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ORIGINAL RESEARCH

Development and Validation of a Prognostic Molecular Phenotype and Clinical Characterization in Grade III Diffuse Gliomas Treatment with Radio-Chemotherapy

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Background: The relationship between molecular phenotype and prognosis in high-grade gliomas (WHO III and IV, HGG) treated with radiotherapy and chemotherapy is not fully understood and needs further exploration.

Methods: The HGG patients following surgery and treatment with radiotherapy and chemotherapy. Univariate and multivariate Cox analyses were used to assess the independent prognostic factors. The nomogram model was established, and its accuracy was determined via the calibration plots.

Results: A total of 215 and 88 patients had grade III glioma and grade IV glioma, respectively. Grade III oligodendroglioma (OG-G3) patients had the longest mPFS and mOS than other grade III pathology, while grade III astrocytoma (AA-G3) patients were close to IDH-1 wildtype glioblastoma (GBM) and had a poor prognosis. The IDH-1 mutant group had a better mPFS and mOS than the IDH-1 wildtype group in all grade III patients, OG-G3 and AA-G3 patients. Furthermore, 1p/19q co-deletion group had a longer mPFS and mOS than 1p/19q non-deletion group in all grade III patients. IDH-1 mutation and 1p/19q co-deletion patients had the best prognosis than other molecular types. Also, the MGMT methylation and IDH-1 mutation or 1p/19q co-deletion group had a longer mPFS and mOS than the MGMT unmethylation and IDH-1 wildtype or 1p/19q non-codeletion of grade III patients. In addition, the low Ki-67 expression group had a better prognosis than high Ki-67 expression group in grade III patients. Univariate and multivariate COX showed that 1p/19q co-deletion and MGMT methylation were the independent prognostic factors for mPFS and mOS. The calibration curve showed that the established nomogram could well predict the survival based on these covariates.

Conclusion: The AA-G3 with IDH-1 wildtype, MGMT unmethylation or 1p/19q non-codeletion patients was resistant to radio-therapy and chemotherapy, has a poor prognosis and needs a more active treatment.

Keywords: glioma, radiotherapy, prognosis, nomogram

Introduction

Glioma originates from glial cells and is the most common primary intracranial tumor in the central nervous system (CNS).[1,2] In addition, new cases and glioma-related deaths have significantly increased. Molecular diagnostics, histology, and immunohistochemistry play a key role in tumor classification according to the fifth edition of the World Health Organization (WHO) classification of tumors of the CNS in 2021. Specifically, grades III–IV are classified as high-grade

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glioma (HGG).[3] HGG has a strong invasiveness and proliferation. In addition, HGG patients experience rapid local recurrence and metastasis even after receiving the maximum safe surgical resection and adjuvant chemo-radiotherapy. The median overall survival (OS) and 5-year survival rate of HGG patients are about 15 months and <5%.[4,5]

Numerous studies have shown that glioma has many molecular subtypes, indicating that effective methods for glioma diagnosis and treatment are necessary.[6,7] Isocitrate dehydrogenase (IDH) is a metabolic enzyme crucial for epigenetic regulation of gene expression and DNA repair. IDH activity depends on NADP+/Mg+ and also yields NADPH.[8] Furthermore, IDH-1 mutation is significantly higher in grades II to III (80%) and less in grade IV (5%) than in IDH-1 wildtype.[9] Moreover, IDH-1 mutant status is crucial for the therapeutic effects in HGG patients. Also, patients with IDH-1 mutation have a better prognosis than IDH1 wildtype.[10] A study also showed that grade II glioma patients with IDH-1 mutation receiving vorasidenib have a longer median progression-free survival (PFS, 27.7 months vs 11.1 months) than placebo.[11] Moreover, low-grade glioma (LGG) patients with IDH-1 mutation have a longer OS and higher rate of response to temozolomide than patients with IDH-1 wildtype.[12] In addition, IDH-mutated diffuse glioma patients receiving radiotherapy with concurrent oral temozolomide have a longer OS (6–10 years vs 1–4 years) than patients with IDH wildtype diffuse glioma.[13] Therefore, IDH1 mutant status is a significant marker for prognosis of chemo-radiotherapy in LGG and HGG patients.

The chromosome arms 1p and 19q (1p/19q) cause human gene imbalanced heterotopy, indicating that 1p and 19q may be key therapeutic factors for HGG patients receiving chemo-radiotherapy.[14] Studies have shown that radiotherapy adjuvant temozolomide can improve the survival of 1p/19q non-co-deleted anaplastic glioma patients.[13] Moreover, radiotherapy adjuvant PCV (procarbacine, lomustine, and vincristine) can significantly improve OS of grade III glioma patients with anaplastic oligodendroglial tumors than mono-radiotherapy in 1p/19q co-deletion patients.[15] Studies have also shown that 1p/19q co-deletion is correlated with IDH mutation status. Notably, IDH mutation and 1p/19q co-deletion are the best prognosis in grade II and III glioma patients.[16] Moreover, Ki-67 is an indicator of cellular proliferation mainly located in the nucleus. Ki-67 expression level is correlated with glioma grading and molecular classification.[17–19] Specifically, high Ki-67 expression is correlated with poor prognosis.[17] However, the relationship among Ki-67 expression, molecular biomarker, and radiotherapy in HGG patients is unclear.

Many studies have shown that molecular marker is correlated with the prognosis of glioma and diagnosis. However, the effect of pathology, IDH1, 1p/19q, Ki-67, and O(6)-methylguanine-DNA methyltransferase (MGMT) on radio-chemotherapy in grade III glioma patients were not full understand and needs further investigation. This retrospective study aimed to analyze the prognostic usefulness of molecular and clinical characterization in HGG following surgery and radio-chemotherapy. Moreover, previous studies have shown that a nomogram can predict treatment efficacy in malignant tumor. In this study, a nomogram was established and validated via the molecular marker and clinical characterization in grade III glioma post-operation adjuvant radiotherapy and chemotherapy.

Patients and Methods

Clinicopathological Characteristics

Patients with histologically confirmed HGG (World Health Organization, WHO, grades III and IV) were retrospectively identified between January 2014 and June 2021. The clinicopathological characteristics included IDH-1 mutant status, 1p/19q co-deletion, Ki-67 expression, MGMT methylation, TP53 mutant status, ATRX deletion and serum biochemical indicators. Moreover, the pathology of glioma included grade III astrocytoma (AA-G3), grade III oligodendroglioma (OG-G3), and IDH-1 wildtype glioblastoma (GBM) etc. The inclusion criteria were: 1) patients with histologically confirmed HGG postoperatively and treated with radiotherapy and chemotherapy; 2) HGG patients preoperatively treated without any anti-tumor treatment; 3) HGG patients who underwent IDH-1, Ki-67, and 1p/19q test.

Post-Treatment Evaluation Criteria and Follow-Up

The HGG patients received surgery, radiotherapy (**54–63G**y), and concurrent chemotherapy (temozolomide or combined with bevacizumab) or adjuvant chemotherapy (temozolomide or combined with bevacizumab or irinotecan, PCV regimen

(procarbacine, lomustine, vincristine)). Post-treatment evaluation was assessed via craniocerebral nuclear magnetic resonance (MRI). The PFS and OS were measured from the starting date of the first-line treatment. The follow-up time for the cut-off date was 31th December 2023.

Glioma Classification and Diagnosis

The immunohistochemistry (IHC) of IDH-1 status, CDKN2A/B, H3 K27, ATRX, MGMT methylation status, Ki-67, TP53 and ATRX, etc., was re-evaluated by two pathologists. The 1p/19q co-deletion status, part of IDH-1 mutant status, CDKN2A/B, H3 K27, TERT, and ATRX, was detected by fluorescence in situ hybridization (FISH) or next-generation sequencing (NGS).

Nomogram Establishment and Validation

The nomogram was established and validated as previously reported.[20] First, grade III glioma patients were randomly divided into training cohort group (n = 150) and validation cohort group (n = 65) (2:1) using R software. Univariate and multivariate Cox proportional hazard regression were used to find the co-variables significantly associated with survival ($P < 0.05$). A nomogram was then established using these covariates. The predictive ability was determined using the calibration curves.

Statistical Analysis

The independent prognostic factors were found via univariate and multivariate Cox analyses. Notably, hazard ratio (HR) and 95% confidence interval (CI) were also calculated. SPSS 22.0 software, GraphPad Prism 9.0 software, and R statistical software version 4.0.0 (http://www.R-project.org) were used for all statistical analyses. P <0.05 was considered a statistically significant difference.

Results

Patients and Data Collection

HGG patients with histologically proven IDH-1, Ki-67, and 1p/19q deletion status following surgery and chemo- or radiotherapy were retrospectively identified. A flowchart for patient selection is shown in Figure 1. Finally, 303 patients, including 215 with grade III glioma and 88 with grade IV, were included in the study. In addition, 129, 60, and 26 patients had AA-G3, OG-G3 and other grade III glioma, respectively. The grade IV glioma (glioblastoma) included 88 patients (Figure 2A). HGG was mainly found in frontal lobe, temporal lobe, parietal lobe, frontal and parietal lobes, temporal and parietal lobes (Figure 2B and Table 1). A total of 223 patients received radiotherapy concurrent and adjuvant temozolomide or combined with bevacizumab or irinotecan. Moreover, 80 patients received radiotherapy concurrent with temozolomide and adjuvant PCV regimen.

