Prognostic value of preoperative inflammatory markers in patients with different molecular subgroups of WHO grade II and III diffuse gliomas

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Abstract

Background: To determine the prognostic implications of these immune indices in WHO Grade II & III gliomas and different molecular subgroups.

Methods: Clinical data from 214 newly diagnosed WHO grade II and III diffuse glioma patients were studied retrospectively. Cut-off values were determined by X-tile software. IDH and TERT promotor mutations were detected by gene sequencing, and 1p19q codeletion was estimated via fluorescence in situ hybridization.

Results: NLR was verified to be an independent prognostic marker for OS in WHO grade II and III diffuse gliomas. NLR level was also associated with OS of IDH mutant subgroup, TERT promotor mutant subgroup, 1p19q intact subgroup and with PFS of 1p19q intact subgroup. LMR level was associated with OS of WHO grade II and III diffuse gliomas and TERT promotor mutant subgroup. dNLR was verified to be an independent prognostic marker for OS in TERT promotor wild-type subgroup, 1p19q intact subgroup, IDH mutant TERT promotor wild-type 1p19q intact subgroup and for PFS of 1p19q intact subgroup. dNLR was associated with OS of WHO grade II and III diffuse gliomas and IDH mutant subgroup. 1p19q codeletion was correlated with low NLR.

Conclusion: Preoperative NLR, LMR and dNLR levels were helpful to forecast prognosis in patients with WHO grade II and III gliomas and different genetic phenotypes.

Background

Diffuse gliomas are the commonest primary cerebral tumors with a tendency to infiltrate surrounding brain tissue[1]. Diffuse gliomas are classified into astrocytoma, oligoastrocytoma and oligodendroglioma based on histological[2]. Lower-grade diffuse gliomas, including grade II and grade III gliomas, present an infiltrative nature and intrinsic tendency to relapse or progress to glioblastoma (GBM). Researchers found different molecular expression, biological characteristics, treatment strategies and prognosis between lower grade glioma and GBM[3].

Recent years, molecular biomarkers have been brought into focus in diagnosis, classification, treatment effect estimating and prognosis of WHO grade II and III diffuse gliomas. Chromosome 1p/19q codeletion has been proved to imply better outcome on account of higher chemo-radiosensitivity in oligodendroglial gliomas [4]. Mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2, which were detected in most of WHO grade II and III diffuse gliomas and secondary GBM, have been verified to be associated with longer OS compared with wild-type [5 6]. Telomerase reverse transcriptase (TERT) promoter region mutations are often discovered in more than 70% of primary GBM and oligodendrogliomas, and less commonly in oligoastrocytomas and astrocytomas [7]. Eckel et al. divided gliomas into five molecular groups according to the above three alterations. The molecular groups were independently associated with characteristic distributions of clinical behavior, acquired genetic alterations, age at diagnosis, and associated germline variants in patients with grade II or III gliomas but not in patients with grade IV gliomas [8].

Except for molecular classification of tumors, it is widely appreciated that inflammation promotes formation and progression of cancers [9]. The preoperative peripheral blood count NLR, dNLR, PLR and LMR have been widely indicated as remarkable prognostic markers in multiple kinds of tumors such as colorectal cancer, non-small cell lung cancer, gastric cancer and so on[10 – 13].

Several studies demonstrated poor outcome in glioma patients with an elevated preoperative NLR[14,15]. On the other hand, some research indicated no significant correlation between NLR level and prognosis of GBM[16] or glioma of grade I to IV[17]. As for the correlation between PLR level and prognosis, previous researches showed different opinions.[15,18] The prognostic role which LMR played in GBM was tested, but no significance was found in previous studies [16], and no study for the correlation between preoperative dNLR and glioma has been performed before. Moreover, up till now rare studies investigating the prognostic effect of NLR, LMR, PRL and dNLR in WHO Grade II and III diffuse gliomas have been published.

Wang et al. made a study of the associations between NLR, LMR, or PLR level and IDH mutations, but no significance was detected [16]. Moreover, they found that high level of PRL was correlated with poor prognosis superior to the low level of NLR for IDH mutant GBM patients. However, IDH mutations were tested immunohistochemically, which could be not as precise as gene sequencing [16].

The aim of the research was to discover the prognostic role of preoperative immune indices in different molecular subgroups of WHO grade II and III diffuse gliomas. We also hoped to identify the prognostic potential of these preoperative inflammatory markers with clinical variables, especially the genetic alterations of WHO grade II and III diffuse gliomas.

