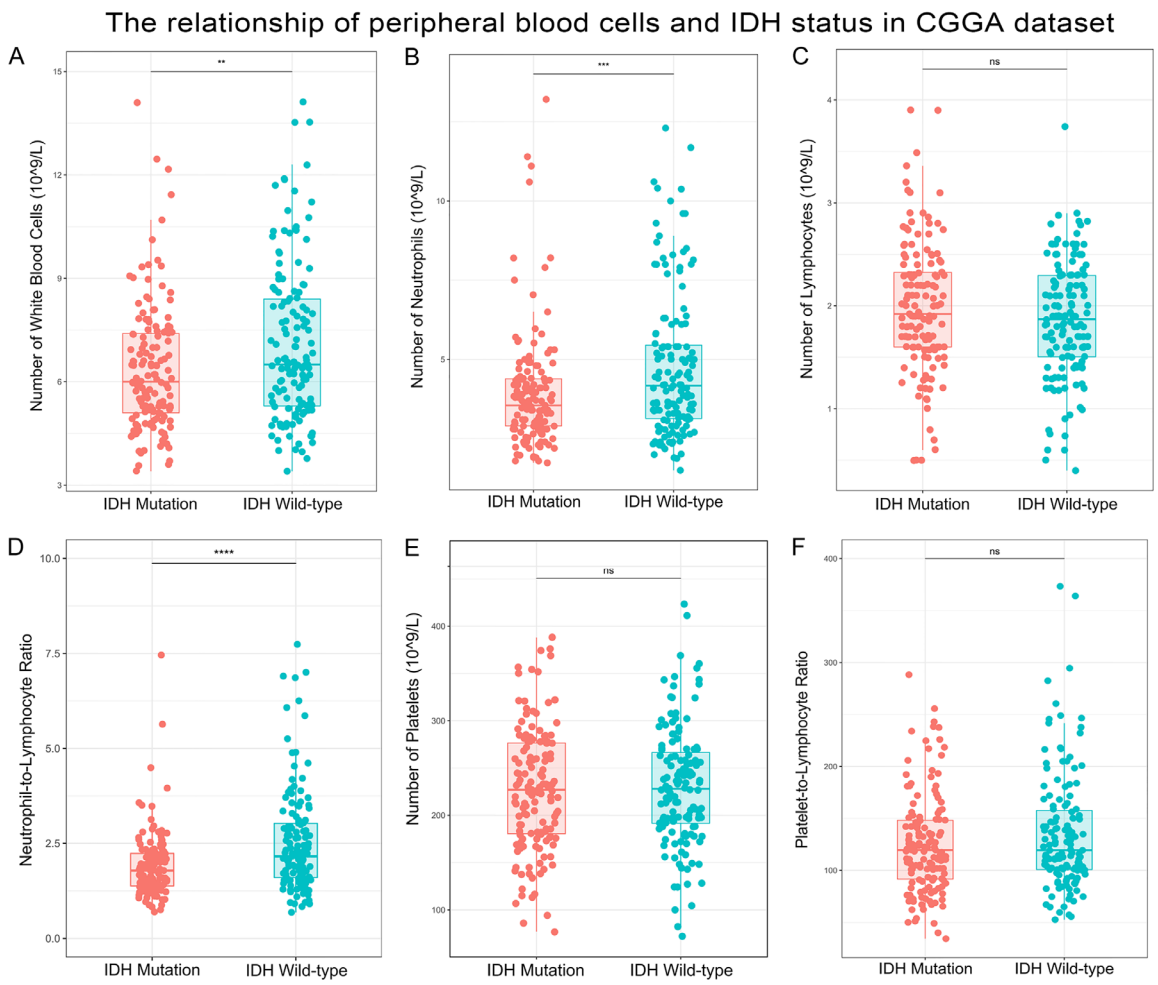

Figure S1. Comparison of six peripheral blood test parameters according to IDH status. B, D. NEU and NLR were significantly increased in IDH wild-type gliomas. A, C, E, F. WBC, LYM, PLT and PLR were not signifcantly difference between IDH mutant group and IDH wild-type group. *, **, ***, and ****indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

