Research Paper

Prognostic value of preoperative hematological markers combined with molecular pathology in patients with diffuse gliomas

Zhen-Yu Zhang[1,*], Yun-Bo Zhan[1,*], Feng-Jiang Zhang[1], Bin Yu[1], Yu-Chen Ji[1], Jin-Qiao Zhou[1], Ya-Hui Bai[1], Yan-Min Wang[1], Li Wang[2], Yan Jing[3], Wen-Chao Duan[1], Chen Sun[1], Tao Sun[1], Hai-Biao Zhao[1], Ke Li[1], Wen-Qing Wang[1], Ruo-Yan Li[4], Hong-Wei Sun[1], Guang Zhai[1], Shu-Kai Wang[1], Xin-Ting Wei[1], Bo Yang[1], Dong-Ming Yan[1], Xian-Zhi Liu[1], Wei-Wei Wang[2]

[1]Department of Neurosurgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
[2]Department of Pathology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
[3]Department of MRI, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
[4]Department of SICU, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
*Equal contribution

Correspondence to: Zhen-Yu Zhang, Xian-Zhi Liu, Wei-Wei Wang; **email:** fcczhangzy1@zzu.edu.cn, fccliuxz@zzu.edu.cn, wangweiwei0086@zzu.edu.cn
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ABSTRACT

The prediction of clinical outcome for patients with infiltrative gliomas is challenging. Although preoperative hematological markers have been proposed as predictors of survival in glioma and other cancers, systematic investigations that combine these data with other relevant clinical variables are needed to improve prognostic accuracy and patient outcomes. We investigated the prognostic value of preoperative hematological markers, alone and in combination with molecular pathology, for the survival of 592 patients with Grade II-IV diffuse gliomas. On univariate analysis, increased neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), and decreased albumin-to-globulin ratio (AGR), all predicted poor prognosis in Grade II/III gliomas. Multivariate analysis incorporating tumor status based on the presence of *IDH* mutations, *TERT* promoter mutations, and 1p/19q codeletion showed that in lower-grade gliomas, high NLR predicted poorer survival for the triple-negative, IDH mutation only, TERT mutation only, and IDH and TERT mutation groups. NLR was an independent prognostic factor in Grade IV glioma. We therefore propose a prognostic model for diffuse gliomas based on the presence of *IDH* and *TERT* promoter mutations, 1p/19q codeletion, and NLR. This model classifies lower-grade gliomas into nine subgroups that can be combined into four main risk groups based on survival projections.

INTRODUCTION

Gliomas are the most common malignant primary brain tumors, accounting for 27% of all central nervous system (CNS) tumors [1]. According to the World Health Organization (WHO) classification of CNS tumors, gliomas are pathologically categorized into four grades, of which Grade II to IV are considered diffusely infiltrating gliomas [2, 3].

Research on molecular alterations in gliomas has revealed three noteworthy biomarkers, namely codeletion

of chromosome arms 1p and 19q (1p/19q codeletion), and mutations in *IDH* and the *TERT* promoter, that can be used to classify Grade II-IV gliomas into five principal molecular groups (triple-positive, *IDH* and *TERT* mutations, *IDH* mutation only, triple-negative, and *TERT* mutation only). These groups are associated with distinct prognosis, germline variants, and median age at diagnosis, highlighting different pathogenic mechanisms [3]. Although most glioma patients receive standard treatments, significant variations in clinical outcomes are often seen due to the heterogeneity of the tumors [4]. Therefore, it is necessary to identify more appropriate and effective biomarkers for predicting prognosis in glioma patients.

Inflammation and immunity are critically involved in glioma initiation and progression [5, 6], and several studies demonstrated that inflammatory response cells such as neutrophils [7], lymphocytes [8] and platelets [9] are associated with the prognosis of cancer patients. In recent years, the prognostic value of preoperative hematological markers, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), median platelet volume (MPV), platelet distribution width (PDW), and albumin-to-globulin ratio (AGR), has been investigated in several cancers, including gliomas [10–17]. However, there is a lack of studies systematically investigating the prognostic value of hematological markers in a large cohort of gliomas, particularly in relation to the different molecular subtypes.

Therefore, we investigated the prognostic value of preoperative hematological markers (NLR, PLR, MLR, MPV, PDW, and AGR), alone and in combination with the 5 glioma molecular groups, on the clinical outcomes of a relatively large cohort (n = 592) of Grade II-IV glioma patients. Based on these findings, we propose a prognostic model for Grade II-IV infiltrative gliomas based on molecular pathology and NLR, and identify for lower-grade (WHO Grade II and III) gliomas, four risk groups with distinct overall survival. Further validation of the model in more extensive cohorts should confirm its usefulness and possibly open the way to new therapeutic strategies.

RESULTS

Clinico-pathological characteristics of the cohort

A total of 592 cases (adult patients, age ≥ 16) of WHO Grade II-IV supratentorial gliomas were analyzed. The median age of the cohort was 42 years (interquartile range = 39–58 years). There were 335 male patients (56.6%) and 257 female patients (43.4%). The cohort included 404 patients (68.2%) with Grade II-III glioma and 188 patients (31.8%) with Grade IV glioma. Median duration of follow-up was 32.0 months. Complete resection was achieved in 456 patients (77%), and incomplete resection was performed in 136 patients (23%). Four hundred and fifty-nine patients (77.5%) received postoperative primary radiation therapy (RT) and 342 patients (57.8%) received postoperative primary chemotherapy (CHT). In patients with astrocytoma, 14 (9.0%) received postoperative primary RT, 10 (6.5%) received postoperative primary CHT, 113 (72.9%) received postoperative primary RT and CHT, and 18 (11.6%) received no postoperative treatment. Among patients with oligodendroglioma or oligoastrocytomas, 31 (12.5%) received postoperative primary RT, 24 (9.7%) received postoperative primary CHT, 166 (66.9%) received postoperative primary RT and CHT, and 27 (10.9%) received no postoperative treatment (Supplementary Table 3). Molecular pathology analyses were available for 573 cases. *IDH* mutations were found in 246 cases (42.9%), mutations in *TERT* promoter were detected in 286 cases (49.9%), and chromosome 1p/19q codeletion was detected in 139 cases (34.4%). Hematological markers were defined in 528/592 cases, as 64 cases were excluded due to conditions that could influence peripheral blood counts. Detailed information on the clinico-pathological features of the cohort is listed in Supplementary Table 1.

Molecular groups

Among the 573 cases of Grade II-IV gliomas, 103 (18.0%) were triple-positive, 19 (3.3%) had mutations in both *IDH* and *TERT*, 108 (18.8%) had *IDH* mutation only, 144 (25.1%) were triple-negative, 155 (27.1%) had *TERT* mutation only, and 44 (7.7%) had other combinations of the three biomarkers (Figure 1A). For lower-grade glioma cases (n = 392), 103 (26.3%) were triple-positive, 19 (4.8%) had both *IDH* and *TERT* mutations, 100 (25.5%) had *IDH* mutation only, 48 (12.24%) had *TERT* mutation only, 78 (19.9%) were triple-negative, and 44 (11.2%) had other combinations (Figure 1B). For Grade IV glioma cases (n = 181), 8 (4.4%) had *IDH* mutation only, 107 (59.1%) had *TERT* mutation only, and 66 (36.5%) were triple-negative (Figure 1C). Univariate survival analysis demonstrated that molecular groups significantly influenced the OS of patients with lower-grade gliomas. The triple-positive group had favorable prognosis, whereas the *TERT* mutation group had a dismal survival expectancy (Figure 1D, univariate analysis in Supplementary Table 2), although this relationship was not found for Grade IV gliomas (Figure 1E, Supplementary Table 5). In subsequent multivariate analysis, molecular group was revealed as an independent prognostic factor in lower-grade gliomas (Table 1).