Log-Rank Survival Analyzed the Prognostic Molecular and Clinical Characterization in Grade III Glioma Glioma

The Prognostic Pathology of Grade III Glioma Patients Treated with Radio-Chemotherapy

Although many treatment options have been recently developed for grade III glioma patients, part of patients still has a poor prognosis. In this study, the correlation between specific pathological types and prognosis in grade III glioma patients was analyzed following surgery and radio-chemotherapy. The grade III patients had a longer mPFS (19.2 vs 9.6 months, P<0.05) and mOS (22.9 vs 16.5 months, P<0.05) than GBM patients (Figure 2C and D). Subgroup analysis revealed that OG-G3 group had the longest mPFS (33.2 vs 12.6 vs 27.15 vs 20.1 vs 9.6 months, P<0.05) and mOS (37.2 vs 19.1 vs 22.75 vs 21.7 vs 17.8 months, P<0.05) than other pathological type; however, mPFS (12.6 vs 9.6 months, P>0.05) and mOS (19.1 vs 17.8 months, P>0.05) of AA-G3 group were similar to that of GBM patients (Figure 2E and F). These findings indicate that AA-G3 has a higher tumor invasion and poor prognosis.

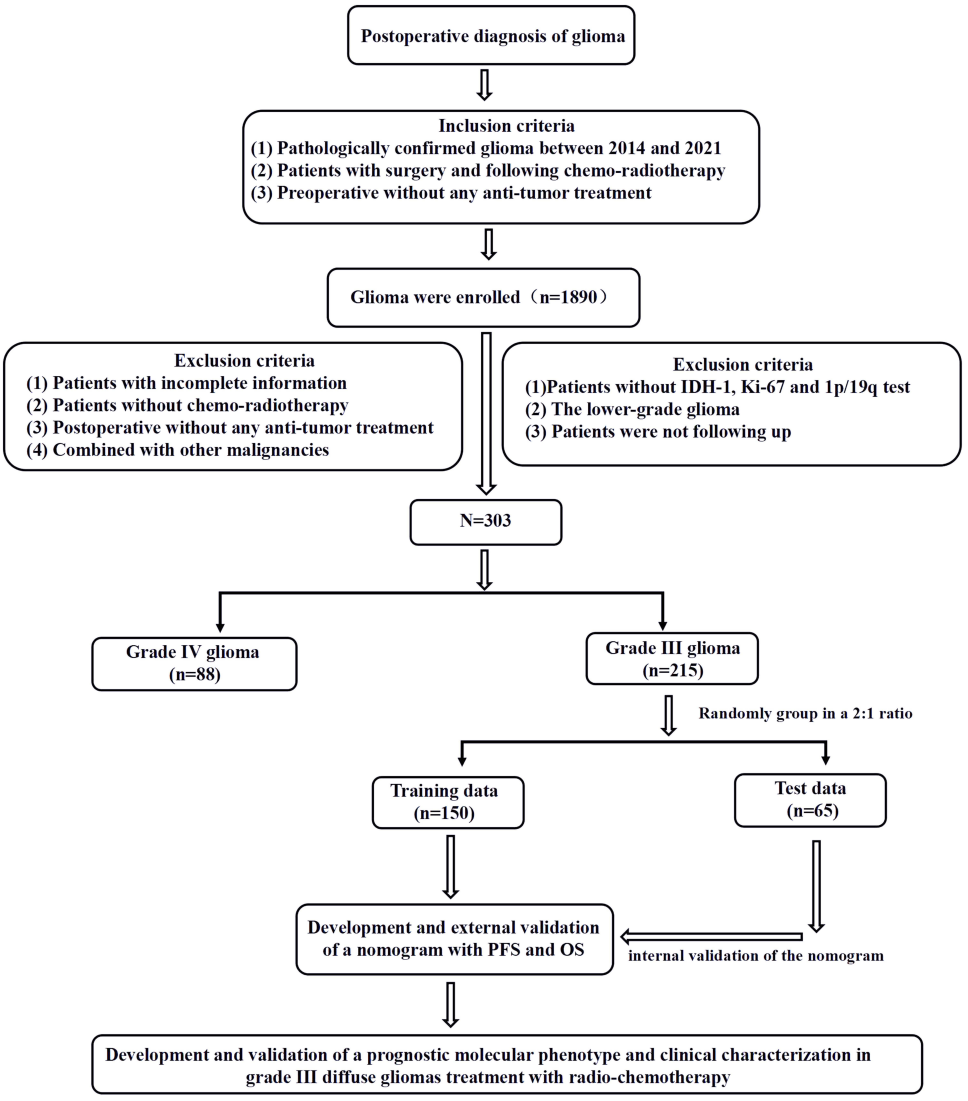

Figure 1 Flowchart showing HGG patient selection.

The Prognostic IDH-1 Mutant Status in Grade III Glioma Patients After Radio-Chemotherapy

IDH-1, a metabolic enzyme, plays key roles in the diagnosis and treatment of HGG patients after radio- and chemotherapy. Herein, the IDH-1 mutation group had longer mPFS (25.1 vs 10.9 months, $P<0.05$) and mOS (33.6 vs 16.6 months, $P<0.05$) than the IDH-1 wildtype group in grade III glioma patients (Figure 3A and B). Additionally, subgroup analysis also revealed that AA-G3 patients with IDH-1 mutation had a longer mPFS (19.2 vs 8.6 months, $P<0.05$) and mOS (23.95 vs 16.6 months, $P<0.05$) than IDH-1 wildtype (Figure 3C and D). The mPFS (38.1 vs 19.2 months, $P<0.05$) and mOS (45.8 vs 20.5 months, $P<0.05$) were better in the IDH-1 mutation group than in the IDH-1 wildtype of OG-G3 patients (Figure 3E and F).

The Prognostic 1p/19q Co-Deletion Status in Grade III Glioma After Radio-Chemotherapy

The 1p and 19q co-deletion causes human gene imbalanced heterotopy, indicating that 1p and 19q may be key therapeutic factors for HGG patients receiving chemo-radiotherapy. In this study, the grade III glioma patients with 1p/19q co-deletion had a longer mPFS (33.2 vs 11.5 months, $P<0.05$) and mOS (38.5 vs 13.9 months, $P<0.05$) than 1p/19q non-codeletion group (Figure 4A and B). Moreover, AA-G3 patients with 1p/19q co-deletion had a longer mPFS

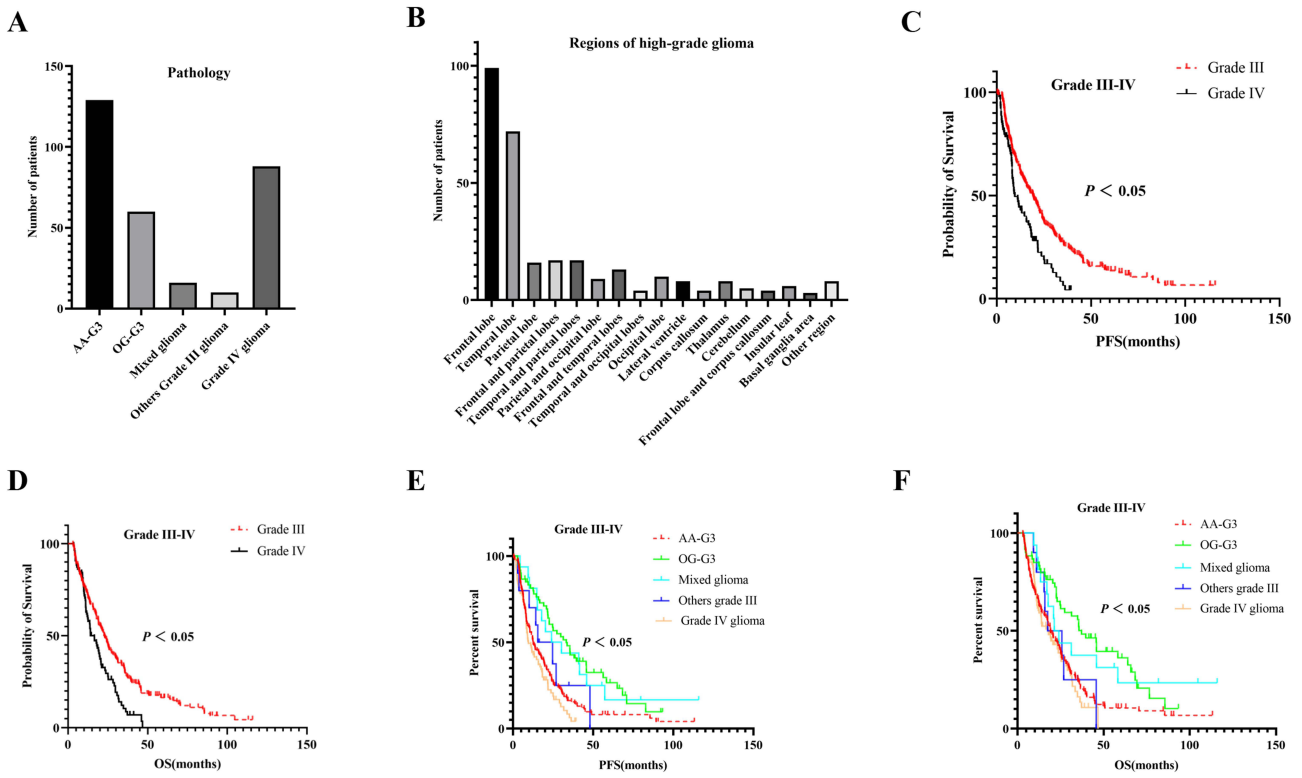

Figure 2 The prognostic pathology in grade III glioma patients after radio-chemotherapy. (**A**) The number of HGG patients with different pathology types. (**B**) The number of HGG patients with tumor lesions. (**C** and **D**) The median PFS and OS in grade III patients after radio-chemotherapy. (**E** and **F**) The survival of HGG patients with different pathology types. HGG, grade III glioma and grade IV glioma (glioblastoma). GBM, glioblastoma; OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