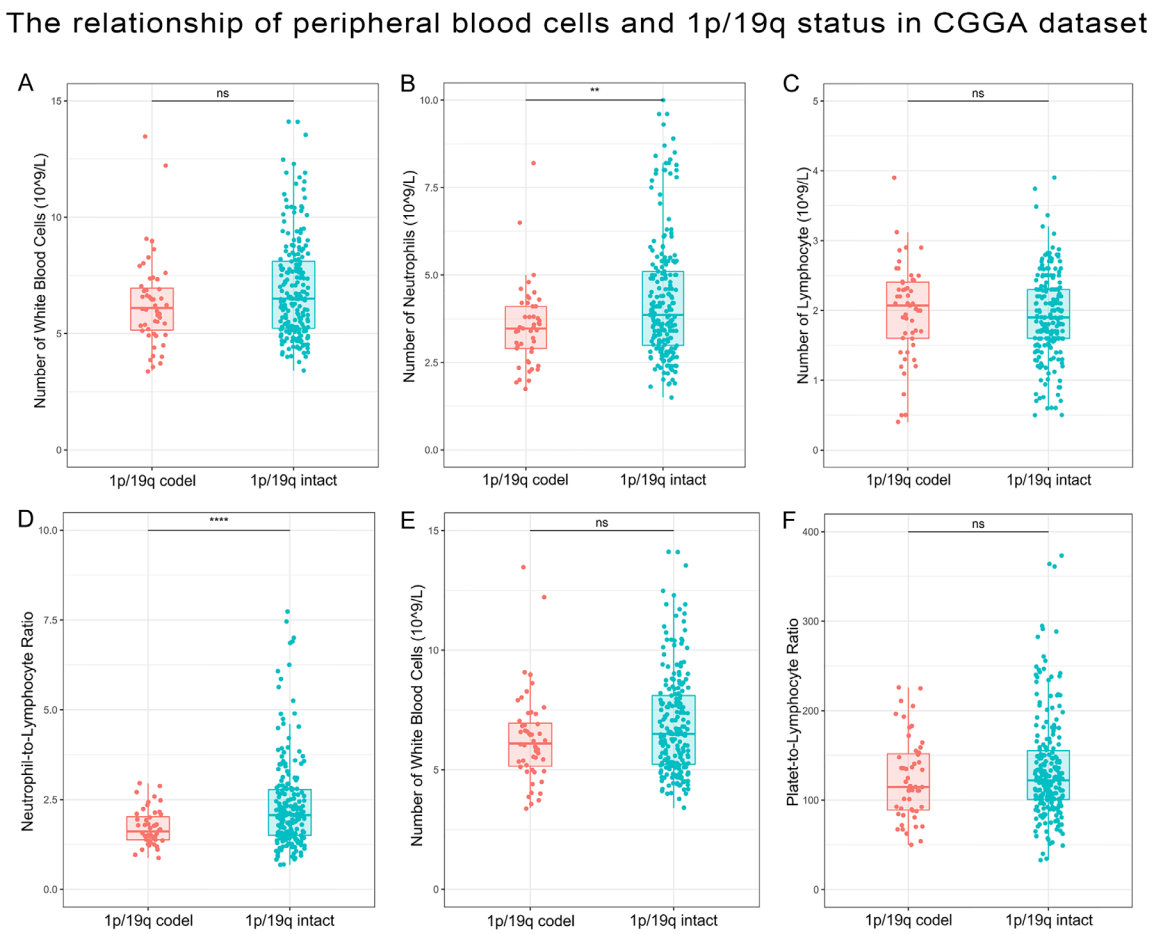

Figure S2. Comparison of six peripheral blood test parameters according to 1p/19q status. B, D. NEU and NLR were significantly increased in 1p/19q intact gliomas. A, C, E, F. WBC, LYM, PLT and PLR were not signifcantly difference between 1p/19q codeletion group and 1p/19q intact group. *, **, ***, and ****indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