Prognostic value of hematological markers in lower-grade and Grade IV gliomas

The prognostic value of the hematological markers was evaluated in lower-grade gliomas and Grade IV gliomas. Optimal cut-off values of NLR, PLR, MLR, MPV, PDW, and AGR were computed by X-tile software. Univariate analysis demonstrated that high NLR ($P < 0.001$), PLR ($P = 0.013$), and MLR ($P = 0.046$), and low AGR ($P = 0.043$) were associated with shorter survival in lower-grade gliomas (Figure 2A–2D), while MPV ($P = 0.204$) and PDW ($P = 0.422$) had no prognostic significance. Because hematological markers were strongly correlated and interfered with each other [14], they were separately analyzed with other potential prognostic factors in multivariate analysis. The latter revealed that NLR ($P = 0.046$, Table 2) is a prognostic factor for lower-grade gliomas independent of age, extent of resection, and adjuvant therapies. Conversely, neither PLR ($P = 0.102$), MLR ($P = 0.188$), nor AGR ($P = 0.621$) were independent prognostic factors (Supplementary Table 4). In Grade IV gliomas, only NLR emerged as a significant prognostic factor in univariate ($P = 0.001$, Figure 2E) and multivariate ($P = 0.002$, Table 3) analyses.

Prognostic value of hematological markers within molecular groups of lower-grade gliomas

Since molecular subtype is an independent factor influencing survival in lower-grade gliomas but not Grade IV glioma, we evaluated the prognostic value of hematological markers for each molecular group in lower-grade gliomas. Optimal cut-off values for NLR, PLR, MLR, and AGR in each group were computed by X-tile software. As shown in Figure 2F–2L, univariate analysis showed that high NLR predicted shorter OS in lower-grade glioma groups defined by *IDH* and *TERT* mutations ($P = 0.009$), *IDH* mutation only ($P = 0.047$), *TERT* mutation only ($P = 0.005$), and in the triple-negative group ($P = 0.005$). In turn, high PLR predicted shorter OS in the *IDH* mutation only ($P = 0.014$) and *TERT* mutation only ($P = 0.001$) groups, while high MLR was associated with shorter OS in gliomas with *IDH* and *TERT* mutations ($P = 0.006$). In contrast, none of the hematological markers impacted OS in the triple-positive group of lower-grade gliomas (Supplementary Figure 1A–1D). Likewise, no prognostic significance was found for PLR and AGR in the *IDH* and *TERT* mutation group (Supplementary Figure 1E, 1F), MLR

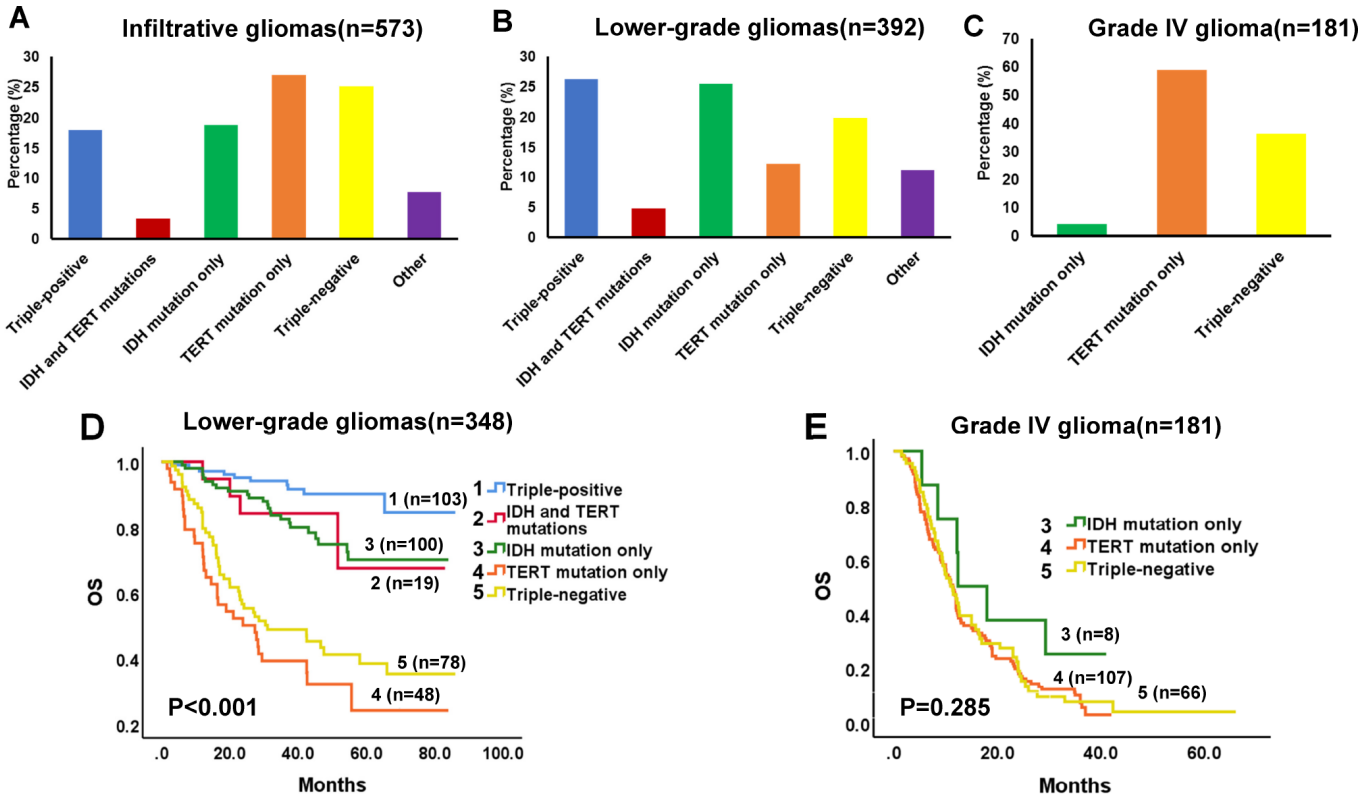

Figure 1. Proportion and Kaplan-Meier survival analyses of molecular groups in diffuse infiltrative gliomas. Survival proportions in infiltrative (Grade II-IV) gliomas (**A**), lower-grade (Grade II-III) gliomas (**B**), and Grade IV glioma (**C**). (**D**) Kaplan-Meier OS curves in lower-grade gliomas. OS estimates for the 5 molecular groups are significantly different ($P < 0.001$). (**E**) Kaplan-Meier OS curves in Grade IV glioma. No differences in OS were detected for the 3 molecular groups ($P = 0.285$).

Table 1. Multivariate analysis of adjusting putative prognostic factors for molecular group (n=348[a]) and risk group (n=348[a]) in lower-grade gliomas.

Factors	OS	
	HR (95%CI)	P-value
Molecular group	1.578 (1.366–1.822)	**<0.001**
Age	1.883 (1.220–2.908)	**0.004**
Extent of resection	1.262 (0.813–1.960)	0.300
RT (Yes or No)	1.770 (1.147–2.732)	**0.010**
Grade (II or III)	3.408 (2.300–5.049)	**<0.001**
KPS (≤80 or >80)	0.693 (0.478–1.006)	0.054
Risk group	1.214 (1.149–1.283)	**<0.001**
Age (≤40 or >40)	1.630 (1.061–2.504)	**0.026**
Extent of resection	1.122 (0.721–1.745)	0.610
RT (Yes or No)	1.772 (1.153–2.724)	**0.009**
KPS (≤80 or >80)	0.742 (0.510–1.079)	0.118
Grade (II or III)	3.112 (2.091–4.634)	**<0.001**

[a]12 cases were excluded due to unavailability of FFPE tissues of the tumors, and 44 cases of other combinations of the three molecular markers were excluded.
Molecular group: the five molecular groups based on the statuses of *IDH* mutations, *TERT* promoter mutations and 1p/19q co-deletion, which include triple positive, *IDH* and *TERT* mutations, *IDH* mutation only, *TERT* mutation only and triple negative).
Risk group: the four groups based on the statuses of *IDH* mutations, *TERT* promoter mutations, 1p/19q codeletion and the levels of NLR in each molecular subgroup
OS: overall survival
RT: radiation therapy, indicating postoperative radiation therapy after first operation
KPS: Karnofsky Performance Status
HR: Hazard-ratio

and AGR in the *IDH* mutation only group (Supplementary Figure 1G, 1H), MLR and AGR in the *TERT* mutation only group (Supplementary Figure 2A, 2B), and PLR, MLR, and AGR in the triple-negative group (Supplementary Figure 2C–2E) of lower-grade gliomas.