Methods

Study population

A retrospective analysis of a cohort of glioma patients who underwent an operation from 2001 to 2013 in our institution was carried out. A total of 214 patients included in the study reached the following rules: Each patient was newly diagnosed, namely without any chemotherapy, radiation or resection. Patients with a history of autoimmunological diseases, current infectious disease, serious heart disease, chronic respiratory disease, allergic disorders, chronic renal insufficiency and chronic atrial fibrillation were excluded. Pathology was confirmed as WHO Grade II or III diffuse glioma by the department of pathology of our institution, according to WHO 2007 classification. Medical records including age at diagnosis, gender, the extent of surgical resection (subtotal resection or total gross resection), pathology after surgery, and overall survival data were reviewed to acquire treatment details and demographic information. Full blood count (FBC) was taken within one week before surgery as part of the standard preoperative workup. Neutrophil, lymphocyte, monocyte, platelet and white blood cell counts were extracted from FBC. NLR was calculated as neutrophil count divided by lymphocyte count. LMR was calculated as lymphocyte count divided by monocyte count. PLR was calculated as platelet count divided by lymphocyte count. dNLR was calculated as neutrophil count divided by white blood cell count with neutrophil count excluded.

Ethical approval was authorized by the local independent Ethics Committee of our institution and written informed consent was obtained from every patient.

Analyses of molecular markers

Glioma DNA was gained from FFPE sections. IDH1 Mutational hotspots at codon 132 and IDH2 at codon 172 were estimated via direct sequencing as previously reported [19]. TERT promoter region mutational hotspots [chr5, 1, 295, 228 (C228T) and 1, 295, 250 (C250T)] were estimated via direct sequencing as previously reported [20]. Chromosome 1p/19q status was estimated via fluorescence in situ hybridization as previously reported [20].

Statistical methods

Data were analyzed using SPSS 19.0.0 software (SPSS Inc., Chicago, USA). The X-tile 3.6.0 software (Yale University, New Haven, CT, USA) was employed to establish the appropriate cut-off values of NLR, LMR, PLR and dNLR[21]. Means and standard deviations (SD) were calculated for normally distributed data. The relationships between preoperative inflammatory markers were analyzed using Spearman's rho for continuous variables. Student's unpaired t-test was employed to compare the variables. PFS was calculated from the date of pathological diagnosis to the date of initial tumor recurrence or progression (radiologically or pathologically). OS was measured from histologic diagnosis to death. Patients who were still alive or lost to follow-up were censored at last follow up. Survival curves were obtained according to the Kaplan–Meier Method. Univariate and multivariate analyses were employed to explore the influence of variables on OS and PFS by Cox proportional hazard method. All tests were two-sided, and a p-value of 0.05 was regarded as significant on analyses.

Results

Cut-off values of preoperative inflammatory parameters for overall survival

By X-tile software, we established the optimal cut-off values for NLR (NLR=2.29), LMR (LMR=4.81), PLR (PLR=112.11) and dNLR (dNLR=1.66). Then we identified the cut-off values from the minimum P value according to the OS (Figure 1). Patients were then divided into different subgroups by the established cut-off values.

Patient characteristics

We enrolled 214 patients in our cohort, of which there were 130 males (60.7%) and 84 females (30.9%). The median age of the cohort at diagnosis was 41 years (range: 5-79 years), and 168 patients (78.5%) were above 50 years old. 162 patients underwent total resections (75.7%), and 52 patients had subtotal resections (24.3%). In the cohort, there were 132 patients being diagnosed with WHO grade II (61.7%) and the other 82 WHO grade III (38.3%), including 65 diffuse astrocytomas (WHO grade II), 21 oligodendrogliomas (WHO grade II), 46 oligoastrocytomas (WHO grade II), 65 anaplastic astrocytomas (WHO grade III), 5 anaplastic oligodendrogliomas (WHO grade III) and 12 anaplastic oligoastrocytomas (WHO grade III). All the patients accepted some form of radiotherapy and/or chemotherapy after the operation.

IDH mutations were detected in 168 (78.5%) cases. TERT promoter mutations were detected in 79 (36.9%) cases. Among the 168 IDH mutant cases, there were 159 cases harboring IDH1 mutations and 9 cases harboring IDH2 mutations. Chromosome 1p/19q codeletion was found in 57(26.6%) glioma tissues.