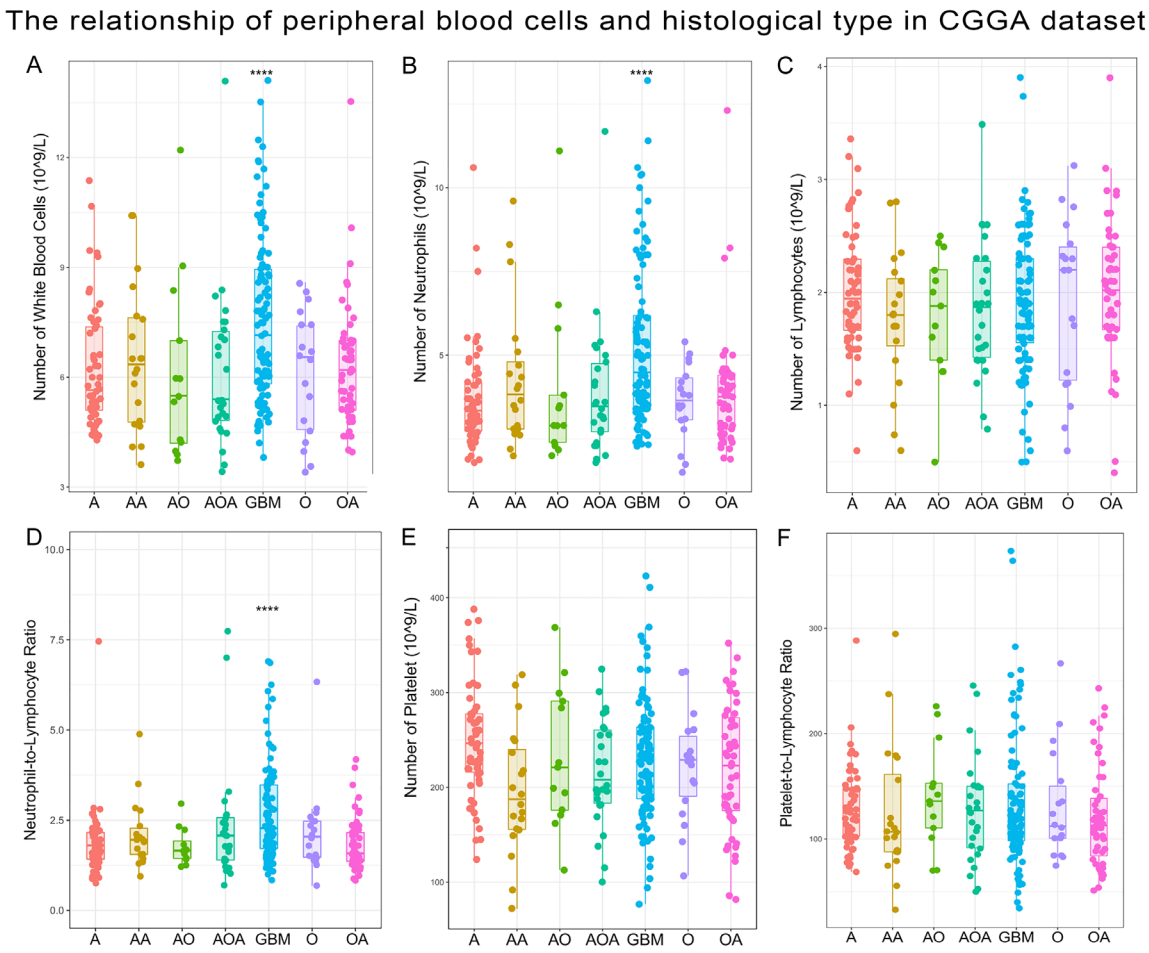

Figure S3. Comparison of six peripheral blood test parameters according to histopathologic classifications. A, B, D. WBC, NEU and NLR were significantly increased in GBM. C, E, F. LYM, PLT and PLR were not significantly difference among histopathologic classifications. *, **, ***, and ****indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.