A glioma prognostic model combining molecular pathology and hematological markers

Based on combined data derived from survival analyses of molecular pathology and hematological markers, we propose a prognostic model to predict survival in glioma patients (Figure 3A). In the model, infiltrative gliomas include lower-grade gliomas and Grade IV glioma. Lower-grade gliomas were divided into 5 primary molecular groups associated with distinct OS [3]. Since NLR is a prognostic factor independent of putative clinical variables in lower-grade gliomas, and predicts survival in 4 tumor subtypes (triple-negative, *IDH* and *TERT* mutations, *IDH* mutation only, and *TERT* mutation only), this hematological marker is proposed to further stratify the prognosis of these 4 molecular groups. In contrast, high NLR arises as an independent predictor of worse survival for Grade IV glioma.

Molecular pathology and NLR stratify lower-grade gliomas into four risk groups

According to the prognostic model proposed in Figure 3A, lower-grade gliomas were categorized into nine subgroups based on the status of *IDH* and *TERT* promoter mutations, 1p/19q codeletion, and NLR. Survival analyses revealed significantly different OS for these nine subgroups ($P < 0.001$, Figure 3B, Supplementary Table 6). Furthermore, subgroups with non-significant differences in OS between them were integrated into individual risk groups: Subgroups 1, 2, and 4 conformed the Low risk group, Subgroups 3, 5, 6, and 8 conformed the Intermediate-I risk group, Subgroup 9 was defined as the Intermediate-II risk group, and Subgroup 7 represented the High risk group (Figure 3B, 3C). Univariate ($P < 0.001$, Figure 3C, Supplementary Table 7) and multivariate ($P < 0.001$, Table 1) analyses yielded significantly different OS for these four risk groups.

DISCUSSION

In the present study, data from a large cohort of gliomas (n = 592) were used to corroborate previous findings on the 5 glioma molecular groups defined by three robust markers, 1p/19q codeletion, *IDH* mutations, and *TERT* promoter mutations [2] and to demonstrate, for lower-grade gliomas, the differential prognostic value of hematological markers in each molecular group. Based on these findings, we propose a prognostic model for infiltrative gliomas that combines molecular and hematological markers.

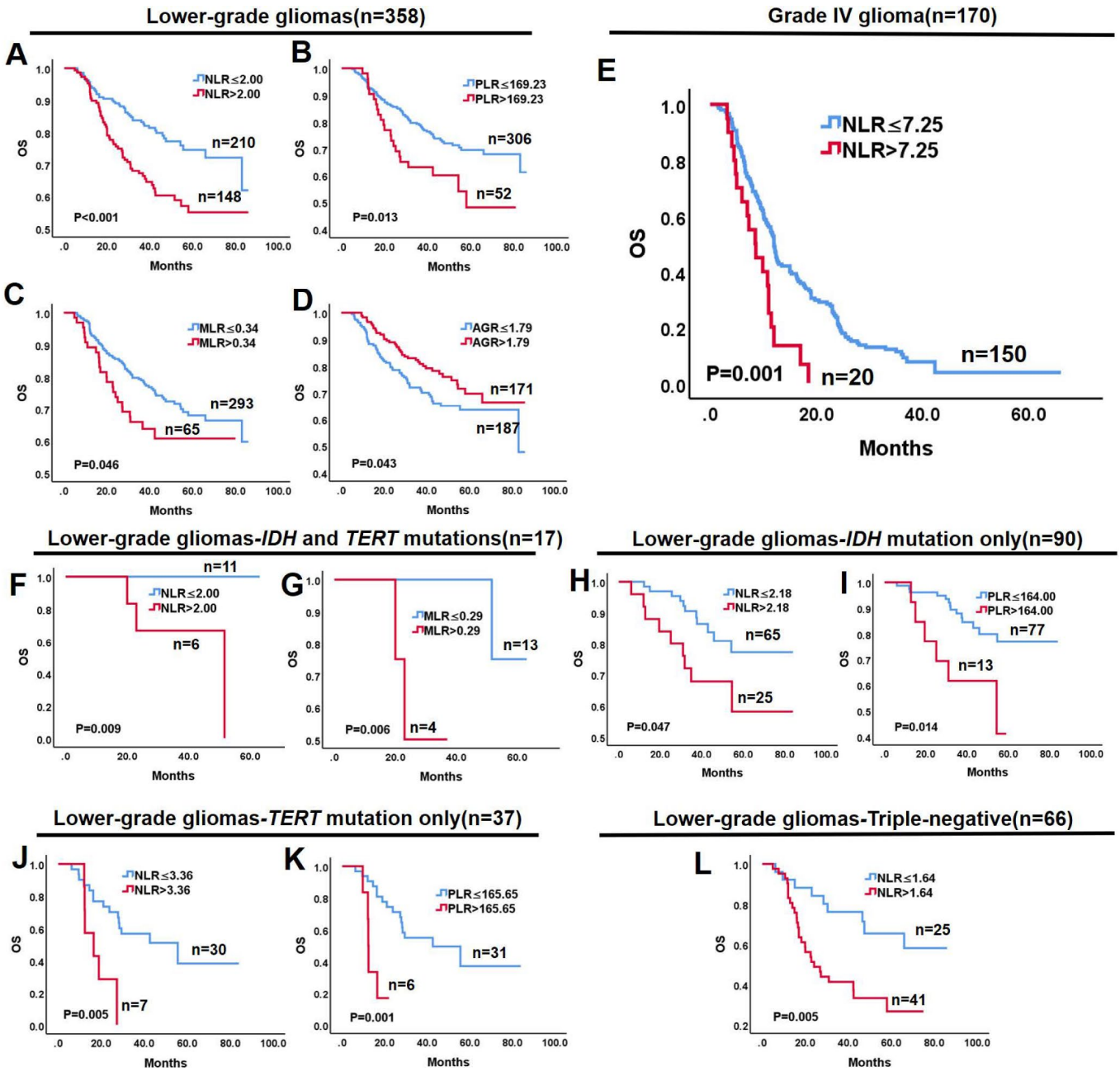

Figure 2. Kaplan-Meier survival curves of glioma subgroups based on hematological markers. Lower-grade gliomas: (**A**) NLR > 2.00 is associated with worse OS (P < 0.001); (**B**) PLR > 169.23 is associated with worse OS (P = 0.013); (**C**) MLR > 0.34 is associated with worse OS (P = 0.046); and (**D**) AGR > 1.79 is associated with better OS (P = 0.043). (**E**) In Grade IV glioma, NLR > 7.25 is associated with better OS (P = 0.001). (**F**, **G**) In the IDH and TERT mutations group of lower-grade gliomas, NLR > 2.00 and MLR > 0.29 predict worse OS (P < 0.009 and P = 0.006, respectively). (**H**, **I**) In the IDH mutation only group of lower-grade gliomas, NLR > 2.18 and PLR > 164.00 predict worse OS (P = 0.047 and P = 0.014, respectively). (**J**, **K**) In the TERT mutation only group of lower-grade gliomas, NLR > 3.36 and PLR > 165.65 predict worse OS (P = 0.005 and P = 0.001, respectively). (**L**) In triple-negative lower-grade gliomas, NLR > 1.64 predicts worse OS.

Table 2. Multivariate analysis of adjusting putative prognostic factors for NLR (n=358[a]) in lower-grade gliomas.

Factors	OS	
	HR (95%CI)	P-value
NLR	1.502 (1.007–2.240)	**0.046**
Age	1.042 (1.024–1.060)	**<0.001**
Extent of resection	0.907 (0.540–1.524)	0.713
RT (Yes or No)	0.860 (0.514–1.440)	0.567
Grade (II or III)	3.746 (2.499–5.618)	**<0.001**
KPS (≤80 or >80)	0.618 (0.416–0.916)	**0.017**

[a]46 cases were excluded due to conditions that could influence hematological makers
OS: overall survival
RT: radiation therapy, indicating postoperative radiation therapy after first operation
KPS: Karnofsky Performance Status
HR: Hazard-ratio

Table 3. Multivariate analysis of adjusting putative prognostic factors for NLR in Grade IV glioma (n=170[a]).