A total of 144 patients (67.3%) were divided into NLR low (≤2.29) group, 113 patients (52.8%) were divided into LMR low (≤4.81) group, 134 patients (62.6%) were divided into PLR low (≤112.11) group and 139 (65.0%) patients were divided into dNLR low (≤1.66) group. (Table 1)

Associations between preoperative inflammatory parameters and other clinical variables

We also examined whether or not certain baseline variables were associated with NLR, LMR, PLR and dNLR level. We found that the PLR level was correlated with gender ($p<0.001$). What's more, 1p19q codeletion represented a low level of NLR ($p=0.045$). No other significant associations were found between inflammatory parameters and clinical variables. (Table 2)

Correlations between preoperative NLR, LMR, PLR and dNLR

Correlations between these parameters were assessed using Spearman analysis. Significant correlations were found between all of these inflammatory parameters. (Table S2)

Prognostic values of preoperative inflammatory parameters for WHO grade II and III diffuse gliomas

During the follow-up period, the median OS of these 214 cases was 5.9 years, and the median PFS was 4.7 years. The relationships between NLR, LMR, PLR, dNLR levels and survival outcomes for WHO grade II and III diffuse gliomas were presented in Figure 2. NLR>2.29 ($p=0.006$), LMR≤4.81 ($p=0.047$) and dNLR>1.66 ($p=0.002$) was associated with poor OS. No significance was found between different PLR level and OS of the cohort. What's more, these inflammatory variables also showed no association with PFS. So higher NLR, higher dNLR and lower LMR foreboded a worse prognosis of WHO grade II and III diffuse gliomas (Figure 2).

Then we performed univariate and multivariate analysis of preoperative inflammatory variables for OS of WHO grade II and III diffuse gliomas. Univariate analysis for OS demonstrated that age ≥50 years ($p=0.012$), glioma WHO grade III ($p<0.001$), IDH wild type ($p<0.001$), 1p19q intact ($p=0.006$), NLR>2.29 ($p=0.004$), LMR≤4.81 ($p=0.036$) and dNLR>1.66 ($p=0.002$) were associated with poor OS. On multivariate analysis for OS, glioma WHO grade III ($p<0.001$), IDH wild type ($p=0.006$), 1p19q intact ($p=0.048$) and NLR>2.29 ($p=0.011$) remained serving as independent prognostic indicators for poor outcome (Table 3). Moreover, univariate Cox regression showed no correlations between these preoperative inflammatory variables and PFS (Table S3).

Prognostic roles of preoperative inflammatory variables for prognosis in IDH mutant or wild-type, TERT promotor mutant or wild-type and 1p19q intact or codeletion subgroups of WHO II and III diffuse gliomas.

Then we divided the cohort into IDH mutant or wild-type subgroups, TERT promotor mutant or wild-type subgroups and 1p19q intact or codeletion subgroups, respectively.

As for IDH mutant or wild-type subgroups, univariate analysis showed prognostic significances of NLR >2.29 ($p=0.016$) and dNLR>1.66 ($p=0.022$) on OS in IDH mutant subgroup (n=168). Subsequent multivariate analysis indicated that only WHO grade III ($p = 0.002$) and TERT promotor mutation ($p = 0.006$) were independent prognostic factors for OS in IDH mutant WHO grade II and III diffuse gliomas. (Figure 3A-3B, Table 3) No significant associations were obtained between these preoperative inflammatory indicators and PFS. (Figure 3A-3B) Data indicated no correlations between these preoperative inflammatory indicators and prognosis of IDH wild-type subgroup of WHO II and III diffuse gliomas (data not shown).

In TERT promotor mutation subgroup (n=79), univariate analysis showed prognostic significances of NLR >2.29 (p=0.049) and LMR>2.29 (p=0.035) on OS, but subsequent multivariate analysis demonstrated that only age>50 (p=0.003), WHO grade III (p = 0.007) and IDH mutation (p <0.001) were independent prognostic factors for OS. No significant associations were obtained between these preoperative inflammatory indicators and PFS. (Figure 3C-3D, Table 4) In TERT promotor wild type subgroup (n=135), univariate analysis showed prognostic significance of dNLR >1.66 (p=0.018) on OS, and subsequent multivariate analysis demonstrated that WHO grade (p = 0.001) and dNLR >1.66 (p=0.024) were independent prognostic factors for OS. No significant associations were observed between these preoperative inflammatory indicators and PFS. (Figure 3E, Table 4)

In 1p19q intact subgroup (n=157), univariate analysis showed prognostic significance of NLR >2.29 (p=0.009) and dNLR >1.66 (p=0.005) on OS, NLR >2.29 (p=0.004) and dNLR >1.66 (p=0.001) on PFS. Subsequent multivariate analysis demonstrated that age>50 (p=0.010), WHO grade III (p = 0.001), IDH mutation (p =0.050) and dNLR >1.66 (p=0.005) were independent prognostic factors for OS, WHO grade III (p <0.001), IDH mutation (p =0.017) and dNLR >1.66 (p=0.004) were independent prognostic factors for PFS. (Figure 3F-3G, Table 5) Data indicated no correlations between these preoperative inflammatory indicators and prognosis of 1p19q codeletion subgroup of WHO II and III diffuse gliomas.