(24.1 vs 9.4 months, $P<0.05$) and mOS (33.7 vs 14.7 months, $P<0.05$) than the 1p/19q non-codeletion group (Figure 4C and D). The mPFS (45.7 vs 12.9 months, $P<0.05$) and mOS (45.8 vs 13.5 months, $P<0.05$) were better in OG-G3 patients with 1p/19q co-deletion group than in the 1p/19q non-codeletion group (Figure 4E and F). Subgroup analyses showed that the IDH-1 mutation and 1p/19q co-deletion group had the longest mPFS (36.5 vs 10.5 vs 10.9 vs 12.2 months,

Table 1 The Lesion and Number of HGG Patients

Glioma Region	Number
Frontal lobe	99 (32.7%)
Temporal lobe	72 (23.8%)
Parietal lobe	16 (5.3%)
Frontal and parietal lobes	17 (5.6%)
Temporal and parietal lobes	17 (5.6%)
Parietal and occipital lobe	9 (3%)
Frontal and temporal lobes	13 (4.3%)
Temporal and occipital lobes	4 (1.3%)
Occipital lobe	10 (3.3%)
Lateral ventricle	8 (2.6%)
Corpus callosum	4 (1.3%)
Thalamus	8 (2.6%)
Cerebellum	5 (1.7%)
Frontal lobe and corpus callosum	4 (1.3%)
Insular leaf	6 (2%)
Basal ganglia area	3 (1%)
Other region	8 (2.6%)

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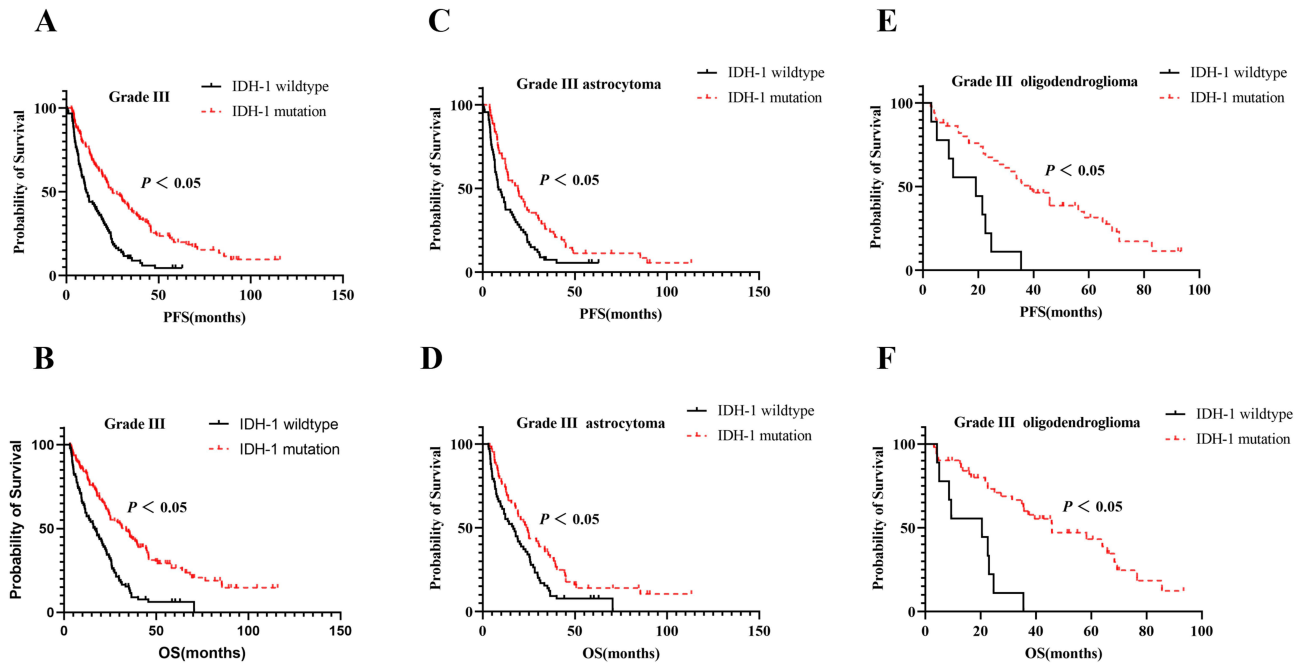

Figure 3 The prognostic IDH-1 mutant status in grade III glioma patients after radio-chemotherapy. The correlation between IDH-1 mutant status and survival in grade III patients (**A** and **B**), AA-G3 patients (**C** and **D**), OG-G3 patients (**E** and **F**) after postoperative and radiotherapy concurrent and chemotherapy or sequential radiotherapy and chemotherapy. OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

P<0.05) and mOS (40.2 vs 15.7 vs 21.4 vs 13.8 months, *P*<0.05) than other groups, while IDH-1 wildtype and 1p/19q non-codeletion group had the shortest survival in grade III glioma patients (Figure 4G and H).

The Prognostic MGMT Methylation Status in Grade III Glioma Patients After Radio-Chemotherapy

MGMT, as a DNA repair enzyme, can reverse DNA damage caused by alkylating agents, leading to tumor resistance to TMZ and nitrosourea. Methylation of MGMT promoter silences MGMT, thus increasing the lethality of the alkylating agent to tumor cells. In this study, the grade III glioma patients with MGMT methylation had a longer mPFS (31.9 vs 12.4 months, *P* < 0.05) and mOS (49.7 vs 17.9 months, *P*< 0.05) than the MGMT unmethylation group (Figure 5A and B). Moreover, AA-G3 patients with MGMT methylation had a longer mPFS (23.5 vs 9.55 months, *P* < 0.05) and mOS (36.5 vs 17.9 months, *P* < 0.05) than the MGMT unmethylation group (Figure 5C and D). Notably, the mPFS (65 vs 21.5 months, *P* >0.05) and mOS (65.8 vs 22.6 months, *P* < 0.05) were better in the OG-G3 patients with MGMT methylation than the MGMT unmethylation group (Figure 5E and F). Furthermore, subgroup analyses showed that the MGMT methylation and IDH-1 mutation group had the longest mPFS (41.5 vs 20.1 vs 10.45 vs 13.1 months, *P*<0.05) and mOS (64 vs 29.75 vs 16.1 vs 19.1 months, *P*<0.05) than other groups (Figure 5G and H), the MGMT methylation and 1p/19q co-deletion group had the longest mPFS (41.5 vs 19.9 vs 10 vs 16.1 months, *P*<0.05) and mOS (64 vs 31.3 vs 13.5 vs 22.6 months, *P*<0.05) than other groups (Figure 5I and J), while MGMT unmethylation, IDH-1 wildtype and 1p/19q non-codeletion group had the shortest survival in grade III glioma patients.

The Prognostic Ki-67 Expression Levels in Grade III Glioma Patients After Radio-Chemotherapy

Ki-67, as a proliferation index, plays an important role in the prognosis and diagnosis of HGG patients. Herein, the cut-off value Ki-67 of 22.5% (ROC curve was established based on glioma grade) had the highest sensitivity and specificity (sensitivity: 84.5%, specificity: 61%, Youden index: 0.455, ROC = 0.763, *P* < 0.001) (Figure 6A and B). Therefore, the patients were divided into low (Ki-67 < 22.5%, n = 140) and high-expression groups (Ki-67 ≥ 22.5%, n = 75) based on Ki-67 expression levels. The high Ki-67 expression group of grade III glioma groups had a worse mPFS (10.2 vs 24.1 months, *P*<0.05) and mOS (15.9 vs 26.0 months, *P*<0.05) than low Ki-67 expression group (Figure 6C and D). Moreover, AA-G3 patients with Ki-67 low-expression group had a longer mPFS (16.7 vs 7.8 months, *P*<0.05) and mOS (22.65 vs 13 months, *P*<0.05) than the high Ki-67 expression group (Figure 6E and F). Notably, the mPFS (39.6 vs