Factors	OS	
	HR (95%CI)	P-value
NLR	2.228 (1.329–3.733)	**0.002**
Extent of resection	2.815 (1.952–4.059)	**<0.001**
RT (Yes or No)	1.213 (0.772–1.907)	0.402
CHT (Yes or No)	1.339 (0.871–2.061)	0.184
Age (≤62 or >62)	1.587(1.103–2.284)	**0.013**

[a]18 cases were excluded due to conditions that could influence hematological makers
OS: overall survival
RT: radiation therapy, indicating postoperative radiation therapy after first operation
CHT: chemotherapy, indicating postoperative chemotherapy after first operation
HR: Hazard-ratio

The involvement of tumor-associated inflammatory cells in carcinogenesis has been firmly established [5, 7]. Cancer cells secrete chemokines and cytokines that attract host inflammatory cells such as neutrophils and lymphocytes, and these cells produce in turn pro-inflammatory cytokines, growth factors, and chemokines that contribute to tumor progression [18–20]. Unlike genetic biomarkers, preoperative hematological markers can be easily calculated from routine blood tests and may have important clinical significance for cancer prognosis. In recent years the prognostic value of NLR, PLR, MLR, and AGR has been investigated and corroborated in several cancers, such as hepatocellular carcinoma [21], pancreatic carcinoma [22], renal carcinoma [11], esophageal cancer [23], gastric carcinoma [24], colorectal cancer [25], lung cancer [26] and gliomas [14–17, 27, 28].

Our study demonstrated that NLR, PLR, MLR, and AGR are prognostic factors in univariate analysis for lower-grade gliomas. Also, for these tumors, multivariate analysis revealed that NLR is an independent prognostic factor after adjusting for age, grade, histology, extent of resection, and adjuvant therapies.

Studies demonstrated that neutrophil-induced immuno-suppression can promote glioma progression, and that certain subsets of T-lymphocytes can instead inhibit it via induction of cytotoxic cell death and cytokine production [20, 29, 30]. Accordingly, Han et al. reported that high neutrophil and low CD3+ T-cell infiltration (elevated NLR) in glioblastomas was correlated with poorer outcomes [15]. Evidence for the importance of PLR in oncogenesis comes from studies showing that platelet activation contributes to tumor angiogenesis, disruption of the extracellular matrix and release of adhesion molecules to promote cancer cell proliferation and metastasis [31, 32]. As for AGR, its relevance in cancer may be related to the antioxidative effects of albumin against carcinogens such as nitrosamines and aflatoxins

[33], and the association of elevated globulin levels with the progression and metastasis of some cancers [34].

Although predicting the clinical outcome of infiltrative gliomas is challenging, considerable progress in the classification of gliomas based on molecular markers has been made in the past several years [2, 3, 35–39]. Particularly, three robust molecular alterations, namely 1p/19q codeletion and *IDH* and *TERT* promoter mutations, were used to categorize five principal molecular groups of gliomas with distinct clinical traits and mechanisms of carcinogenesis [2]. Chromosome 1p/19q codeletion is associated with oligodendrogliomas, sensitivity to adjuvant therapies, and favorable survival [35, 40]. Mutations in *IDH* genes (*IDH*1 and *IDH*2) have been revealed in the majority of lower-grade gliomas and in secondary glioblastoma multiforme, and predict better survival [36, 41]. In a previous study we demonstrated that *TERT* promoter mutations could identify among lower-grade gliomas a group of *IDH*-mutated-1p/19q-intact tumors with better survival and a subset of *IDH* wild-type tumors with worse prognosis [42]. At present, the classification of infiltrative gliomas based on these three molecular markers is routinely conducted and of vital significance in clinical practice [3]. Based on this scheme, through multivariate survival analysis on 573 adult infiltrative gliomas we confirmed in lower-grade

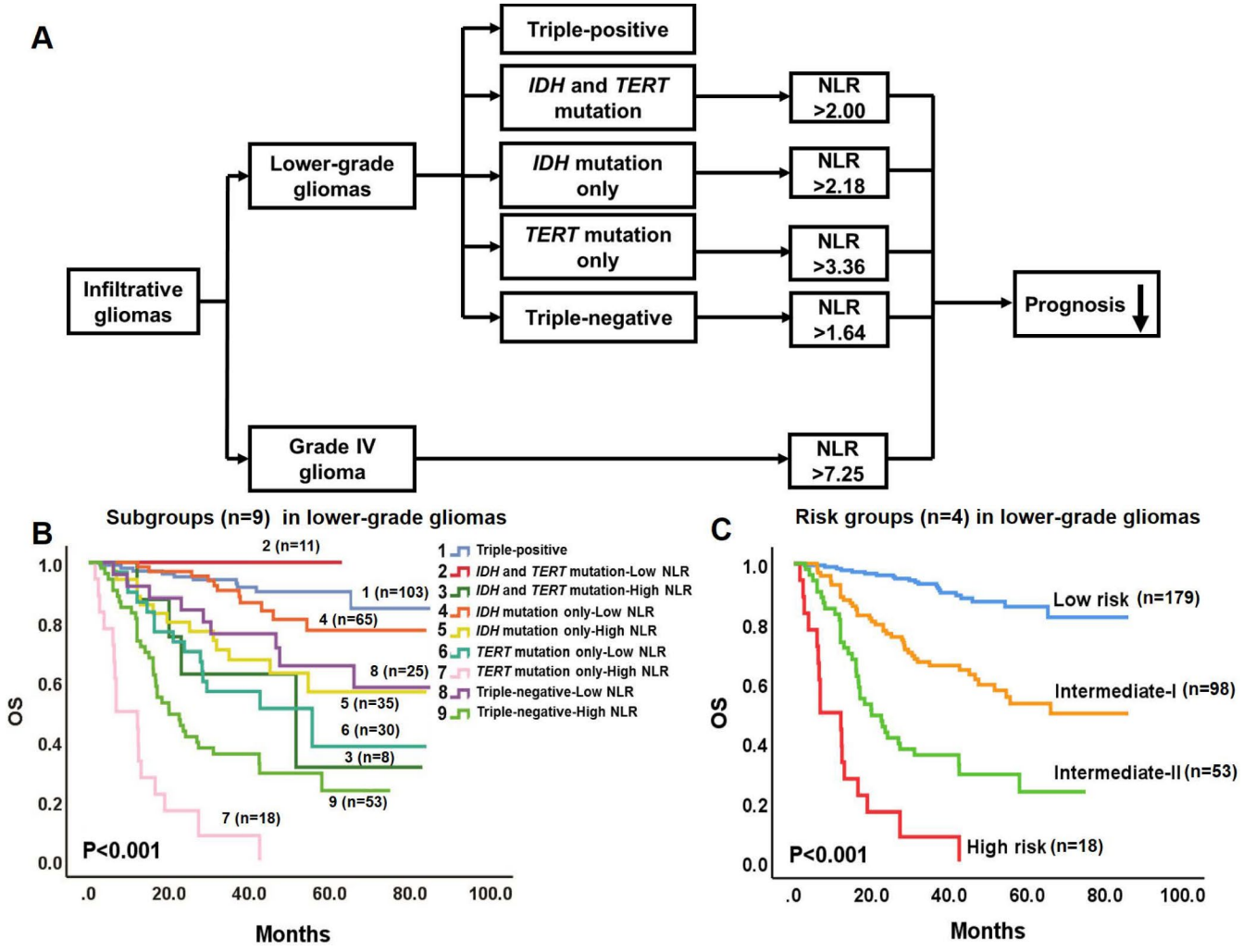

Figure 3. Prognostic model combining molecular pathology and hematological markers for gliomas. (**A**) In the model, infiltrative gliomas include lower-grade gliomas and Grade IV glioma. Lower-grade gliomas are divided into 5 molecular groups with different OS. NLR is proposed to further stratify the OS of 4 of these groups (triple-positive tumors are excepted). In Grade IV glioma, high NLR independently predicts worse OS. (**B**) Kaplan-Meier OS curves of the 9 subgroups of lower-grade gliomas. The OS of the 9 subgroups are significantly different (P < 0.001). (**C**) Kaplan-Meier OS curves of the 4 risk groups in lower-grade gliomas: Subgroups 1, 2, and 4 were integrated into a Low risk group; Subgroups 3, 5, 6, and 8 were integrated into an Intermediate-I risk group; Subgroup 9 comprises the Intermediate-II risk group; and Subgroup 7 constitutes the High risk group. The OS of the 4 risk groups differs significantly (P < 0.001).

tumors the prognostic significance of the principal molecular groups independent of age, histology, and clinical variables. Our results further corroborate the findings reported by Eckel-Passow et al. [3] while adding several key clinical variables omitted in their research. Meanwhile, consistent with Eckel-Passow et al., for WHO Grade IV gliomas the molecular groups lacked independent prognostic significance.