Prognostic roles of preoperative variables for prognosis in 5 molecular subgroups of WHO II and III diffuse gliomas.

Depending on a previous study [8], we divided the cohort into five molecular groups: triple-negative, mutation in TERT only, mutation in IDH only, mutations in both IDH and TERT, and triple-positive (mutations in both IDH and TERT plus 1p/19q codeletion). In IDH mutant only subgroup (n=88), we found dNLR >1.66 to be associated with OS (p=0.037) (Figure 3H, Table S1). No other correlations were observed significantly between the other four subgroups and these preoperative inflammatory variables (data not shown).

Discussion

As far as we know, our study is the first to evaluate the value of preoperative NLR, LMR, PLR and dNLR for prognosis in WHO Grade II and III gliomas and different molecular subgroups. It is also the first published article to investigate the correlation between preoperative NLR, LMR, PLR, dNLR values and degree of IDH mutation, TERT promotor mutation and 1p19q codeletion.

Tumor invasion ability is dependent both on the intrinsic characteristics and microenvironment around the tumor[22]. An abnormal phenotype of the tumor may stimulate an influx of inflammatory cells into tissues around the tumor [23]. These inflammations may give rise to the increasing of neutrophils and platelets and decreasing of lymphocytes along with the advancement of cancer[24].

Several studies had verified the prognostic role of NLR in various tumors; a meta-analysis of more than100 studies including 40,559 patients proved NLR to be a promising prognostic marker in solid tumors [25]. A high NLR represents both an enhanced neutrophil-mediated inflammatory response and a weaken lymphocyte-dependent antitumor effects, which contributes to cancer progression and poor prognosis[26]. The concrete mechanism for the prognostic role of NLR is unclear, but it may be partly explained by neutrophilia, an inflammatory response which could suppress the tumor immunity by inhibiting the cytolytic reactions of immune cells such as activated T cells, lymphocytes, and natural killer cells[27]. On the other hand, a relative lymphocytopenia may represent a lower level of CD4 + T-helper lymphocytes, giving rise to a reduced lymphocyte-dependent antitumor reaction[28].

Increased preoperative NLR shows a prognostic significance in glioblastoma patients. Bambury et al. were the first researchers to raise the theory that NLR ≤ 4 portended better outcome independent of other well-known prognostic factors for GBM [14]. Others drew similar conclusions in GBM [15]or glioma of grade I to IV [18]. Conversely, some research indicated no association between NLR level and prognosis of GBM[16] or glioma of grade I to IV[17]. Previous researchers used a traditional way to choose the best cut-off value of NLR relying on the receiver-operator characteristic analyses, which failed to take survival time into consideration. So, the accuracy could not be guaranteed. We adopted X-tile software which was designed specially to explore the most accurate cut-off values depending on survival tree analysis and further test for the prognostic specificity across patients within different clinicopathologic profiles. The ultima cut-off for NLR was 2.29, which was proved to be an independent prognostic factor for OS of WHO grade II and III diffuse gliomas via univariate and multivariate Cox regression analysis. We also validated the pre-published cut-off value of 4 in GBM and glioma of grade I to IV but did not find a significant association with clinical outcome in our cohort (Supplement Figure S1). So, the pre-published cut-off value of 4 may be not suitable for WHO grade II and III diffuse gliomas.

Increased level of LMR has been indicated as a better prognostic marker for multiple tumors such as soft tissue sarcoma, renal cell carcinoma and urothelial carcinoma [29]. The prognostic role which LMR played in GBM was tested, but no significance was found in a previous study [16]. dNLR was also not yet proved a significant prognostic marker for glioma. In our study, we discovered positive correlations of LMR ≤ 4.81, dNLR > 1.66 with poor outcome of WHO grade II and III diffuse gliomas but could not be confirmed by multivariate Cox regression analysis.

As for PLR, its decreased level has been demonstrated to be a prognostic factor for multiple tumors [30]. We defined the optimal cut-off of PLR to be 112.11 by X-tile software but failed to verify a significant correlation between PLR value and WHO grade II and III diffuse gliomas.

Correlations between these parameters were assessed using Spearman analyses. We detected the correlation among the four preoperative inflammatory variables and discovered a significant correlation between every two markers of them. However, these inflammatory markers revealed different degrees of prognostic significance for WHO grade II and III diffuse gliomas.

Data provided compelling evidence that NLR, LMR, PLR and dNLR values corresponded with a glial brain tumor grading [31,32]. We did not draw similar conclusions. In our study, the cut-off value we established for NLR, LMR and dNLR were correlated with prognosis but not diverse significantly between grade II and III gliomas.