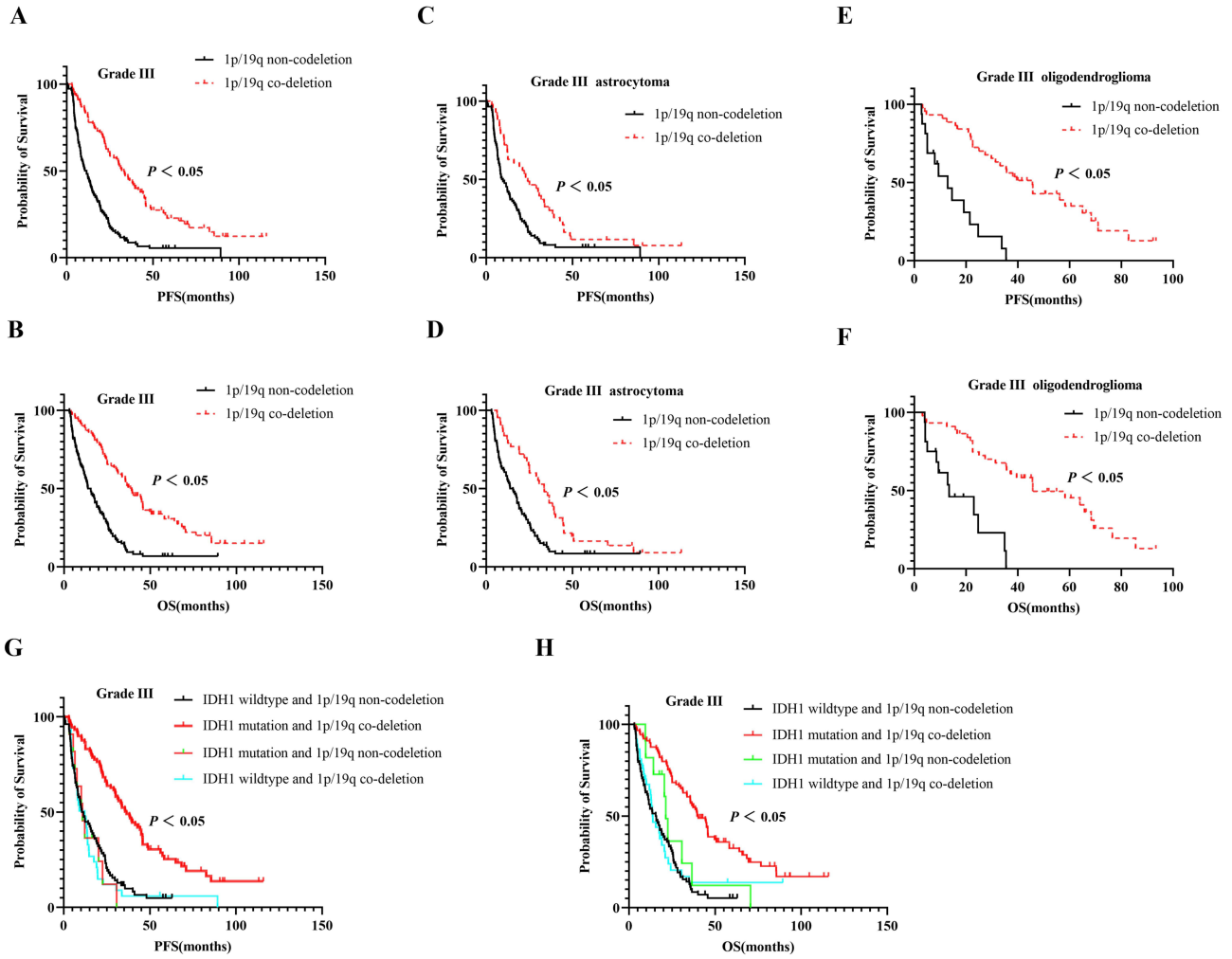

Figure 4 The prognostic 1p/19q co-deletion status in grade III glioma patients after radio-chemotherapy. The correlation between 1p/19q co-deletion status and survival in grade III patients (**A** and **B**), AA-G3 patients (**C** and **D**), OG-G3 patients (**E** and **F**) after radio-chemotherapy. Subgroup analysis was used to compare the survival of IDH-1 mutation and 1p/19q co-deletion group, IDH-1 mutation and 1p/19q non-codeletion group, IDH-1 wildtype and 1p/19q co-deletion group, IDH-1 wildtype and 1p/19q non-codeletion group in grade III patients (**G** and **H**). OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

15.9 months, $P<0.05$) and mOS (45.8 vs 20.5 months, $P<0.05$) were better in the OG-G3 patients with low Ki-67 expression than the high Ki-67 expression group (Figure 6G and H).

The Prognostic Other Clinical Characterization in Grade III Glioma Patients After Radio-Chemotherapy

The young grade III glioma patients (<60 years) had a worse mOS (25 vs 17.75 months, $P<0.05$) than older patients (Figure 7B), while mPFS was not significantly different (20.3 vs 16 months, $P>0.05$, Figure 7A). The serum low ALB group also had a poorer mPFS (12.9 vs 21.5 months, $P<0.05$) and mOS (19.1 vs 24.6 months, $P<0.05$) than the high ALB group (Figure 7C and D). The serum low LDH group had a longer mPFS (21.7 vs 11.5 months, $P<0.05$) and mOS (25.7 vs 12.6 months, $P<0.05$) than the high LDH group (Figure 7E and F). The serum high monocyte group had a longer mPFS (25.7 vs 12.6 months, $P<0.05$) and mOS (24.7 vs 20.8 months, $P<0.05$) than the low monocyte group (Figure 7G and H).

Univariate and Multivariate Survival Analyses in the Primary Cohort

A total of 215 grade III glioma patients were included in this study. Univariate COX analysis showed that pathology, IDH-1 mutation status, 1p/19q co-deletion status, Ki-67 expression levels, MGMT methylation status, serum ALB, serum monocyte count, and serum LDH were correlated with mPFS (Table 2 and Figure 8A, $P<0.05$). In addition, age, pathology, IDH-1

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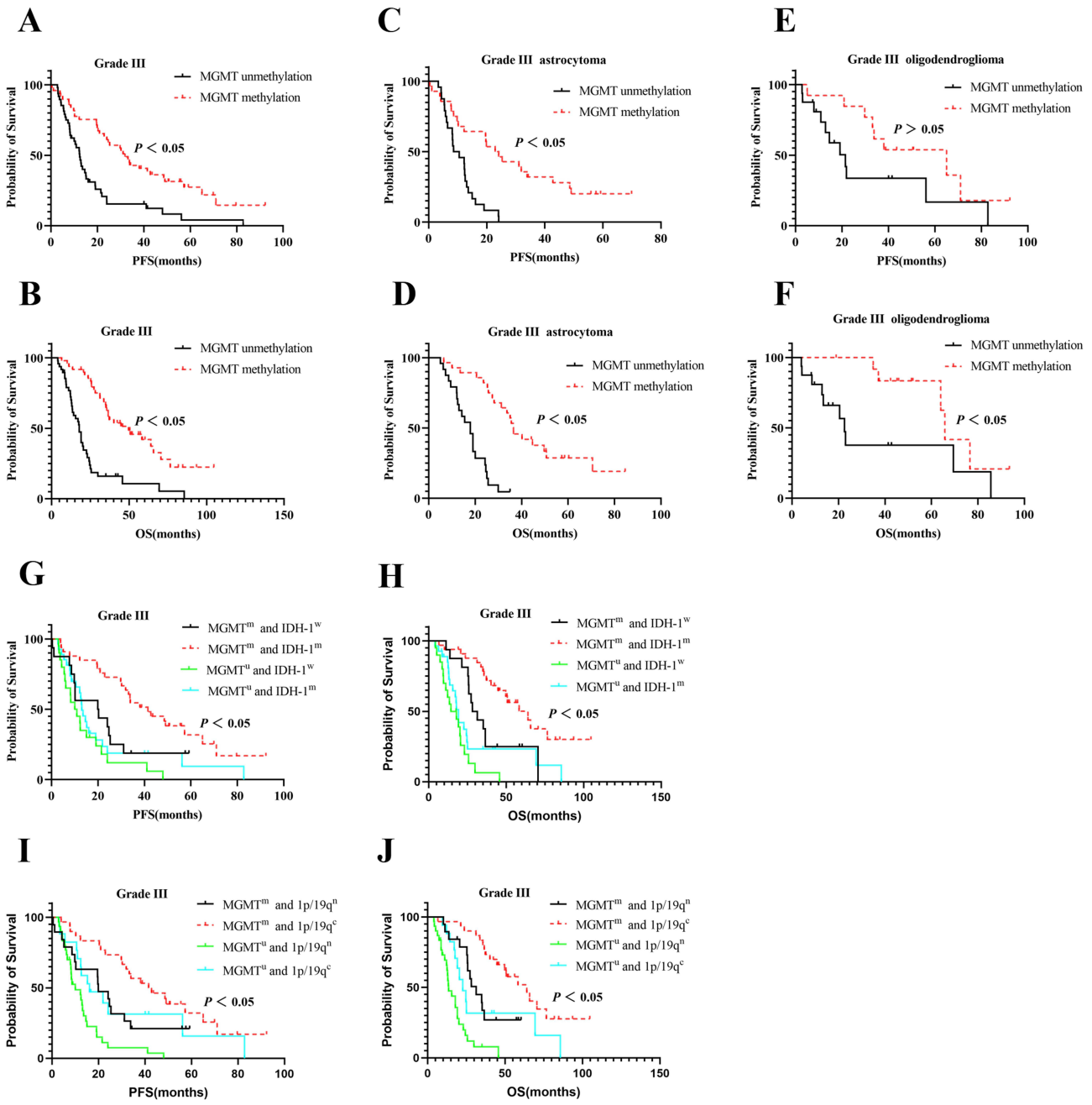

Figure 5 The prognostic MGMT methylation status in grade III glioma patients after radio-chemotherapy. The correlation between MGMT methylation status and survival in grade III patients (**A** and **B**), AA-G3 patients (**C** and **D**), OG-G3 patients (**E** and **F**); Subgroup analysis was used to compare the survival of MGMT methylation with IDH-1 mutation (**G** and **H**) or 1p/19q non-codeletion (**I** and **J**) in grade III glioma patients. $MGMT^{m}$ and $IDH\text{-}1^{w}$, MGMT methylation and IDH-1 wildtype; $MGMT^{m}$ and $IDH\text{-}1^{m}$, MGMT methylation and IDH-1 mutation; $MGMT^{u}$ and $IDH\text{-}1^{w}$, MGMT un-methylation and IDH-1 wildtype; $MGMT^{u}$ and $IDH\text{-}1^{m}$, MGMT un-methylation and IDH-1 mutation; $MGMT^{m}$ and $1p/19q^{n}$, MGMT methylation and 1p/19q non-codeletion; $MGMT^{m}$ and $1p/19q^{c}$, MGMT methylation and 1p/19q co-deletion group; $MGMT^{u}$ and $1p/19q^{n}$, MGMT un-methylation and 1p/19q non-codeletion; $MGMT^{u}$ and $1p/19q^{c}$, MGMT un-methylation and 1p/19q co-deletion group. OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

mutation status, 1p/19q co-deletion status, Ki-67 expression levels, MGMT methylation status, serum monocyte count, and serum LDH were correlated with mOS (Table 2 and Figure 8B, $P<0.05$). Furthermore, multivariate COX analysis showed that pathology, 1p/19q co-deletion, low Ki-67 expression levels, MGMT methylation and higher serum ALB were the independent prognostic factors for PFS (Table 3 and Figure 8C, $P < 0.05$). Moreover, MGMT methylation, 1p/19q co-deletion, low Ki-67 expression levels, and serum LDH were the independent prognostic factors for OS (Table 3 and Figure 8D, $P<0.05$).