We investigated for the first time, to the best of our knowledge, the prognostic value of hematological markers within the 5 primary glioma molecular groups and found that for lower-grade gliomas, high NLR and MLR predicted worse survival in the *IDH* and *TERT* mutations group, high NLR and PLR predicted worse survival in the *IDH* mutation only and *TERT* mutation only groups, and high NLR was associated with shorter survival in the triple-negative group. Interestingly, no predictive value was found for any hematological marker in triple-positive tumors. We speculate that any potential contribution to prognosis may be masked by the favorable survival characteristic of lower-grade gliomas within this molecular group. The differential prognostic values found for these hematological markers may be related to distinct immune micro-environments associated with specific molecular groups. For example, Qian et al. reported that immune responses in lower-grade gliomas are regulated by *IDH* mutations [43]. In Grade IV glioma, NLR was revealed as an independent prognostic factor in multivariate analysis, while the predictive values of PLR, MLR, and AGR were not significant in univariate analyses. We therefore developed a prognostic model for infiltrative gliomas by combining molecular and hematological markers. The model identified four risk groups based on molecular pathology and NLR in lower-grade gliomas, and two risk groups based on NLR in Grade IV glioma. The model only requires information of routine preoperative blood tests and molecular analysis of 1p/19q codeletion and *IDH* and *TERT* promoter mutations, which is also available in most medical centers. We think the model can be used readily and easily in the clinic, after corroboration from a multi-center, prospective clinical trial.

The present study has some limitations. First, due to the retrospective nature of the study, systematic bias might influence the accuracy of the results. Second, although the current study enrolled a relatively large sample size, it was carried out in a single research center. Thus, multi-center, prospective studies are necessary to corroborate our findings. Lastly, more extensive research is needed to clarify the detailed mechanisms through which hematological markers influence the prognosis of molecular groups in gliomas.

In summary, our study corroborates the prognostic significance of glioma subtypes based on 1p/19q codeletion and *IDH* and *TERT* promoter mutations in a large Chinese cohort. Moreover, we propose a novel prognostic model for diffuse infiltrative gliomas that combines molecular pathology and hematological markers, and may increase prognostic accuracy and improve patient outcomes.

MATERIALS AND METHODS

Study cohort

This study was approved by the Human Scientific Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Five hundred and ninety-two cases of infiltrative gliomas (WHO II, III and IV) with complete follow-up data were enrolled in the study. Patients in the cohort were surgically treated in the First Affiliated Hospital of Zhengzhou University from 2011 to 2016. The diagnosis was made by pathological examination and centrally reviewed by two pathologists according to the 2016 WHO classification of tumors of the CNS [2]. All patients enrolled in the current study were treatment-naïve (i.e. neither surgical resection, chemotherapy, nor radiotherapy were administered before the first operation). For survival analysis of hematological markers, patients with hematological diseases, serious infections, surgery, trauma, and anti-coagulant therapy were excluded. All clinical data, including gender, age, preoperative Karnofsky Performance Status (KPS) score, extent of resection, histological grade, and adjuvant therapies were collected from the medical record system. Follow-up data were acquired by telephone or out-patient follow-up. Overall survival (OS) was calculated as the time interval between the date of surgery and the date of death or the end of follow-up.

Molecular classification

Formalin-fixed, paraffin embedded (FFPE) tissues were available in 573 cases. The detection of molecular markers was centrally conducted with standardized protocols. Mutational hotspots in *IDH*1, *IDH*2, and the *TERT* promoter were detected by Sanger sequencing. Chromosome 1p/19q status was evaluated by fluorescence in situ hybridization in all WHO Grade II and Grade III gliomas. Detailed protocols are described in the Supplementary Materials. According to the status of the three molecular markers, infiltrative gliomas were categorized into five principal groups: triple-positive (mutations in *TERT* promoter and *IDH*, plus 1p/19q codeletion); mutations in both *TERT* and *IDH*; mutation in *IDH* only; mutation in *TERT* only; and triple-negative [3].

Hematological markers

Routine preoperative blood and hepatic function tests prior to the first surgical resection were centrally performed at the Department of Clinical Laboratory within 2 hours of blood sample collection. Blood test results included neutrophil, lymphocyte, mononuclear cell, and platelet counts, as well as mean platelet volume and platelet distribution width. Results of the hepatic function test included albumin and globulin levels to calculate AGR. Hematological markers included: NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, MLR = monocyte-to-lymphocyte ratio, MPV = median platelet volume, PDW = platelet distribution width, and AGR = albumin-to-globulin ratio.

Statistical methods

SPSS 19.0 (IBM Corp., Armonk, NY, USA), GraphPad Prism 6.0 (Graph-pad Inc, La Jolla, USA) and X-tile 3.6.1 (http://medicine.yale.edu/lab/rimm/research/software.aspx) were used to analyze the data. The Kaplan-Meier method and the log-rank test were used to calculate survival rates. Post-hoc Bonferroni test was used for multiple comparisons. Multivariate analysis using Cox regression was performed to evaluate independent prognostic factors. $P < 0.05$ was considered statistically significant.

AUTHOR CONTRIBUTIONS

Research conception and design, manuscript revision and approval: Zhen-yu Zhang, Xian-zhi Liu, Wei-wei Wang; Material and data collection, statistical analysis, drafting of the manuscript: Zhen-yu Zhang, Yun-bo Zhan; Molecular pathology: Wei-wei Wang, Li Wang; Acquisition of tissue specimens and assessment of clinical outcomes: Feng-jiang Zhang, Bin-Yu, Yu-chen Ji, Jin-qiao Zhou, Ya-hui Bai, Yan-min Wang, Yan Jing, Wen-chao Duan, Chen Sun, Tao Sun, Hai-biao Zhao, Ke Li, Wen-qing Wang, Ruo-yan Li, Hong-wei Sun, Guang Zhai, Shu-kai Wang, Xin-ting Wei, Bo Yang, Dong-ming Yan.

CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

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SUPPLEMENTARY MATERIALS

Supplementary Methods

Mutation analysis of *IDH*1/*IDH*2

Mutational hotspots of *IDH1/IDH2* were evaluated by direct sequencing. Tissues from representative tumor area (the proportion of tumor cells>20%) were scrapped off from dewaxed sections and treated with PCR reaction solution A 10μl (reaction mixture containing 1μl of cell lysate, 0.3mM of each dNTP, 2.5mM $MgCl_2$, 0.3μM of each primer and 0.2U of KAPA HiFi HotStart DNA Polymerase (Kapa Biosystems Inc., Wilmington, USA)), Shrimp Alkaline Phosphatase (SAP) enzyme (NEB, Ipswich, MA, USA) 2μl and BigDye (BigDye™ Terminator v3.1 Cycle Sequencing Kit, Thermo Fisher Scientific, Waltham, MA, USA) 1μl for centrifugation at 2000 rpm for 10 sec. The crude cell lysate was centrifuged and supernatant was used for subsequent PCR analysis. The forward primer primers (*IDH*1-F:5'-CGGTCTTCAGAGAAGCCATT-3',*IDH*1-R:5'-CACATTATTGCCAACATGAC-3',*IDH*2-F:5'-AGCCCATCATCTGCAAAAAC-3',*IDH*2-R:5'-CTAGGCGAGGAGCTCCAGT-3') were used to amplify the region of mutational hotspots of *IDH1/IDH2*. ①PCR was performed was initiated at 95°C for 5 min, followed by 40 cycles of 95°C for 20 sec, 57°C for 30 sec and 72°C for 1min, and a final extension of 72°C for 5 min and 10°C for 10 min. ②5μl PCR products were then mixed with 2μl SAP enzyme and reacted at 37°C for 40min and then at 80° C for 15min. ③ Then 18μl PCR reaction solution C (CWBIO, Beijing, Chima), 1μl products from ② step, and 1μl BigDye were mixed and reacted at 96°C for 1 min, followed by 30 cycles of 96°C for 10 sec, 50° C for 5 sec and 60° C for 2 min, and a final extension of 25°C for 1 min and 10°C for 10 min. Then 50μl natrium asceticism-ethanol mixture (3M NaAc: ethanol=1:15) were added and the mixture was centrifuged for 30min (12000 rpm, 4°C), with the supernatant being discarded. Then 70μl 75% ethanol were added and the mixture was centrifugated for 15min (12000 rpm, 4°C), with the supernatant being discarded. After complete volatilization of the ethanol at room temperature, 12μl Hi-Di™ Formamide (Thermo Fisher Scientific, Waltham, MA, USA) were added into the precipitate to dissolve the DNA. The dissolved products were sequenced on Applied Biosystems™ 3500DxGenetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), and analyzed by Chromas software (Technelysium, South Brisbane, Australia). The sequencing results were compared with wild-type sequences of IDH1/IDH2 for analysis.