IDH mutations, TERT promotor mutations and 1p19q codeletions are important milestones in genomics of glioma in recent years. Multiple studies have confirmed that IDH mutation predicts better prognosis in gliomas [33]. TERT promoter mutations also represented a longer survival in combination with IDH mutations. Chromosome 1p/19q codeletion has been proved to imply better outcome on account of enhanced chemo-radiosensitivity in oligodendroglial tumors [4]. These molecular markers have been proved to regulate glioma immune microenvironment. IDH mutant glioma cells acquire resistance to NK cells through epigenetic silencing of NKG2D ligands ULBP1 and ULBP3, which could inhibit the immune function of glioma. [34] Another research indicated that methylation level of immune checkpoints genes PD-1 was significantly associated with TERT promotor mutation degree in IDH mutant WHO grade II and III gliomas. [35] Inflammatory responses were identified to belong to high-risk categories for clinical outcome of 1p/19q codeletion glioma patients. [36] Wang demonstrated no association between NLR, PLR, or LMR and IDH mutations. In our cohort, we verified a positive correlation between a low level of NLR and 1p19q codeletion, and also found no association between inflammatory variables with IDH and TERT promotor mutation. Perhaps NLR could serve as an inexpensive and routinely detected marker for 1p19q codeletion in future.

Then we divided the cohort into IDH mutant or wild-type subgroups, TERT promotor mutant or wild-type subgroups and 1p19q intact or codeletion subgroups, respectively. NLR ≤ 2.29 was indicated to be a prognostic factor for longer OS in IDH mutant, TERT promotor mutant and 1p19q intact subgroups, and for longer PFS in 1p19q intact subgroup of WHO II and III diffuse gliomas. In TERT promotor mutant subgroup, data verified a correlation between LMR > 4.81 and better outcome. dNLR ≤ 1.66 was proved to be correlated with better OS in IDH mutant subgroup and verified to be an independent prognostic factor for OS in TERT promotor wild type subgroup. dNLR ≤ 1.66 was also demonstrated to be an independent prognostic factor for both PFS and OS in 1p19q intact subgroup of WHO II and III diffuse gliomas.

According to Eckel's definition [8], we divided gliomas into five molecular groups: triple-negative, mutation in TERT only, mutation in IDH only, mutations in both IDH and TERT, and triple-positive (mutations in both IDH and TERT plus 1p/19q codeletion). dNLR ≤ 1.66 was demonstrated to be an independent prognostic factor for OS In IDH mutant only subgroup of WHO II and III diffuse gliomas.

This study has several limitations. The total number of this patient cohort was small, especially after being divided into each subgroup. Distribution was uneven thus the credibility of multivariate analyses was poor in one subgroup. Moreover, this study was a retrospective study, protocols of treatment such as adjuvant genotoxic therapies were not consistent, which could bring in bias to data analysis.

Conclusions

We demonstrated that pre-treatment NLR was superior to LMR and dNLR as a prognostic in patients with WHO grade II and III diffuse gliomas. What's more, NLR level may reflect the degree of 1p19q codeletion in grade II and III diffuse gliomas.

Preoperative NLR, LMR, dNLR are all reproducible, easily measured, and inexpensive markers from a complete blood count that can be easily incorporated into the routine clinical practice. These inflammatory variables may serve as cost-effective prognostic biomarkers and be used to speculate the molecular phenotype for different subgroups of WHO grade II and III gliomas.

Declarations

Ethics approval and consent to participate

This study was submitted to the Ethical Review Committee of Huashan Hospital, Fudan University for approval and clearance. Accordingly, the study has been checked for ethical issue and permission letter was obtained. Written consent was taken from each patient after they read and signed the consent form.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study were included in this published article and its supplementary information files are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no competing interests.

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Authors' contributions

Zengxin Qi, Jiajun Cai, Chao Tang, Liqin Lang: have developed proposal, data collection supervision, manuscript writing

Xiangda Meng, Shengyong Cai: have participated in proposal development, data collection, data analysis and manuscript writing.

All the authors read and approved the final manuscript.