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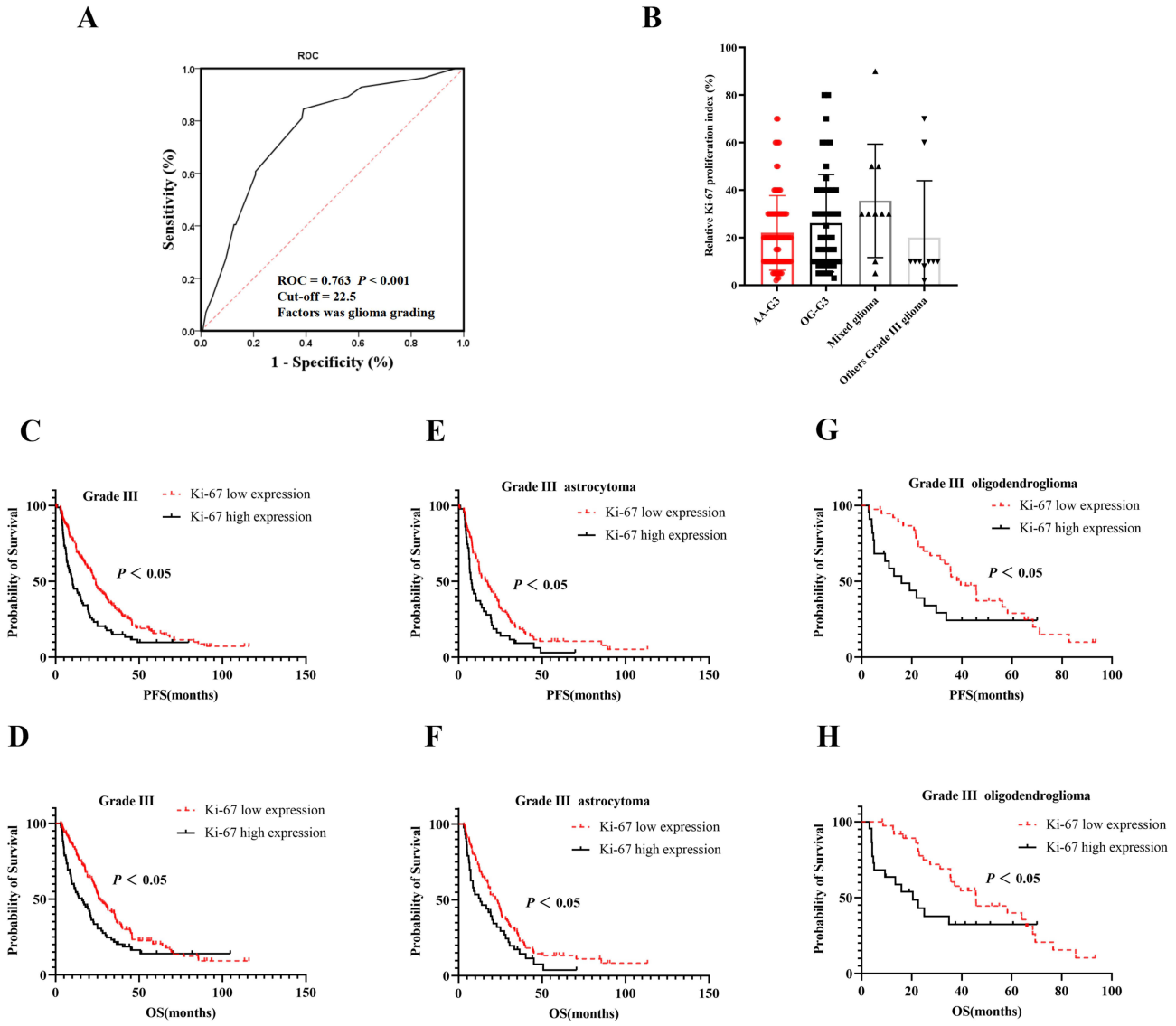

Figure 6 The prognostic Ki-67 expression levels in grade III glioma patients after radio-chemotherapy. (**A**) Ki-67 ROC curve for cut-off points based on glioma grade. (**B**) The expression of Ki-67 proliferation index in grade III glioma patients. The correlation between Ki-67 expression and survival in grade III patients (**C** and **D**), AA-G3 patients (**E** and **F**), OG-G3 patients (**G** and **H**) after radio-chemotherapy. OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

Development and Internal Validation of the Nomogram

The grade III glioma patients were randomly divided into training cohort group (n = 150) and validation cohort group (n = 65) (2:1) using R software, and the number of patients in those two groups had no significant (Table 4, $P>0.05$). The nomogram was developed based on multivariate Cox analyses of key variables ($P < 0.05$), including five variables for PFS and four variables for OS. Every variable was calculated, and the total prognostic scores (from 0 to 450) were determined (Figure 9A and B). Results showed that 1p/19q co-deletion status and MGMT methylation had the most significant contribution to predicted points for PFS (12-, 24- and 36-month) and OS (12-, 36- and 60-month) among HGG patients ($P<0.05$). Moreover, the calibration plots showed that the 12-, 24- and 36-month PFS (Figure 9C, D and E) as well as the 12-, 36- and 60-month OS (Figure 9F, G and H) probabilities predicted by the nomogram fitted well the actually observed values in grade III glioma in the internal validation cohort.

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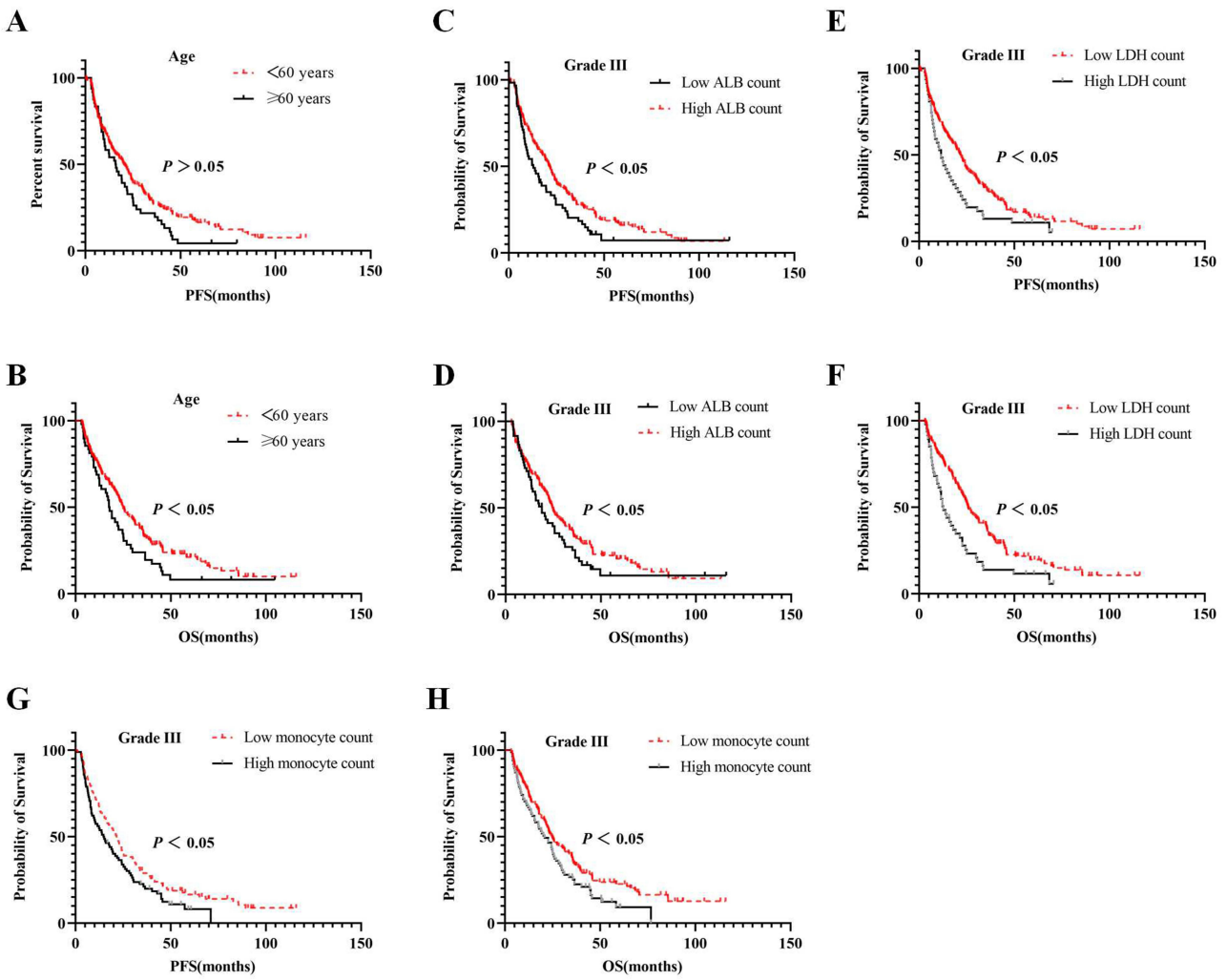

Figure 7 The prognostic other clinical characterization in grade III glioma patients after radio-chemotherapy. The effect of ages (**A** and **B**), serum ALB count (**C** and **D**), serum LDH count (**E** and **F**) and serum monocyte count (**G** and **H**) on survival in grade III glioma patients after radio-chemotherapy. ALB, albumin; LDH, lactate dehydrogenase.

Discussion

HGG is strongly invasive and proliferative. Notably, HGG patients rapidly experience local recurrence and metastasis even after receiving maximum safe surgical resection and adjuvant chemo-radiotherapy. The median OS and 5-year survival rate of HGG patients are 15 months and <5%, respectively.[4,5] Additionally, more precise, effective, and personalized therapy regimens are found in the last decade, and those new treatment options are also improved the median PFS and OS in HGG patients.[21] Therefore, it is important to analyze the relationship between molecular markers and radio-chemotherapy, and explore novel treatment strategies for HGG patients.