Mutation analysis of *TERT* promoter

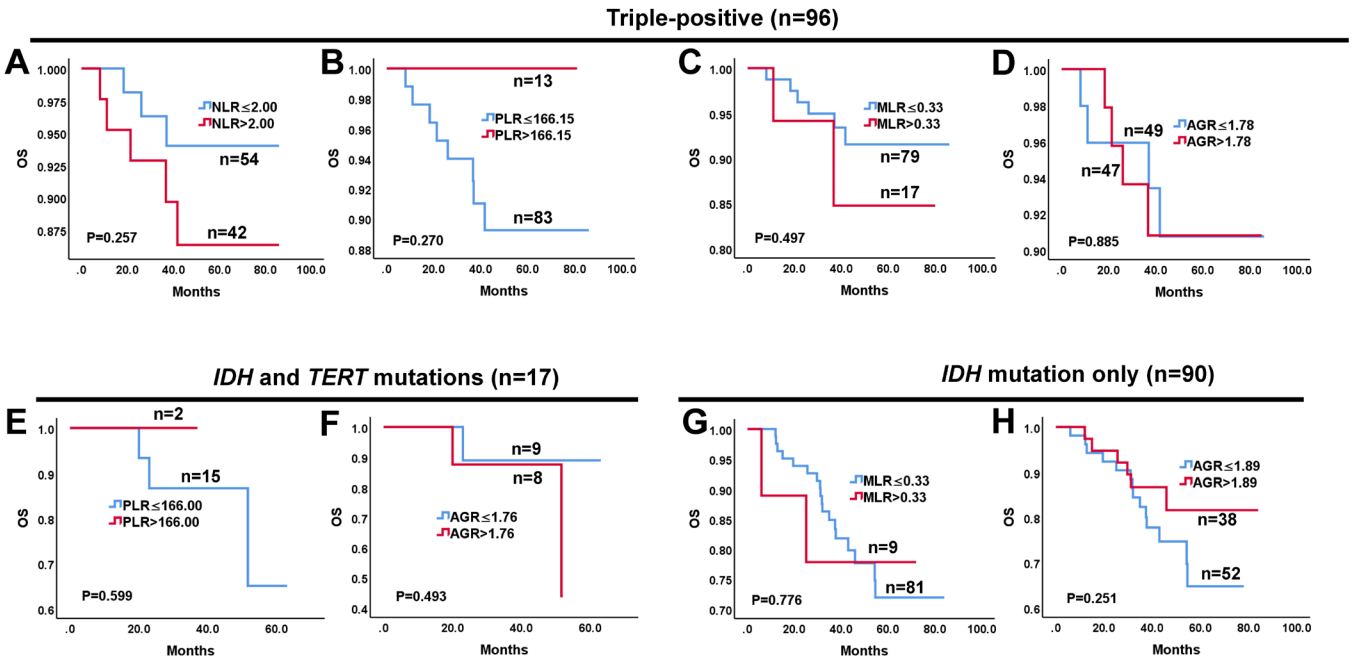
Tissues sample were prepared according to the "Mutation Analysis of *IDH*1/*IDH*2 protocol" protocol previous described. The crude cell lysate was centrifuged and supernatant was used for subsequent PCR analysis. The forward primer *TERT*-F (5'-GTCCTGCCCCTTCACCTT-3') and reverse primer *TERT*-R (5'-CAGCGCTGCCTGAAACTC-3') were used to amplify a 163bp fragment spanning the two mutational hotspots [chr5, 1,295,228 (C228T) and 1,295,250 (C250T)] in *TERT* promoter region. ①PCR was performed was initiated at 95°C for 5 min, followed by 40 cycles of 95°C for 20 sec, 57°C for 30 sec and 72°C for 1min, and a final extension of 72°C for 5 min and 10°C for 10 min. ②5μl PCR products were then mixed with SAP enzyme and reacted at 37°C for 40 min and then at 80° C for 15 min. ④Then 18μl PCR reaction solution C, 1μl products from ② step, and 1μl BigDye were mixed and reacted at 96°C for 1 min, followed by 30 cycles of 96°C for 10 sec, 50° C for 5 sec and 60° C for 2min, and a final extension of 25°C for 1 min and 10°C for 10 min. The following steps were performed according to the "Mutation Analysis of *IDH*1/*IDH*2 protocol" protocol previous described. The sequencing results were compared with wild-type sequences of TERT for analysis.

Chromosome 1p/19q status by Fluorescence in Situ Hybridization (FISH)