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Abbreviations

OS, overall survival;

PFS, progression-free survival;

STR, subtotal resection;

GTR, gross total resection;

GBM, glioblastoma;

LMR, lymphocyte to monocyte ratio;

NLR, neutrophil to lymphocyte ratio;

PLR, platelet to lymphocyte ratio;

dNLR, derived NLR;

IDH, isocitrate dehydrogenase

TERT, telomerase reverse transcriptase

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Tables

Table 1. Clinical characteristics of the patient cohort (n=214)

Variable	Median (range)	Patients number (%)
Age at diagnosis (years)	41(5-79)	
≤50		168(78.5)
>50		46(21.5)
Gender		
Male		130(60.7)
Female		84(39.3)
WHO Grade		
□		132 (61.7)
□		82(38.3)
Histology		
Astrocytic		130(60.7)
Oligodendroglial/Oligoastrocytic		84(39.3)
Extent of resection		
GTR		162(75.7)
STR		52(24.3)
IDH mutation		
Mutant		168(78.5)
Wild-type		46(21.5)
TERT promoter mutation		
Mutant		79(36.9)
Wild-type		135(63.1)
1p/19q codeletion		
Yes		57(26.6)
No		157(73.4)
NLR	1.89(0.62-32.17)	
≤2.29		144(67.3)
>2.29		70(32.7)
LMR	4.76(0.83-30.17)	
≤4.81		113(52.8)
>4.81		101(47.2)
PLR	100.34(32.87-389.27)	
≤112.11		134(62.6)
>112.11		80(37.4)
dNLR	1.45(0.52-13.93)	
≤1.66		139(65.0)
>1.66		75(35.0)

Abbreviations: GTR, gross total resection; STR, subtotal resection; IDH, isocitrate dehydrogenase; TERT, telomerase reverse transcriptase; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; dNLR, derived neutrophil to lymphocyte ratio

Table 2 Assessment for correlations between NLR, LMR, PLR, dNLR and other clinical variables

Variables	Stratification	NLR		P	LMR		P	PLR		P	dNLR		P
		≤2.29	>2.29		≤4.81	>4.81		≤112.11	>112.11		≤1.66	>1.66	
Age	≤50	112	56	0.313	89	79	0.991	107	61	0.579	110	58	0.179
	>50	31	15		24	22		27	19		29	17	
Gender	male	87	44	0.959	71	60	0.299	94	37	**<0.001***	87	44	0.2319
	female	56	27		42	41		40	43		52	31	
WHO Grade	II	90	42	0.331	67	65	0.705	80	52	0.581	90	42	0.447
	III	53	29		46	36		54	28		49	33	
Extent of resection	GTR	107	55	0.8469	84	78	0.902	103	59	0.467	104	58	0.699
	STR	36	16		29	23		31	21		35	17	
IDH mutation	Mutant	115	53	0.806	87	81	0.797	104	64	0.644	114	54	0.763
	Wild-type	28	18		26	20		30	16		25	21	
TERT promoter mutation	Mutant	54	25	0.429	42	37	0.492	45	34	0.716	55	24	0.357
	Wild-type	89	46		71	64		89	46		84	51	
1p/19q codeletion	Yes	36	21	**0.045***	29	28	0.255	34	23	0.209	37	20	0.134
	No	107	50		84	73		100	57		102	55	

Abbreviations: GTR, gross total resection; STR, subtotal resection; IDH, isocitrate dehydrogenase; TERT, telomerase reverse transcriptase; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; dNLR, derived neutrophil to lymphocyte ratio

Table 3. Univariate and multivariate Cox regression analysis of different prognostic parameters for overall survival in All and IDH mutation (+) WHO grade II and III diffuse glioma patients

	Variables	Univariate			Multivariate		
		HR	95%CI	P	HR	95%CI	P
All Patients	Age(≤50 vs >50)	1.936	1.156-3.244	**0.012***	1.642	0.967-2.788	0.067
	Gender (male vs female)	1.215	0.748-1.973	0.432			
	WHO Grade(II vs III)	0.277	0.165-0.463	**<0.001***	3.000	1.720-5.234	**<0.001***
	Extent of resection (GTR vs STR)	1.085	0.630-1.869	0.768			
	IDH (mutation vs no)	3.238	1.976-5.305	**<0.001***	0.464	0.270-0.800	**0.006***
	TERT promotor (mutation vs no)	1.393	0.857-2.265	0.181			
	1p19q (codeletion vs no)	0.452	0.255-0.800	**0.006***	1.876	1.006-3.497	**0.048***
	NLR (≤2.29 vs >2.29)	1.968	1.242-3.117	**0.004***	0.550	0.346-0.872	**0.011***
	LMR (≤4.81 vs >4.81)	1.672	1.035-2.700	**0.036***	0.850	0.489-1.480	0.566
	PLR (≤112.11 vs >112.11)	1.417	0.864-2.323	0.167			
	dNLR (≤1.66 vs >1.66)	0.487	0.308-0.772	**0.002***	1.414	0.533-3.754	0.487
IDH mut. (+) Patients	Age(≤50 vs >50)	1.825	0.950-3.505	0.071			
	Gender (male vs female)	0.754	0.409-1.389	0.364			
	WHO Grade(II vs III)	2.678	1.347-5.325	**0.005***	0.325	0.159-0.663	**0.002***
	Extent of resection (GTR vs STR)	1.323	0.699-2.502	0.390			
	TERT promotor (mutation vs no)	0.454	0.247-0.836	**0.011***	2.403	1.293-4.467	**0.006***
	1p19q (codeletion vs no)	1.848	1.004-3.401	**0.048***	1.587	0.762-3.304	0.217
	NLR (≤2.29 vs >2.29)	1.994	1.137-3.496	**0.016***	1.665	0.438-6.329	0.454
	LMR (≤4.81 vs >4.81)	0.654	0.366-1.169	0.152			
	PLR (≤112.11 vs >112.11)	0.668	0.363-1.227	0.194			
	dNLR (≤1.66 vs >1.66)	1.929	1.098-3.390	**0.022***	1.097	0.285-4.232	0.893