The median OS of grade III and grade IV glioma patients were 2–5 years and 12–18 months, respectively.[22–24] Herein, in order to further the different pathology effect on survival, AA-G3, OG-G3 and glioblastoma patients were included to further assess their effect on survival. AA-G3 had the highest incidence rate. Furthermore, OG-G3 patients had the longest median PFS and OS than other pathology of glioma. However, the median survival time was not significantly different between the AA-G3 and glioblastoma patients. These findings indicate that AA-G3 has a higher tumor invasion and poor prognosis even after surgery and radio-chemotherapy.

IDH is crucial for epigenetic regulation of gene expression and DNA repair. IDH1 mutant status is a key marker for prognosis and diagnosis of glioma.[6–11,25] IDH mutations are heterozygous missense mutations found in almost 70–80%

Table 2 Univariate COX Analysis of Primary Cohort for Radio-Chemotherapy in Grade III Glioma Patients

Characteristic		N	Primary Cohort(mPFS)			Primary Cohort(mOS)		
			Months	HR(95% CI)	*P*	Months	HR(95% CI)	*P*
Gender	Male	119	16.6	Ref	0.277	22.9	Ref	0.341
	Female	96	19.9	0.85(0.635–1.139)		24.3	0.865(0.641–1.166)	
Age(years)	≤60	167	20.3	Ref	0.53	25.0	Ref	0.034
	>60	48	16.0	1.398(0.996–1.962)		17.75	1.453(1.028–2.052)	
Pathology	Grade III astrocytoma	129	12.6	Ref	<0.001	19.1	Ref	0.001
	Grade III oligodendroglioma	60	33.2	0.478(0.337–0.678)	<0.001	37.2	0.504(0.351–0.725)	<0.001
	Mixed glioma	16	27.15	0.511(0.288–0.908)	0.022	22.75	0.557(0.306–1.014)	0.056
	Others grade III	10	20.1	0.836(0.408–1.713)	0.625	21.7	0.976(0.476–2.003)	0.947
ECOG PS	0–1	88	22.1	Ref	0.08	25.3	Ref	0.072
	2–3	127	17.4	1.303(0.969–1.754)		21.3	1.324(0.975–1.797)	
IDH-1 mutation	Wildtype	89	10.9	Ref	<0.001	16.6	Ref	<0.001
	Mutation	126	25.1	0.44(0.325–0.596)		33.6	0.42(0.308–0.573)	
1p/19q codeletion	Non-deletion	115	11.5	Ref	<0.001	13.9	Ref	<0.001
	Deletion	100	33.2	0.358(0.264–0.486)		38.5	0.333(0.242–0.458)	
IDH1 and 1p/19q	$IDH1^{w}$ and $1p/19q^{n}$	78	10.5	Ref	<0.001	15.7	Ref	<0.001
	$IDH1^{m}$ and $1p/19q^{d}$	89	36.5	0.329(0.233–0.464)	<0.001	40.2	0.299(0.21–0.427)	<0.001
	$IDH1^{m}$ and $1p/19q^{n}$	37	12.2	1.139(0.755–1.719)	0.535	13.8	0.906(0.587–1.397)	0.654
	$IDH1^{w}$ and $1p/19q^{d}$	11	10.9	1.243(0.639–2.415)	0.522	21.4	0.63(0.314–1.262)	0.192
Ki-67[a]	≤ 22.5%	140	24.1	Ref	<0.001	26.0	Ref	<0.001
	> 22.5%	75	10.2	1.724(1.271–2.338)		15.9	1.556(1.138–2.127)	
ATRX	Non-deletion	10	10.1	Ref	0.012	12.2	Ref	0.247
	Deletion	47	13.9	1.083(0.506–2.318)	0.838	20.5	1.246(0.553–2.807)	0.596
	Unknown	158	22.1	0.642(0.313–1.315)	0.225	24.9	0.903(0.421–1.937)	0.794
MGMT	Non-methylation	47	12.4	Ref	<0.001	17.9	Ref	<0.001
	Methylation	49	31.9	0.402(0.255–0.634)	<0.001	49.7	0.318(0.195–0.519)	<0.001
	Unknown	119	17.7	0.694(0.482–1.0)	0.05	18.9	0.86(0.591–1.251)	0.43
TP53	Wildtype	39	16.6	Ref	0.202	24.3	Ref	0.309
	Mutation	57	14.8	1.34(0.846–2.123)	0.212	20.1	1.369(0.846–2.218)	0.201
	Unknown	119	22.1	0.989(0.655–1.494)	0.96	25.1	1.069(0.694–1.647)	0.762
EMA	Negative	95	16.1	Ref	0.591	22.6	Ref	0.599
	Positive	26	12.3	1.19(0.751–1.884)	0.459	17.9	1.208(0.754–1.936)	0.431
	Unknown	94	22.1	0.938(0.688–1.279)	0.685	25.5	0.95(0.69–1.308)	0.754

(*Continued*)

Table 2 (Continued).

Characteristic		N	Primary Cohort(mPFS)			Primary Cohort(mOS)		
			Months	HR(95% CI)	*P*	Months	HR(95% CI)	*P*
GFAP	Low expression	6	5.1	Ref	0.004	5.1	Ref	0.015
	High expression	103	13.7	0.645(0.261–1.592)	0.341	19.3	0.613(0.248–1.516)	0.29
	Unknown	106	24.1	0.408(0.165–1.011)	0.053	30.1	0.417(0.168–1.034)	0.059
Vimentin	Low expression	14	16.6	Ref	0.183	18.9	Ref	0.123
	High expression	76	13.9	1.03(0.528–2.007)	0.932	19.1	0.956(0.474–1.926)	0.899
	Unknown	125	22.6	0.778(0.405–1.496)	0.452	25.5	0.701(0.352–1.396)	0.312
Monocyte (10^9/L)[b]	≤0.44	130	21.5	Ref	0.037	24.6	Ref	0.028
	>0.44	85	14.9	1.373(1.02–1.85)		20.7	1.411(1.037–1.92)	
NLR ratio	≤4.2	158	19.9	Ref	0.258	25.0	Ref	0.054
	>4.2	57	13.9	1.206(0.872–1.669)		17.9	1.382(0.995–1.92)	
PLR ratio	≤166	146	19.8	Ref	0.97	25.1	Ref	0.366
	>166	69	17.9	1.006(0.737–1.374)		19.3	1.158(0.843–1.59)	
ALB(g/L)[c]	< 40	59	12.9	Ref	0.032	19.1	Ref	0.108
	≥ 40	156	21.5	0.701(0.507–0.969)		24.6	0.76(0.544–1.062)	
LDH (U/L) [c]	≤250	168	21.7	Ref	0.019	25.7	Ref	0.001
	>250	47	11.5	1.516(1.071–2.144)		12.6	1.812(1.272–2.581)	

Abbreviations: IDH1, isocitrate dehydrogenase-1; 1p/19q co-deletion, chromosome 1 and the long arm of chromosome 19; ATRX, alpha-thalassemia/mental retardation syndrome X-linked; Ki-67, nuclear proliferation antigen 67; GFAP, glial fibrillary acidic protein; EMA, epithelial membrane antigen; ECOG PS, eastern cooperative oncology group performance Status; MGMT: O(6)-methylguanine-DNA methyltransferase; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; ALB, albumin; LDH, lactate dehydrogenase; WHO, World Health Organization; $IDH1^w$ and $1p/19q^n$, IDH-1 wildtype and 1p/19q non-codeletion; $IDH1^m$ and $1p/19q^d$, IDH-1 mutation and 1p/19q codeletion; $IDH1^m$ and $1p/19q^n$, IDH-1 mutation and 1p/19q non-codeletion. $IDH1^w$ and $1p/19q^d$, IDH-1 wildtype and 1p/19q codeletion. a=the cut-off points was used by ROC curve (according to grade III and grade IV), b=the cut-off points was used mean value, c=the cut-off points was used relevant assay kits, and all those factors divided into high and low groups for statistical analysis.

of grade II–IV glioma patients.[26,27] Herein, patients with IDH-1 mutation had better survival than the IDH-1 wildtype group after radiotherapy with concurrent oral temozolomide treatment.[13] Although many studies have shown that IDH-1 is a key biomarker for HGG, other studies have shown that several therapeutic opportunities and biology were controversially results, especially in grade III diffuse glioma. In the present study, patients with histologically confirmed HGG were retrospectively identified to better understand the therapeutic effects and prognosis of HGG patients, especially in grade III diffuse gliomas. Results showed that grade III diffuse glioma patients with IDH-1 mutation had better survival than the IDH-1 wildtype group. Additionally, AA-G3 and OG-G3 patients with IDH-1 mutation had a longer PFS and OS than the IDH-1 wildtype group. Notably, the AA-G3 patients with IDH-1 wildtype had a worse prognosis, although these patients receiving radio-chemotherapy were remainly experienced rapid drug resistance and disease progression.