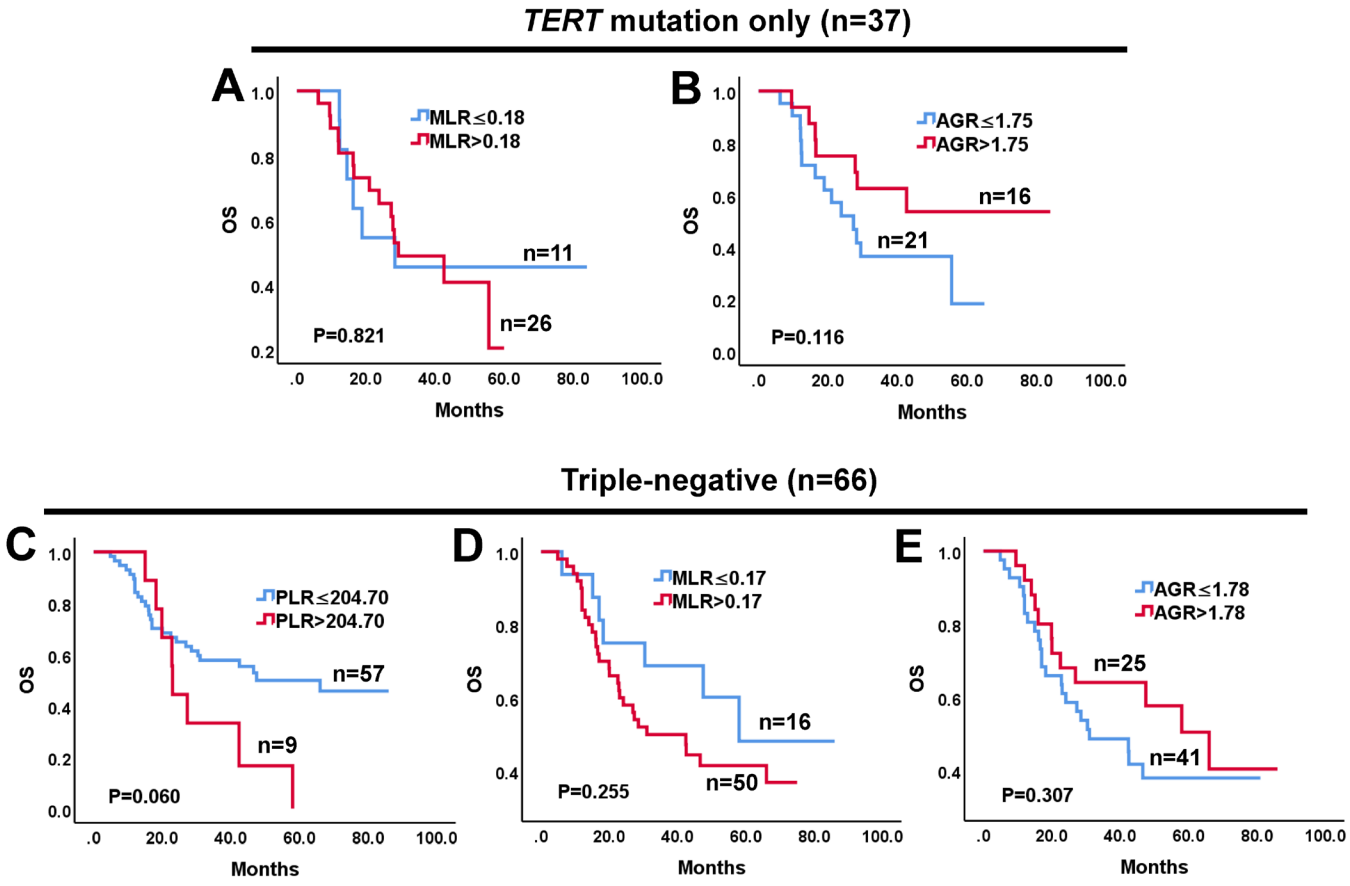
Chromosome 1p/19q status was examined by fluorescence in situ hybridization. 4μm thick FFPE sections were baked at 65°C for 2-3h and deparaffinized in xylene for 10 minutes for 2 times. The sections were hydrated by 100% ethanol for 2 min, 85% ethanol for 2 min and 70% ethanol for 2 min orderly, and then immerged in deionized water for 3 min. The sections were processed with citrate repair solution in (pH6.0) for 4 min in high pressure condition, and then rinsed in 2×SSC solution for 5 min for 2 times. The sections were immerged in protease K fluid (200μg/ml) and incubated for 2 min at 37°C, and then rinsed in 2×SSC solution for 5 min for 2 times. 10μl probes (GP Medical Technologies, Beijing, China) mixture was added to the hybridization zone of the section, and the denaturation and hybridization process was carried out on the ThermoBrite®hybridization instrument (Leica Biosystems, Nussloch, Germany), with denaturation temperature at 83°C for 5 min and hybridization temperature at 42°C for 16h. Sections were immerged in 0.4×SSC plus 0.3% NP-40 cleaning solution (65±1°C) and vibrated for 3 sec. Sections were then retrieved 2 min later and put into 0.1% NP-40 plus 2×SSC cleaning solution at room temperature, vibrated for 3 sec and cleansed for 1 min. Then the sections were immerged in 70% ethanol for 3 min and dried avoiding light at room temperature. 15μl DAPI redyeing agent was added into the hybridization zone of the section, and the section was placed avoiding light for 10 min. At

last, the section was placed under the BX51TRF fluorescence microscope (Olympus, Tokyo, Japan) for analysis by expert pathologist (Dr. Wei-wei Wang). Hybridizing signals in at least 100 non-overlapping nuclei were counted. The loci interrogated were 1p36.3 (RP11-62M23 labeled red)/1q25.3-q31.1 (RP11-162L13 labeled green) and 19q13.3 (CTD-2571L23 labeled red)/19p12 (RP11-420K14 labeled green). A sample was considered 1p or 19q deleted according to the ratio of number of red signal to green signal. In 1p36 or 19q13, positive loss of heterozygosity (LOH) was determined when the ratio of number of red signal to green signal was less than 0.7.

Supplementary Figures



Supplementary Figure 1. Kaplan-Meier overall survival curves of subgroups divided by hematological markers in triple-positive, *IDH* and *TERT* mutations, and *IDH* mutation only molecular groups of lower-grade gliomas. (**A**–**D**) In triple-positive group of lower-grade gliomas, the OS of patients with NLR>2.00 or PLR>166.15 or MLR>0.33 or AGR>1.78does not significantly differ from that of patients with NLR≤2.00 or MLR≤0.29or MLR≤0.33 or AGR≤1.78 (NLR P=0.257, PLR P=0.270, MLR P=0.497, AGR P=0.885). (**E**, **F**) In *IDH* and *TERT* mutations group of lower-grade gliomas, the OS of patients with PLR>166.00 or AGR>1.76 does not significantly differ from that of patients with PLR≤166.00 or AGR≤1.76 (PLR P=0.599, AGR P=0.493). (**G**, **H**) In *IDH* mutation only group of lower-grade gliomas, the OS of patients with MLR>0.33 or AGR>1.89 does not significantly differ from that of patients with MLR≤0.33 or AGR≤1.89 (MLR P=0.776, AGR P=0.251).



Supplementary Figure 2. Kaplan-Meier overall survival curves of subgroups divided by hematological markers in *TERT* mutation only, and triple-negative groups of lower-grade gliomas. (**A**–**B**) In *TERT* mutation only group of lower-grade gliomas, the OS of patients with MLR>0.18 or AGR>1.75 does not significantly differ from that of patients with MLR≤0.18 or AGR≤1.75 (MLR P=0.821, AGR P=0.116). (**C**–**E**) In triple-negative group of lower-grade gliomas, the OS of patients with PLR>204.70 or MLR>0.17 or AGR>1.78 does not significantly differ from that of patients with PLR≤204.70 or MLR≤0.17 or AGR≤1.78 (PLR P=0.060, MLR P=0.255, AGR P=0.307).

Supplementary Tables

Supplementary Table 1. Clinico-pathological features of the cohort (n=592).

Baseline characteristics	Number
Gender	
Male	335 (56.59%)
Female	257 (43.41%)
Age	
Mean ±SD	48.26±1.503
Median (range)	42 (16-82)
Extent of resection	
Complete	456 (77.03%)
Incomplete	136 (22.97%)
RT	
Yes	459 (77.53%)
No	133 (22.47%)
CHT	
TMZ	186 (31.42%)
NMST/FMST	156 (26.35%)
No	250 (42.23%)
Tumor grade	
II	282 (47.64%)
III	122 (20.61%)
IV	188 (31.76%)
IDH mutations	
IDH1 mutation	235 (41.01%)
R132C	2 (0.35%)
R132G	1 (0.17%)
R132H	227 (39.62%)
R132S	2 (0.35%)
R133H	1 (0.17%)
R134H	1 (0.17%)
R135H	1 (0.17%)
IDH2 mutation	11 (1.92%)
R132S	1 (0.17%)
R172G	1 (0.17%)
R172K	5 (0.87%)
R172W	4 (0.70%)
IDH wild type	327 (57.07%)
TERT promoter mutations	
TERT promoter mutation	286 (49.91%)

C228T	212 (37.00%)
C250T	74 (12.91%)
TERT promoter wild type	287 (50.09%)
1p/19q deletion	
Only 1p deletion	15 (2.62%)
Only 19q deletion	35 (6.11%)
1p/19q codeletion	139 (24.26%)
1p/19q intact	384 (67.02%)
Hematological marker (n=528) [a]	
NLR (Mean ±SD)	3.02±2.81
PLR (Mean ±SD)	129.28±62.17
MLR (Mean ±SD)	0.28±0.13
AGR (Mean ±SD)	1.77±0.36
MPV (Mean ±SD)	8.46±0.05
PDW (Mean ±SD)	16.47±0.02
Molecular group	
Grade II-IV (n=573) [b]	
Triple-positive	103 (17.98%)
IDH and *TERT* mutations	19 (3.32%)
IDH mutation only	108 (18.85%)
TERT mutation only	155 (27.05%)
Triple-negative	144 (25.13%)
Other	44 (7.7%)
Lower-grade gliomas (n=392)	
Triple-positive	103 (26.28%)
IDH and *TERT* mutations	19 (4.85%)
IDH mutation only	100 (25.51%)
TERT mutation only	48 (12.24%)
Triple-negative	78 (19.90%)
Other	44 (11.22%)
Grade IV glioma (n=181)	
IDH mutation only	8 (4.42%)
TERT mutation only	107 (59.12%)
Triple-negative	66 (36.46%)

[a]64 cases were excluded from the 592 cases due to conditions that could influence hematological makers
[b]19 cases were excluded from the 592 cases due to unavailability of FFPE tissues of the tumors.
RT: radiation therapy, indicating postoperative radiation therapy after first operation
CHT: chemotherapy, indicating postoperative chemotherapy after first operation
TMZ: temozolomide, FMST: fotemustine, NMST: nimustine

Supplementary Table 2. Univariate analysis of prognostic factors for OS in lower-grade gliomas (n=404).