Abbreviations: GTR, gross total resection; STR, subtotal resection; IDH, isocitrate dehydrogenase; TERT, telomerase reverse transcriptase; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; dNLR, derived neutrophil to lymphocyte ratio

Table 4. Univariate and multivariate Cox regression analysis of different prognostic parameters for overall survival in TERT promotor mutant and wild type WHO grade II and III diffuse glioma patients

	Variables	Univariate			Multivariate		
		HR	95%CI	P	HR	95%CI	P
TERT promotor mutation (+)	Age(≤50 vs >50)	5.118	2.178-12.026	**<0.001***	4.395	1.671-11.558	**0.003***
	Gender (male vs female)	0.538	0.222-1.304	0.170			
	WHO Grade(II vs III)	7.762	3.328-18.104	**<0.001***	4.290	1.499-12.281	**0.007***
	Extent of resection (GTR vs STR)	0.775	0.265-2.264	0.642			
	IDH (mutation vs no)	0.046	0.018-0.121	**<0.001***	0.050	0.011-0.233	**<0.001***
	1p19q(codeletion vs no)	2.481	1.119-5.501	**0.025***	0.740	0.215-2.550	0.634
	NLR (≤2.29 vs >2.29)	2.224	1.005-4.921	**0.049***	0.573	0.184-1.785	0.337
	LMR (≤4.81 vs >4.81)	0.389	0.162-0.934	**0.035***	0.571	0.178-1.828	0.345
	PLR (≤112.11 vs >112.11)	0.473	0.197-1.135	0.094			
	dNLR (≤1.66 vs >1.66)	2.001	0.887-4.512	0.095			
TERT promotor mutation (-)	Age(≤50 vs >50)	1.259	0.610-2.601	0.533			
	Gender (male vs female)	1.094	0.608-1.969	0.764			
	WHO Grade(II vs III)	3.534	1.731-7.251	**0.001***	3.528	1.713-7.265	**0.001***
	Extent of resection (GTR vs STR)	1.255	0.663-2.374	0.486			
	IDH (mutation vs no)	0.617	0.331-1.151	0.129			
	1p19q (codeletion vs no)	1.794	0.643-5.005	0.264			
	NLR (≤2.29 vs >2.29)	1.670	0.947-2.942	0.076			
	LMR (≤4.81 vs >4.81)	0.779	0.437-1.390	0.398			
	PLR (≤112.11 vs >112.11)	0.875	0.480-1.594	0.662			
	dNLR (≤1.66 vs >1.66)	1.987	1.127-3.504	**0.018***	1.925	1.090-3.399	**0.024***

Abbreviations: GTR, gross total resection; STR, subtotal resection; IDH, isocitrate dehydrogenase; TERT, telomerase reverse transcriptase; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; dNLR, derived neutrophil to lymphocyte ratio

Table 5. Univariate and multivariate Cox regression analysis of different prognostic parameters for overall survival and progression free survival in 1p19q intact WHO grade II and III diffuse glioma patients