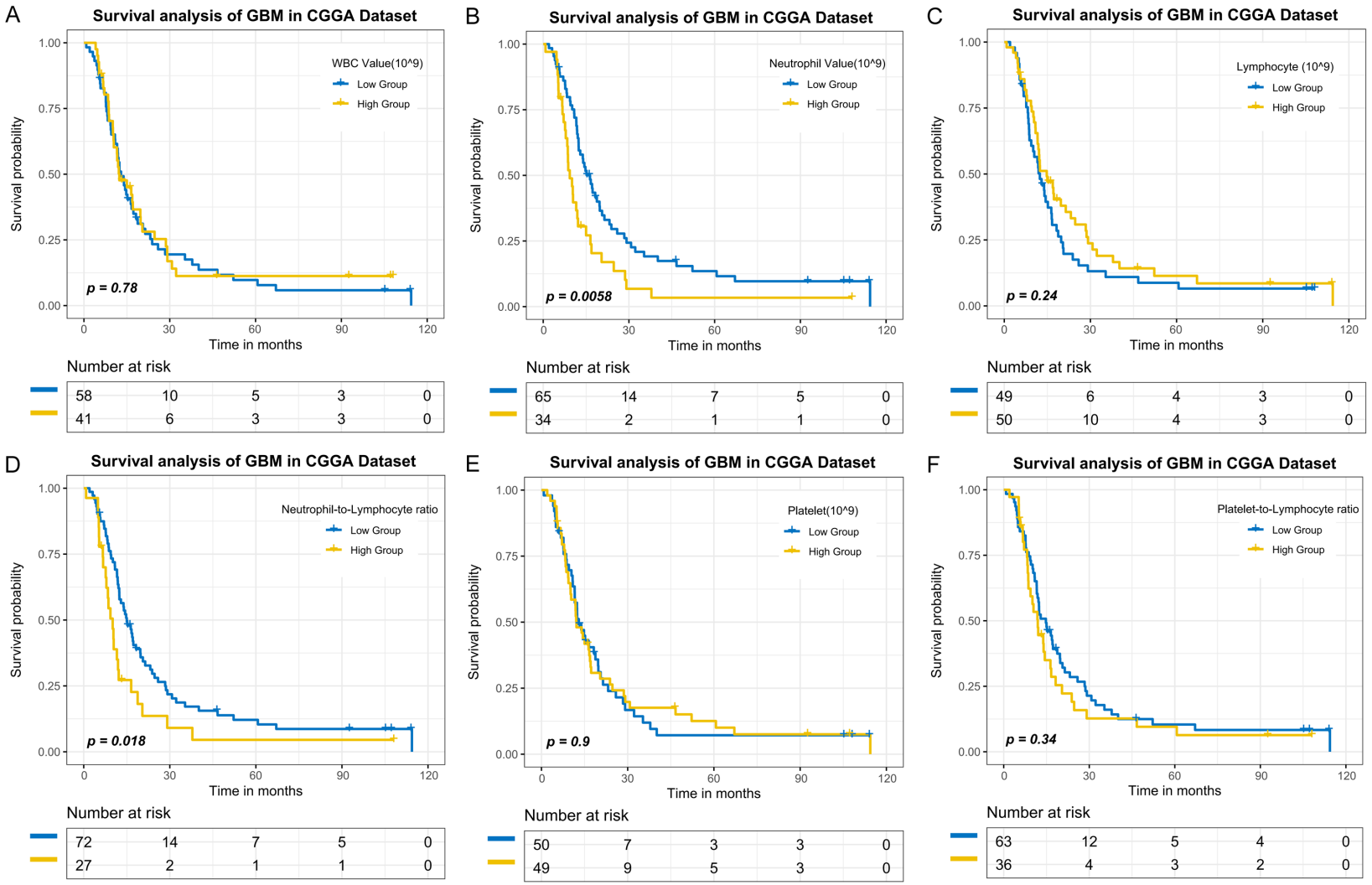

Figure S4. Survival analysis of six peripheral blood test parameters in GBM. B, D. Kaplan-Meier survival analysis showed that higher level of NEU and NLR conferred a significantly worse prognosis in GBM. A, C, E, F. WBC, LYM, PLT and PLR were not prognostic factors for GBM patients.