Factors	No. of cases	5-year OS (%)	P-value
Sex			
Male	228	59.6	P=0.276
Female	176	66.8	
Age			
≤40	146	73.7	**P<0.001**
>40	258	56.1	
KPS			
≤80	149	56.7	**P=0.019**
>80	255	66.7	
Extent of resection			
Gross total	326	65.2	**P=0.004**
Subtotal	78	49.9	
RT			
Yes	324	65.9	**P=0.019**
No	80	48.1	
CHT			
Yes	313	64.7	P=0.129
No	91	54.4	
Grade			
II	282	76.3	**P<0.001**
III	122	27.8	
Molecular group(n=348) [a, b]			
Triple-positive	103	90.2	**P<0.001**
IDH and *TERT* mutations	19	67.4	
IDH mutation only	100	70.0	
TERT mutation only	48	24.0	
Triple-negative	78	38.3	

[a] 12 cases were excluded due to unavailability of FFPE tissues of the tumors, and 44 cases of other combinations of the three molecular markers were excluded.

[b] multiple comparisons for molecular groups are listed in Supplementary Table 3

OS: overall survival; KPS: Karnofsky Performance Status; RT: radiation therapy, indicating postoperative radiation therapy after first operation; CHT: chemotherapy, indicating postoperative chemotherapy after first operation

Supplementary Table 3. The classification of radiotherapy, chemotherapy and chemotherapy program in WHO II-III gliomas (n=403).

	Astrocytoma (%)	Oligodendroglioma or Oligoastrocytomas (%)
Only RT	14 (9.03)	31 (12.5)
Only CHT	10 (6.45)	24 (9.68)
RT and CHT	113 (72.90)	166 (66.94)
No RT nor CHT	18 (11.61)	27 (10.89)
CHT program		
TMZ	36 (29.27)	68 (35.79)
FMST/NMST	49 (39.84)	81 (42.63)
NA	38 (30.89)	41 (21.58)

RT: radiotherapy; CHT: chemotherapy; TMZ: temozolomide; FMST: fotemustine; NMST: nimustine; NA: not available

Supplementary Table 4. Univariate analysis of molecular groups of lower-grade gliomas with multiple comparisons (n=348[a]).

Molecular group 1 vs Molecular group 2	No. of cases	5-year OS (%)	P-value[b]
Triple-positive vs *IDH* and *TERT* mutations	103 vs 19	90.2 vs 67.4	P=0.096
Triple-positive vs *IDH* mutation only	103 vs 100	90.2 vs 70.0	P=0.009
Triple-positive vs *TERT* mutation only	103 vs 48	90.2 vs 24.0	**P<0.001**
Triple-positive vs Triple negative	103 vs 78	90.2 vs 38.3	**P<0.001**
IDH and *TERT* mutations vs *IDH* mutation only	19 vs 100	67.4 vs 70.1	P=0.994
IDH and *TERT* mutations vs *TERT* mutation only	19 vs 48	67.4 vs 24.0	**P=0.002**
IDH and *TERT* mutations vs Triple negative	19 vs 78	67.4 vs 38.3	P=0.011
IDH mutation only vs *TERT* mutation only	100 vs 48	70.0 vs 24.0	**P<0.001**
IDH mutation only vs Triple negative	100 vs 78	70.0 vs 38.3	**P<0.001**
TERT mutation only vs Triple negative	48 vs 78	24.0 vs 38.3	P=0.162

[a]12 cases were excluded due to unavailability of FFPE tissues of the tumors, and 44 cases of other combinations of the three molecular markers were excluded.
[b]To correct for multiple comparisons, a Bonferroni adjusted P value of 0.05/10(number of times of comparisons) =0.005 was adopted as the significance threshold

Supplementary Table 5. Univariate analysis of prognostic factors for OS in Grade IV glioma (n=188).

Factors	No. of cases	5-year OS (%)	P-value
Sex			
Male	107	4.8	P=0.488
Female	81	2.2	
Age			
≤62	50	3.5	**P=0.037**
>62	138	4.0	
KPS			
≤80	45	9.7	P=0.151
>80	143	2.6	
Extent of resection			
Gross total	130	4.4	**P<0.001**
Subtotal	58	1.9	
RT			
Yes	135	6.6	**P=0.006**
No	53	6.3	
CHT			
Yes	125	0.0	**P<0.001**
No	63	3.2	
Molecular group (n=181)[a]			
IDH mutation only	8	25.0	P=0.285
TERT mutation only	107	2.7	
Triple-negative	66	3.7	

[a]7 cases were excluded due to unavailability of FFPE tissues of the tumors.
OS: overall survival; KPS: Karnofsky Performance Status; RT: radiation therapy, indicating postoperative radiation therapy after first operation CHT: chemotherapy, indicating postoperative chemotherapy after first operation

Supplementary Table 6. P-value in the univariate analysis of subgroups of lower-grade gliomas with multiple comparisons (n=348[a]).

Subgroup 1 vs Subgroup 2	P-value[b]
Triple positive vs IDH and TERT mutation-Low NLR	**P<0.001**
Triple positive vs IDH mutation only-Low NLR	**P<0.001**
Triple positive vs TERT mutation only-High NLR	**P<0.001**
Triple positive vs TERT mutation only-Low NLR	**P<0.001**
Triple positive vs Triple-negative-Low NLR	**P<0.001**
IDH and TERT mutation-High NLR vs TERT mutation only-Low NLR	**P<0.001**
IDH and TERT mutation-High NLR vs Triple-negative-Low NLR	**P=0.001**
IDH and TERT mutation-Low NLR vs Triple-negative-Low NLR	**P<0.001**
IDH mutation only-High NLR vs TERT mutation only-High NLR	**P<0.001**
IDH mutation only-High NLR vs TERT mutation only-Low NLR	**P<0.001**
IDH mutation only-High NLR vs Triple-negative-Low NLR	**P<0.001**
IDH mutation only-Low NLR vs TERT mutation only-Low NLR	**P<0.001**
IDH mutation only-Low NLR vs Triple-negative-Low NLR	**P=0.001**
TERT mutation only-High NLR vs TERT mutation only-Low NLR	**P<0.001**
TERT mutation only-Low NLR vs Triple-negative-High NLR	**P<0.001**
TERT mutation only-Low NLR vs Triple-negative-Low NLR	**P<0.001**
Triple-negative-High NLR vs Triple-negative-Low NLR	**P=0.001**

[a]12 cases were excluded due to unavailability of FFPE tissues of the tumors, and 44 cases of other combinations of the three molecular markers were excluded.
[b]To correct for multiple comparisons, a Bonferroni adjusted P value of 0.05/36(number of times of comparisons) =0.0014 was adopted as the significance threshold
We removed the date that P>0.05 for a more streamlined form.

Supplementary Table 7. Univariate analysis of risk group of lower-grade gliomas with multiple comparisons (n=348[a]).

Risk group 1 vs Risk group 2	No.of cases	5-year OS (%)	P-value[b]
Low risk vs Intermediate-I	179 vs 98	85.5 vs 53.0	**P<0.001**
Low risk vs High risk	179 vs 18	85.5 vs 0.0	**P<0.001**
Low risk vs Intermediate-II	179 vs 53	85.5 vs 23.4	**P<0.001**
Intermediate-I vs High risk	98 vs 18	53.0 vs 0.0	**P<0.001**
Intermediate-I vs Intermediate-II	98 vs 53	53.0 vs 23.4	**P<0.001**
High risk vs Intermediate-II	18 vs 53	0.0 vs 23.4	**P<0.001**

[a]12 cases were excluded due to unavailability of FFPE tissues of the tumors, and 44 cases of other combinations of the three molecular markers were excluded.
[b]To correct for multiple comparisons, a Bonferroni adjusted P value of 0.05/6(number of times of comparisons) =0.0083 was adopted as the significance threshold