Glioma patients with 1p/19q non-codeletion have a worse outcome than the 1p/19q co-deletion group.[14] CATNON study showed that radiotherapy adjuvant temozolomide improves survival of 1p/19q non-co-deleted anaplastic glioma patients.[13] Although many studies have proven that 1p/19q co-deletion status is correlated with radio-chemotherapy in HGG patients, many biological behaviors and prognosis of grade III glioma are also unknown. In the present study, grade III diffuse glioma patients with 1p/19q co-deletion had a better survival time than the 1p/19q non-codeletion group. Studies have also shown that 1p/19q co-deletion is correlated with IDH mutation status. Herein, grade II and III glioma

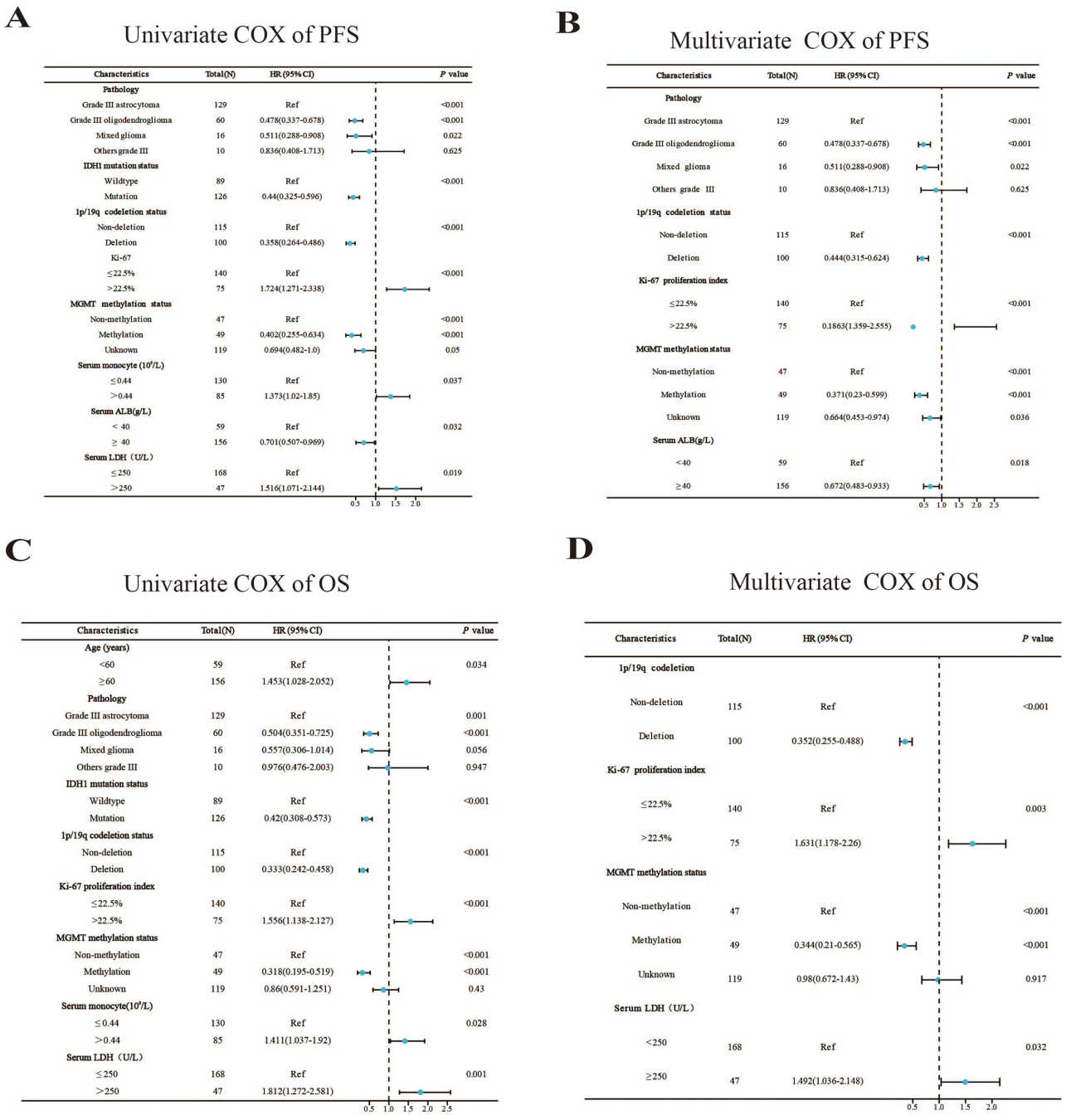

Figure 8 The relationship between clinical characterization and molecular types in grade III glioma patients after radio-chemotherapy. (**A–D**) The key factors found in the univariate and multivariate survival analyses were used to draw a forest map. OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

patients with IDH mutation and 1p/19q co-deletion had the best prognosis.[16] Additionally, 1p/19q co-deletion and IDH-1 mutation patients had significantly better survival than other groups after radiotherapy and adjuvant chemotherapy. Specifically, grade III diffuse glioma patients with 1p/19q non-codeletion and IDH-1 wildtype experienced rapid disease progression and radiotherapy resistance, indicating that the 1p/19q non-codeletion and IDH-1 wildtype patients need more treatment strategies.

MGMT is a DNA repair enzyme and promotes methylation in glioma cells. MGMT can also reverse DNA damage caused by alkylating agents, leading to tumor resistance to TMZ and nitrosourea.[28] Notably, MGMT methylation can predict the effect of alkylating agents in low-grade gliomas and glioblastoma.[29–31] MGMT promoter methylation also has

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Table 3 Multivariate COX Analysis of Primary Cohort for Radio-Chemotherapy in Grade III Glioma Patients

Characteristic	Groups	Primary Cohort(mPFS)		Primary Cohort(mOS)	
		HR(95% CI)	*P*	HR(95% CI)	*P*
Pathology	Grade III astrocytoma	Ref	0.029		
	Grade III oligodendroglioma	0.595(0.403–0.877)	0.009		
	Mixed glioma	0.659(0.365–1.191)	0.167		
	Grade III others	0.557(0.266–1.166)	0.121		
1p/19q codeletion	Non-deletion	Ref	<0.001	Ref	<0.001
	Deletion	0.444(0.315–0.624)		0.352(0.255–0.488)	
Ki-67	≤ 22.5%	Ref	<0.001	Ref	0.003
	> 22.5%	1.863(1.359–2.555)		1.631(1.178–2.26)	
MGMT	Non-methylation	Ref	<0.001	Ref	<0.001
	Methylation	0.371(0.23–0.599)	<0.001	0.344(0.21–0.565)	<0.001
	Unknown	0.664(0.453–0.974)	0.036	0.98(0.672–1.43)	0.917
ALB	< 40	Ref	0.018		
	≥ 40	0.672(0.483–0.933)			
LDH (U/L)	≤250			Ref	0.032
	>250			1.492(1.036–2.148)	

Table 4 Demographic and Clinical-Pathological Characteristics of the Training Cohort and Validation Cohort

Characteristic	Groups	Primary Cohort (n=215, %)	Training Cohort (n=150, %)	Validation Cohort (n=65, %)	*P* value
Gender	Male	119	80	39	0.366
	Female	96	70	26	
Age (years)	<60	167	116	51	0.855
	≥60	48	34	14	
Pathology	Grade III astrocytoma	129	91	38	0.572
	Grade III oligodendroglioma	60	43	17	
	Mixed glioma	16	11	5	
	Others grade III	10	5	5	
ECOG PS	0–1	88	56	32	0.103
	2–3	127	94	33	
IDH1 mutation	Wildtype	89	62	27	0.978
	Mutation	126	88	38	
1p/19q codeletion	Deletion	115	81	34	0.819
	Non-deletion	100	69	31	
IDH1 and 1p/19q	$IDH1^{w}$ and $1p/19q^{n}$	78	53	25	0.631
	$IDH1^{m}$ and $1p/19q^{d}$	89	60	29	
	$IDH1^{m}$ and $1p/19q^{n}$	37	28	9	
	$IDH1^{w}$ and $1p/19q^{d}$	11	9	2	

(*Continued*)

Table 4 (Continued).

Characteristic	Groups	Primary Cohort (n=215, %)	Training Cohort (n=150, %)	Validation Cohort (n=65, %)	*P* value
Ki-67	≤22.5%	140	103	37	0.143
	> 22.5%	75	47	28	
ATRX	Deletion	10	7	3	0.508
	Non-deletion	47	36	11	
	Unknown	158	107	51	
MGMT	Non-methylation	47	33	14	0.358
	Methylation	49	38	11	
	Unknown	119	79	40	
TP53	Wildtype	39	26	13	0.546
	Mutation	57	43	14	
	Unknown	119	81	38	
EMA	Negative	95	68	27	0.818
	Positive	26	17	9	
	Unknown	94	65	29	
GFAP	Low expression	6	5	1	0.694
	High expression	103	70	33	
	Unknown	106	75	31	
Vimentin	Low expression	14	11	3	0.748
	High expression	76	52	24	
	Unknown	125	87	38	
Monocyte (10^9/L)	≤0.44	130	89	41	0.606
	>0.44	85	61	24	
NLR	≤ 4.2	158	108	50	0.453
	> 4.2	57	42	15	
PLR	≤ 166	146	101	45	0.784
	>166	69	49	20	
ALB	< 40	59	41	18	0.957
	≥ 40	156	109	47	
LDH (U/L)	≤250	168	116	52	0.664
	>250	47	34	13	

Abbreviations: IDH1, isocitrate dehydrogenase-1; 1p/19q co-deletion, chromosome 1 and the long arm of chromosome 19; ATRX, alpha-thalassemia/mental retardation syndrome X-linked; Ki-67, nuclear proliferation antigen 67; GFAP, glial fibrillary acidic protein; EMA, epithelial membrane antigen; ECOG PS, eastern cooperative oncology group performance Status; MGMT: O(6)-methylguanine-DNA methyltransferase; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; ALU, albumin; LDH, lactate dehydrogenase; WHO, World Health Organization; $IDH1^w$ and $1p/19q^n$, IDH-1 wildtype and 1p/19q non-codeletion; $IDH1^m$ and $1p/19q^d$, IDH-1 mutation and 1p/19q codeletion; $IDH1^m$ and $1p/19q^n$, IDH-1 mutation and 1p/19q non-codeletion. $IDH1^w$ and $1p/19q^d$, IDH-1 wildtype and 1p/19q codeletion.