	Variables	Univariate			Multivariate		
		HR	95%CI	P	HR	95%CI	P
OS	Age(≤50 vs >50)	2.372	1.412-3.985	**0.001***	2.006	1.180-3.412	**0.010***
	Gender (male vs female)	0.776	0.469-1.285	0.325			
	WHO Grade(II vs III)	3.366	1.851-6.119	**<0.001***	2.919	1.566-5.441	**0.001***
	Extent of resection (GTR vs STR)	1.381	0.824-2.315	0.221			
	IDH (mutation vs no)	0.470	0.285-0.778	**0.003***	0.592	0.350-1.001	**0.050***
	TERT promotor (mutation vs no)	1.089	0.621-1.912	0.766			
	NLR (≤2.29 vs >2.29)	1.901	1.171-3.086	**0.009***	1.397	0.522-3.738	0.505
	LMR (≤4.81 vs >4.81)	0.737	0.452-1.201	0.220			
	PLR (≤112.11 vs >112.11)	0.881	0.534-1.453	0.619			
	dNLR (≤1.66 vs >1.66)	2.000	1.232-3.248	**0.005***	0.495	0.304-0.805	**0.005***
PFS	Age(≤50 vs >50)	1.927	1.080-3.439	**0.026***	0.694	0.379-1.269	0.235
	Gender (male vs female)	0.811	0.471-1.398	0.452			
	WHO Grade(II vs III)	4.812	2.522-9.183	**<0.001***	0.254	0.129-0.501	**<0.001***
	Extent of resection (GTR vs STR)	0.964	0.528-1.759	0.904			
	IDH (mutation vs no)	0.383	0.227-0.648	**<0.001***	1.941	1.125-3.350	**0.017***
	TERT Promotor (mutation vs no)	1.061	0.581-1.936	0.848			
	NLR (≤2.29 vs >2.29)	0.466	0.278-0.781	**0.004***	1.156	0.373-3.584	0.802
	LMR (≤4.81 vs >4.81)	0.601	0.349-1.033	0.065			
	PLR (≤112.11 vs >112.11)	0.672	0.385-1.174	0.163			
	dNLR (≤1.66 vs >1.66)	2.492	1.485-4.182	**0.001***	0.458	0.271-0.776	**0.004***

Abbreviations: GTR, gross total resection; STR, subtotal resection; IDH, isocitrate dehydrogenase; TERT, telomerase reverse transcriptase; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; dNLR, derived neutrophil to lymphocyte ratio

Figures

Figure 1

X-tile analysis of OS were performed using patients' data to determine the optimal cut-off values for NLR, LMR, PLR and dNLR. The sample of patients was equally divided into training and validation sets. X-tile plots of training sets are shown in the left panels, with plots of matched validation sets shown in the smaller inset. The optimal cut-off values highlighted by the black circles in left panels are shown in histograms of the entire cohort (middle panels), and Kaplan-Meier plots are displayed in right panels. Blue represents the low-level group, and gray represents the high-level group. P values were determined by the cut-off values defined in training sets and applying them to validation sets. A. The optimal cut-off value for NLR in terms of OS was 2.29. B. The optimal cut-off value for LMR in terms of OS was 4.81. C. The optimal cut-off value for PLR in terms of OS was 121.11. D. The optimal cut-off value for dNLR in terms of OS was 1.66.

Figure 2

Kaplan-Meier survival curves of preoperative inflammatory markers for OS and PFS in WHO grade II and III diffuse gliomas. A. Low level of NLR was associated with significantly longer OS (p=0.006), but not correlated with better PFS (p=0.065). B. High level of LMR was associated with significantly longer OS (p=0.047), but not correlated with better PFS (p=0.459). C. No significance was found between PLR level and OS (p=0.112) or PFS (p=0.300) of the cohort. D. Low level of dNLR was associated with significantly longer OS (p=0.002), but not correlated with better PFS (p=0.071).

Figure 3

Kaplan-Meier survival curves of preoperative inflammatory markers for OS and PFS in IDH mutant or wild type, TERT promotor mutant or wild type and 1p19q intact or codeletion subgroups A. Low level of NLR was associated with significantly longer OS (p=0.014), but not correlated with better PFS (p=0.207) in IDH mutant subgroup. B. Low level of dNLR was associated with significantly longer OS (p=0.020), but not correlated with better PFS (p=0.404) in IDH mutant subgroup. C. Low level of NLR was associated with significantly longer OS (p=0.042), but not correlated with better PFS (p=0.126) in TERT promotor mutant subgroup. D. High level of LMR was associated with significantly longer OS (p=0.028), but not correlated with better PFS (p=0.436) in TERT promotor mutant subgroup. E. Low level of dNLR was associated with significantly longer OS (p=0.015), but not correlated with better PFS (p=0.211) in TERT promotor wild type subgroup. F. Low level of NLR was associated with significantly longer OS (p=0.003) and better PFS (p=0.008) in 1p19q intact subgroup. G. Low level of dNLR was associated with significantly longer OS (p=0.001) and better PFS (p=0.004) in 1p19q intact subgroup. H. Kaplan-Meier survival curves of dNLR for OS and PFS in IDH mutation (+) TERT promotor mutation (-) 1p19q codeletion (-) WHO grade II and III diffuse gliomas: Low level of dNLR was associated with significantly longer OS (p=0.032), but not correlated with better PFS (p=0.182).

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