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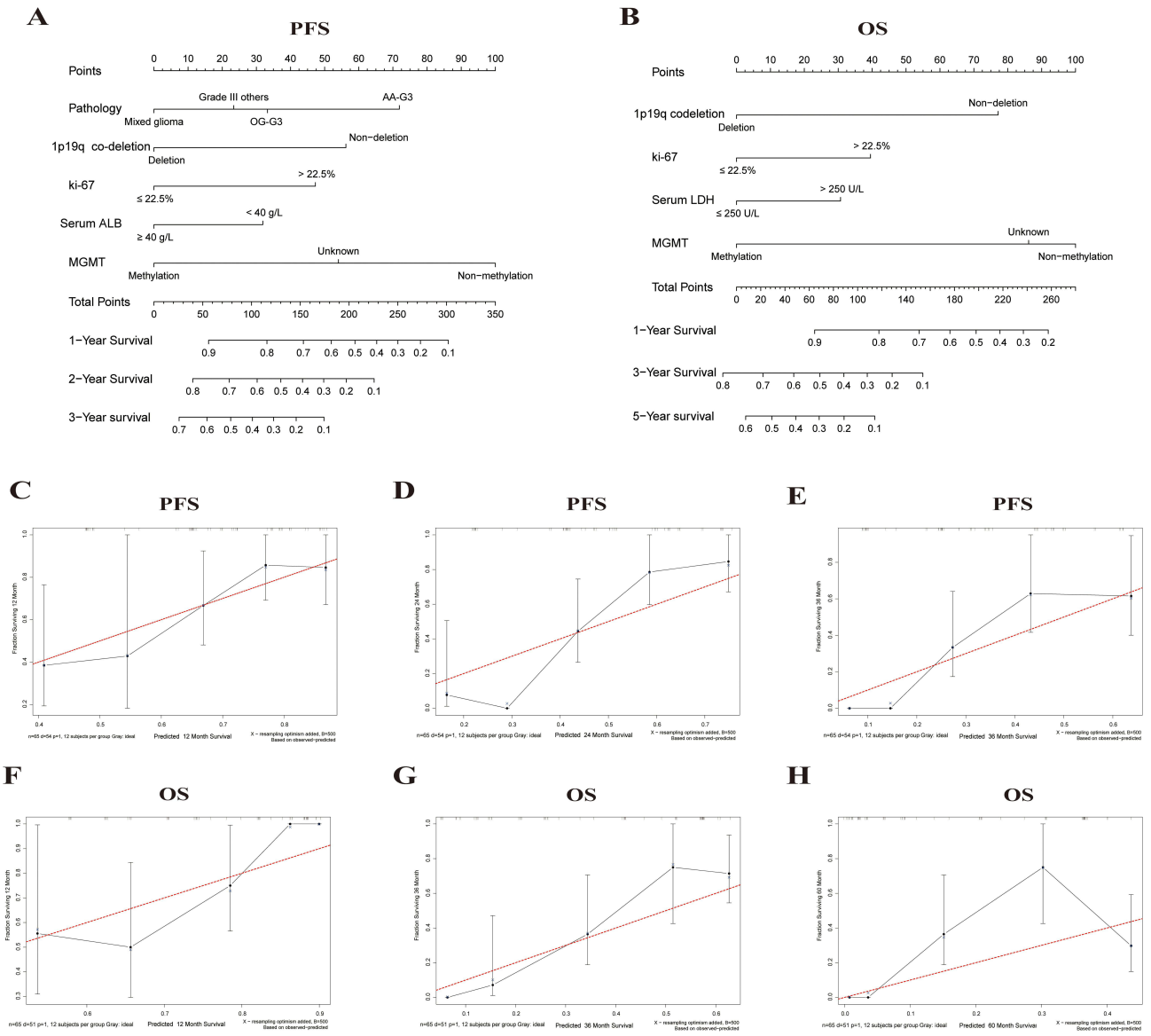

Figure 9 Nomogram development and internal validation. (**A** and **B**) The key factors (statistically significant factors) in multivariate COX analysis were incorporated into the nomogram, then a nomogram was established for PFS and OS. (**C–E**) The internal validation cohort. The calibration plots used to evaluate and validate the 12-, 24- and 36-month PFS probabilities predicted by the nomogram. (**F–H**) The calibration plots used to evaluate and validate 12-, 36- and 60-month OS probabilities predicted by the nomogram. OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma.

a good prognosis for TMZ, but not radiotherapy.[32,33] The EORTC26951 study showed that MGMT methylation is associated with better survival in anaplastic oligodendroglioma patients compared with MGMT non-methylation after radiotherapy alone or sequential radiotherapy and chemotherapy (procarbacine, lomustine, vincristine) group. However, MGMT methylation showed no effect on glioblastoma patients.[34] Notably, the effect of MGMT methylation status on radio-chemotherapy in grade III glioma patients is not fully understand. In this study, grade III diffuse glioma patients with MGMT methylation had a better prognosis than the MGMT non-methylation group, and the MGMT methylation and IDH-1 mutation or 1p/19q co-deletion patients had the best prognosis than other molecular phenotype. Moreover, the MGMT non-methylation with 1p/19q non-codeletion or IDH-1 wildtype experienced rapid disease progression and radiotherapy resistance, and a new treatment strategies need to found, especially in grade III astrocytoma patients. At the present results, as the clinical studies have reported that MGMT methylation is associated with a longer PFS after radiotherapy and chemotherapy (PVC).[35,36]

Ki-67 is an indicator of cellular proliferation and is mainly located in the nucleus. Ki-67 expression level is correlated with glioma grading and molecular classification.[17–19] Specifically, a high Ki-67 expression is associated with poor prognosis.[17] Moreover, our previous study reported that Ki-67 expression is an independent risk factor of glioma grading. Herein, HGG patients had higher Ki-67 expression than lower-grade glioma (LGG) patients. Moreover, the established nomogram could predict HGG.[18] Therefore, our study further shown that grade III glioma patients with low Ki-67 expression had better survival than the high Ki-67 expression group after radio-chemotherapy. The basic hematological and clinicopathological data of a nomogram can predict drug efficacy for malignancy tumors.[37–39] Our previous study found that Ki-67 expression can predict glioma grading.[18] In this study, results showed that IDH-1, Ki-67, 1p/19q status, and clinicopathological data of HGG patients could well predict the survival of grade III glioma patients. Nonetheless, the prognostic molecular and clinical characterization enhances grade III glioma diagnosis and treatment.

However, this study has some limitations. First, the grade III glioma patients received the treatment regimen were not uniformed, including radiotherapy concurrent with chemotherpy or sequential radiotherapy and chemotherapy (temozolomide, procarbacine, lomustine, vincristine), which may lead to different therapeutic effects. Second, this is single centre study retrospectively analyzing the relationship between molecular and radio-chemotherapy in HGG. Therefore, a larger sample size and multicenter research is needed to verify the results. Third, the correlation between preoperative related complications (seizures) and molecular subtypes was not followed up and analyzed in HGG patients. Fourth, this study retrospectively identified the glioma patients between January 2014 and June 2021, and most of the classification and diagnosis of glioma according to the World Health Organization (WHO) classification of tumors of the CNS in 2016 (2016 CNS WHO), however, not the new fifth edition of 2021 WHO CNS 5, may be a key limitation in our study.

Conclusion

In summary, grade III oligodendroglioma had the longest survival time than other grade III pathology patients, while grade III astrocytoma patients were close to IDH-1 wildtype GBM and with a poorer prognosis. Moreover, IDH-1 mutation and 1p/19q co-deletion patients had the best prognosis than other molecular types. Notably, 1p/19q non-codeletion and IDH-1 wildtype patients experienced rapid progressive disease and radiotherapy resistance in HGG and grade III glioma patients. The grade III glioma patients with MGMT methylation had a better prognosis than the MGMT non-methylation patients. Interestingly, it was found that a nomogram established by MGMT, Ki-67, 1p/19q status and clinicopathological data could well predict the curative effect of radio-chemotherapy in grade III glioma patients. Therefore, it is imperative to clarify the predictive and prognostic molecular type in grade III glioma after radio-chemotherapy and explore new molecular markers and treatment strategies for HGG patients.

Abbreviations

HGG, High-grade glioma; LGG, low-grade glioma; WHO, World Health Organization; GBM, glioblastoma; OG-G3, grade III oligodendroglioma; AA-G3, grade III astrocytoma; OS, overall survival; PFS, Progression free survival; IDH1, isocitrate dehydrogenase-1; 1p/19q co-deletion, chromosome 1 and the long arm of chromosome 19; ATRX, alpha-thalassemia/mental retardation syndrome X-linked; Ki-67, nuclear proliferation antigen 67; GFAP, glial fibrillary acidic protein; EMA, epithelial membrane antigen; MGMT, O(6)-methylguanine-DNA methyltransferase; ECOG PS, eastern cooperative oncology group performance Status; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; ALB, albumin; LDH, lactate dehydrogenase; $IDH1^{w}$ and $1p/19q^{n}$, IDH-1 wildtype and 1p/19q non-codeletion; $IDH1^{m}$ and $1p/19q^{d}$, IDH-1 mutation and 1p/19q codeletion; $IDH1^{m}$ and $1p/19q^{n}$, IDH-1 mutation and 1p/19q non-codeletion. $IDH1^{w}$ and $1p/19q^{d}$, IDH-1 wildtype and 1p/19q codeletion; CNS, central nervous system; C-index, concordance index; HR, hazard ratio; CI, confidence interval.

Data Sharing Statement

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Human Ethics and Consent to Participate Declarations

This retrospective study has been reviewed and approved by the ethics institution committee of the First Affiliated Hospital of Nanchang University and Gaoxin Branch of the First Affiliated Hospital of Nanchang University, and the ethics committee approved our study to not need consent from the study participants. Our study of ethics is also in accordance with the declaration of Helsinki, and all the patients' data were kept confidential.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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